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The Synthesis and Biological Evaluation of Novel Derivatives from the Hop Component Humulone

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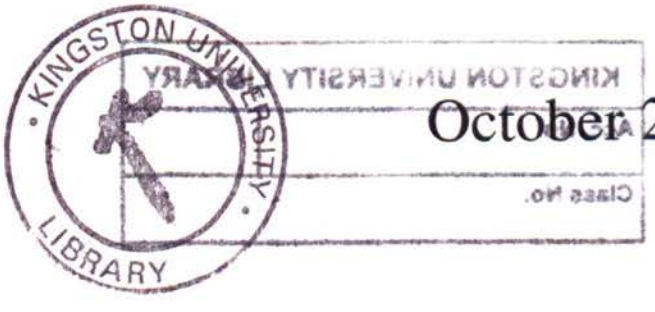
A thesis submitted in partial fulfilment of the requirements of Kingston University for the degree of Doctor of Philosophy

In partial collaboration with

BOTANIX



October 2006

Acknowledgements

Firstly I would like to thank my first supervisor, Dr. Elizabeth Tyrrell, without whose tireless efforts to keep this project running, despite the loss of industrial funding, this project would never have been completed. I would also like to thank Dr. Anthony Skinner, my second supervisor, for all of his help and advice during the project.

I would like to thank all of my friends and family for their support during the time I spent working on my thesis. In particular I would like to thank my parents for their financial support during my Undergraduate Degree and their emotional support during these works presented herein.

I would also like to express my deep gratitude to my partner Leanne, who has endured my extended absences during this project.

I am in debt to all of the technical and teaching staff at Kingston University for all of their help, I am also grateful for all of the help I have had from my colleagues in the research lab, over the final year of this project.

I would like to thank Botanix Ltd for their funding the early stages of the project and Kingston University for their financial support during the final year of my studies. I would also like to thank the EPSRC MS service at Swansea. Finally I would like to thank Jane Hawks from the Chemistry Department at Kings College, London for the INADEQUATE NMR spectra and Kuldip Singh at the Chemistry Department at the University of Leicester for collecting the X-ray data for lupulone, colupulone and the THP ethers of humulone.

He was a wise man who invented beer

- Plato

Beer is proof that God loves us and wants us to be happy.

- Benjamin Franklin

He's great. I love him. He's hops. He's barley. He's protein. He's a meal.

- Denis Leary

Abstract:

Hops have been used in the brewing of alcoholic beverage for over 3000 years. Due to new consumer preferences towards sweeter drinks or 'Alcopops', beer consumption has taken a drastic decline. This has caused the economic value of hops as a commodity to decline, therefore new applications for this material are required.

The hop extracts humulone (8) and lupulone (22) exist in several tautomeric forms. We present unequivocal evidence for the existence of the major tautomer of lupulone, colupulone and a derivative of humulone in polar media. The evidence has been obtained from single X-ray crystallographic data and computer modelling as well as ^{1}H and ^{13}C NMR spectroscopy. The first 2D INADEQUATE spectrum of the tautomer of lupulone provides unambiguous chemical shift assignments for all of the structural carbon atoms.

This project has also involved the investigation into known and novel derivatives of the major constituents of hops, humulone (8) and lupulone (22), as possible bactericides. The main mode of action of these compounds activity against bacterial cells is their ability to chelate metal ions and allow passive diffusion of these

complexes out of cells. A number of chemical derivatives have been synthesised and, using a model developed as part of this research, tested for their potential to pass metal ions from aqueous to non-polar organic media. In addition to this some of the novel compounds have been screened on bacterial samples to determine the structure activity relationship of the known and novel derivatives.

Chapter 1 covers the background of humulone (8) and the hop acids.

Chapter 2 covers the biology of the hop acids including biological activity.

Chapter 3 covers the structural analysis of the hop acids collected during this project and the derivatisation work.

Chapter 4 covers the physical property data and biological data collected during this project.

Chapter 5 gives a brief summary of the achievements of this project and future directions.

Chapter 6 covers the experimental techniques and collected data.

Contents:

Abbreviations:

ABC	ATP- Binding Cassette
ATP	Adenosine triphosphate
COSY	Correlated Spectroscopy
DCC	Dicyclohexylcarbodiimide
DCHA	Dicyclohexylamine
DCM	Dichloromethane
DIPEA	Diisopropylethylamine
DMAP	Dimethylamino pyridine
DMF	Dimethyl formamide
DMS	Dimethylsulfate
DMSO	Dimethylsulfoxide
GC	Gas Chromatography
HETCOR	Heteronuclear Correlation Experiment
HHBA	Hexahydro – β – acids
HMPA	Hexamethylphosphoramide
HPLC	High Performance Column Chromatography
INADEQUATE	Incredible Natural Abundance Double Quantum Transfer Experiment
LAB	Lactic Acid Bacteria
LDA	Lithium diisopropylamide
mCPBA	*meta*-Chloroperbenzoic acid
MEM	Methoxyethoxymethyl
MIC	Minimum Inhibitory Concentration
MOM	Methoxymethyl
NMR	Nuclear Magnetic Resonance
ORD	Optical Rotatory Dispersion
PTC	Phase Transfer Catalyst
PPTS	Pyridinium *para*-toluene sulfonate
TCD	Tricyclodeoxyhumulone

TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
UV	Ultra Violet
XRD	X-ray Diffraction

Chapter 1

Introduction

1.0 Introduction

1.1 Aims of the Project

This project has focused on new applications for hop extracts, namely humulone (8) and its derivatives.

The aims of this project are as follows:

- To develop an improved method for the isolation of large quantities of humulone (8).

- To obtain pure crystalline derivatives of humulone (8) in order to obtain single crystal X-ray diffraction data.

- To spectroscopically characterise humulone (8) and lupulone (22) using advanced spectroscopy.

- To develop a method for resolving racemic ($^{+}/_{-}$) humulone (8).

- Synthesise humulone derivatives in order to provide an indepth chemical study and for biological screening.

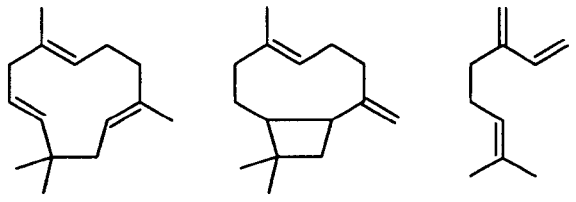
- To biologically evaluate a library of the above compounds.

1.2 Hop Acids

Hops have been used for many years in the brewing industry, along with malt they give beer its unique flavour and aroma. The use of hops in brewing can be dated back to ancient Egypt, some 3000 years ago, where beer was also traditionally flavoured using different herbs such as rosemary or thyme [1]. However, it was discovered that if hops were used in brewing, they not only gave a bitter taste to the brew but the beer would have a longer storage time and therefore could be transported much greater distances without spoilage. The observation of the preservative value of hops predates pasteurisation [2]. Today the consumer has become accustomed to the 'unwanted' bitter flavour consequently all beers and lagers are brewed using hops to a greater or lesser degree [3].

There are many varieties of hops and it was these differences in composition that gave each brewer a method of keeping the brew 'constant'. This was not always the case and often a batch of beer would have problems such as turbidity or off flavour that led scientists to analyse the composition of hops in order to determine the factors that gave beer its aroma and taste. Modern developments in hop extraction and fractionation have allowed brewers to 'fine tune' the desired taste and aroma for the brew more readily, without the use of varietal differences.

As is the case with many botanicals used in the food and beverage industry the aroma comes from the 'essential oil' of the plant. Much research has been carried out into the analysis of this volatile oil, which comprises mostly of the terpenes humulene (1), caryophyllene (2) and myrcene (3) (Figure 1) [4] along with a number of other terpenes, sesquiterpenes and terpenoids, the ratios or presence of which depends greatly on the variety [5].



(1) (2) (3)

Fig 1 Humulene (1) caryophyllene (2) and myrcene (3)

The essential oil found in hops is, however, not bitter. This begs the question as to where does the bitterness arise? Experiments with solvent extracted hops, traditionally from either chloroform or benzene, showed that many hop constituents could be dissolved into alkaline solutions. This suggested that the compounds were acidic. Further separation produced two distinct fractions:

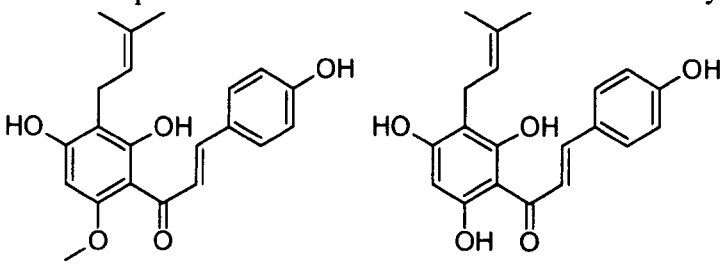
i) Hard resin (1.2.1)

ii) Soft resin (1.2.2)

1.2.1 The Hard Resin

The hard resin, named so due to the physical properties of the extract, consists of compounds that are insoluble in petroleum ether [6]. They consist mostly of polyphenols, flavonoids and chalcones [7]. Along with the essential oil constituents, the presence of these vary considerably with variety. The main components of the hard resins can be separated into just two fractions:

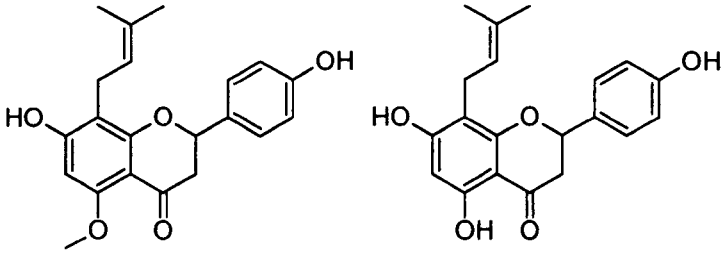
i) Chalcones such xanthohumol [8] (4) and desmethylxanthohumol (5) [9] (Figure 2), these compounds constitute around 0.1 – 0.5% of the dry weight of hops.



(4) (5)

Fig 2 Xanthohumol (4) and desmethylxanthohumol (5) respectively

ii) Flavonoids such as isoxanthohumol [9] (6) and 8-prenylnaringenin [10] (7) (Figure 3), these constitute between 0.005 and 0.01% of the dry weight of hops.



(6) (7)

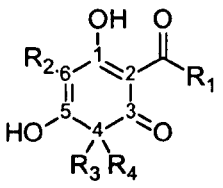
Fig 3 Isoxanthohumol (6) and 8-prenylnaringenin (7) respectively

The flavonoids are converted into chalcones under basic conditions [11] and it is distinctly possible that the presence of the chalcones is merely an artefact of extraction.

These compounds have their uses outside of the brewing industry, for example 8-prenylnaringenin (7) is a potent phytoestrogen used in the treatment of the menopause [12] and xanthohumol (4) has been shown to have anticancer activity [13]. They are, however, considered a nuisance to the brewers as they cause small water soluble proteins to flocculate and hence precipitate out of solution giving the beer a cloudy appearance. However they may be removed from beer made from whole hops by filtering through a bed of polyvinylpolypyrrolidone (PVPP).

1.2.2 The Soft Resin

The soft resin, named after their physical properties, are the extract of hops obtained using petroleum as the solvent [6]. Purification of the alkaline extracts of the soft resins allowed scientists to isolate a range of compounds from the various fractions. The major fraction was separated into the α- and β-acids, both of which have the basic general structure (Figure 4):

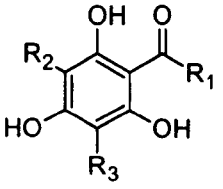


α-acids	β–acids
R_1 = varies	R_1 = varies
$R_2 = R_3 = CH_2CHC(CH_3)_2$	$R_2 = R_3 = R_4 = CH_2CHC(CH_3)_2$
R_4 = OH	

The stereochemistry about C4 will be discussed later

Fig 4 General structure for the hop acids

The similarity in their structures suggest that the α-and β-acids are formed *via* a similar biosynthetic pathway. This is supported by the analysis of extracts taken from immature hops showing the presence of a set of compounds known as the deoxy α-acids [14] (Figure 5).



R_1 = varies

$R_2 = R_3 = CH_2CHC(CH_3)_2$

Fig 5 General structure for the deoxy-α-acids

In the final stage of biosynthesis the deoxy-α-acids are oxidised to the α-acids and *iso*prenylated to the β-acids [15].

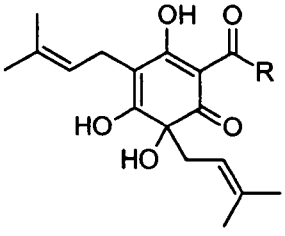
Of all of the natural compounds that are found in hops the α-acids are by far the most important as far as brewing is concerned. The β-acids although useful in brewing, are not as commercially useful as the α-acids. In recent years hop varieties that contain more α-acids than β-acids have been grown selectively by the brewing industry.

1.3.1. The α-Acids

The only characteristic that distinguishes the various α-acids is the nature of the substituent R_1 (Figure 6). There are five known α-acids:

i) n- humulone, abbreviated to just humulone (8)
ii) cohumulone (9)
iii) adhumulone (10)
iv) prehumulone (11)
v) posthumulone (12)

These have the following general structure (Figure 6):



(8) Humulone R = $CH_2CH(CH_3)_2$
(9) Cohumulone R = $CH(CH_3)_2$
(10) Adhumulone R = $CH(CH_3)CH_2CH_3$
(11) Prehumulone R = $CH_2CH_2CH(CH_3)_2$
(12) Posthumulone R = CH_2CH_3

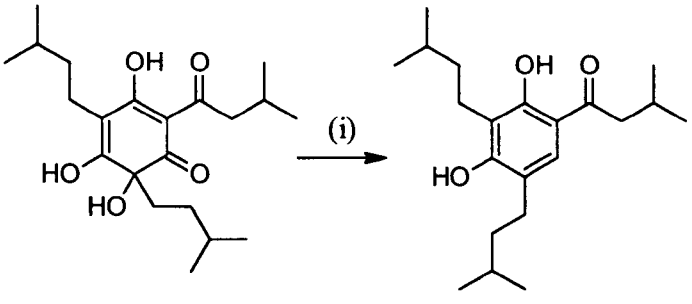
Fig 6 General structure of the α-acids

The structure of these compounds was the subject of some debate over the last century. It was not until the 1950s that a definitive structure was proposed [16], and verified a few years later with their syntheses [17].

Other than slight differences in polarity these compounds all share the same chemistry. The most abundant α-acid is humulone (8), which ranges from 35 – 70% of the total α-acid content. Very few varieties of hops have more cohumulone (9) so as a rule humulone (8) is at least 50% or above [18]. This has made humulone (8) an ideal substrate for investigating the chemistry of these compounds.

1.3.2 Humulone (8)

The tautomerisation pattern of humulone (8) was determined by perhydrogenation of tetrahydrohumulone (13) to give 2,4-di-(3-methyl)butyl-6-(3-methyl)butanoylresorcinol [19, 20] (14).

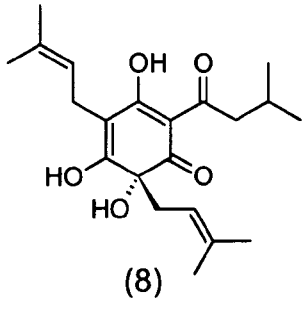


(13) (14)

Reagents: i) PtO_2, H_2PtCl_6, MeOH, H_2

Scheme 1 Perhydrogenation of tetrahydrohumulone

Humulone (8), along with the other α-acids is optically active, by virtue of a stereogenic centre at (C4) which has been assigned as (-)-(R)-humulone (Figure7) [19,20]

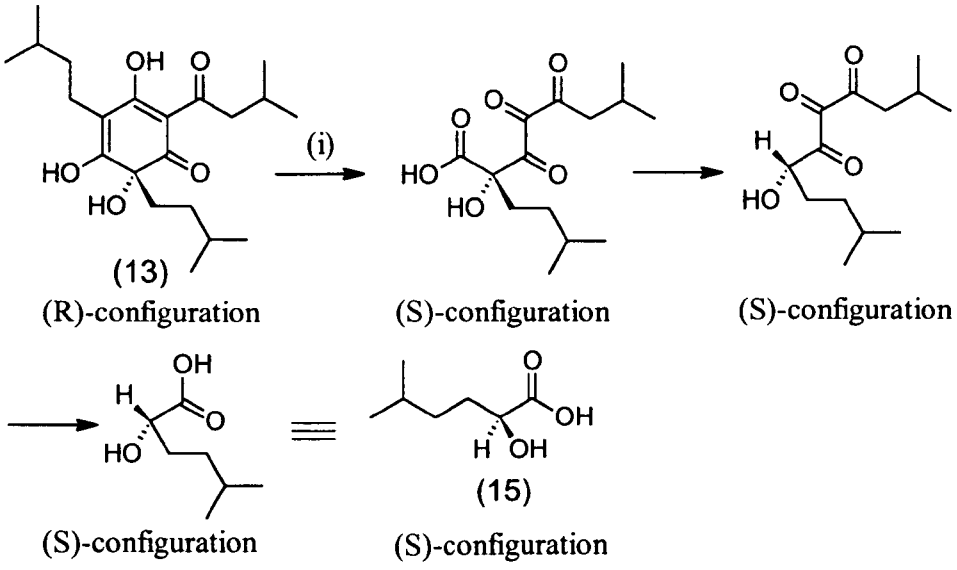


(8)

Fig 7 Accepted absolute configuration of (-)-(R)- humulone (8)

The optical rotation of humulone appears to be solvent dependant, for instance in isooctane the specific optical rotation is -245°. However in basic solution the rotation is positive, for example in one equivalent of methanolic sodium hydroxide the specific rotation is + 32° and in piperidine the rotation is found to be +53° [21]. The absolute configuration was determined *via* ozonolytic degradation of the

hydrogenated derivative (13), which gave the 2-hydroxy acid (15) in which the stereochemistry of this compound was retained [19,20] (Scheme 2).



Reagents i) O_3, Zn, HOAc

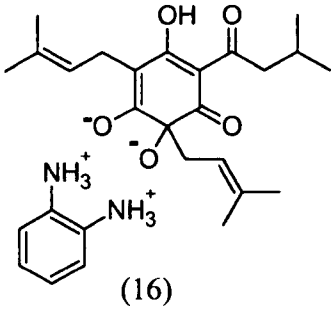
Scheme 2 Ozonolysis of terahydrohumulone (13)

The techniques employed in the determination of this proposed configuration are dated and leave some doubt as to the accuracy, however, no contrary evidence has been published to date and this is the universally accepted structure [19].

1.3.3. Isolation and Purification of (-) - (R) – Humulone (8)

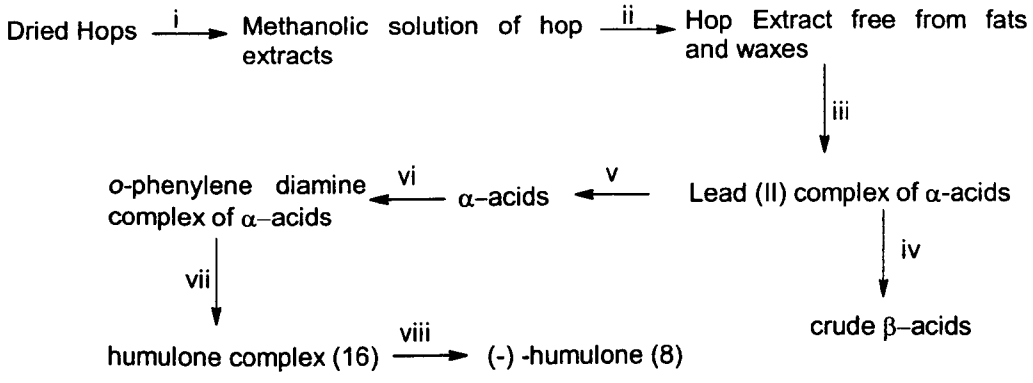
In order to study the chemistry of this compound it was first necessary to isolate it from hops. This is not an easy task as also contained within hops are a number of compounds with similar properties to those of humulone (8), for example, the other α-acids and the β-acids. Many methods have been used throughout the last few decades. However, many pre-date such purification techniques as liquid chromatography and the products were not characterised properly using modern spectroscopy so the purity of the products remains in doubt. All methods involve preparation of an extract of hops *via* solvent extraction. Humulone is freely soluble in a number of solvents. Methanol is often the solvent of choice. The purification

step involves selective complexation [22] of the α-acids followed by recrystallisation of the diamine complex (Figure 8). Decomposition of this complex yields humulone (8) (Scheme 3).



(16)

Fig 8 1,2-Phenylenediaminium humulate (16)



Reagents i) MeOH, ii) 10% wt water, 4°C centrifugation iii) $Pb(OAc)_2$, MeOH iv) NaOH (1M) / H_2SO_4 (1M), hexane v) H_2SO_4 (10% in methanol), hexane vi) *ortho*-phenylenediamine, benzene vii) 7 – 10 re-crystallisation steps from benzene viii) diethyl ether, HCl (2M).

Scheme 3 Isolation and purification of natural humulone (8)

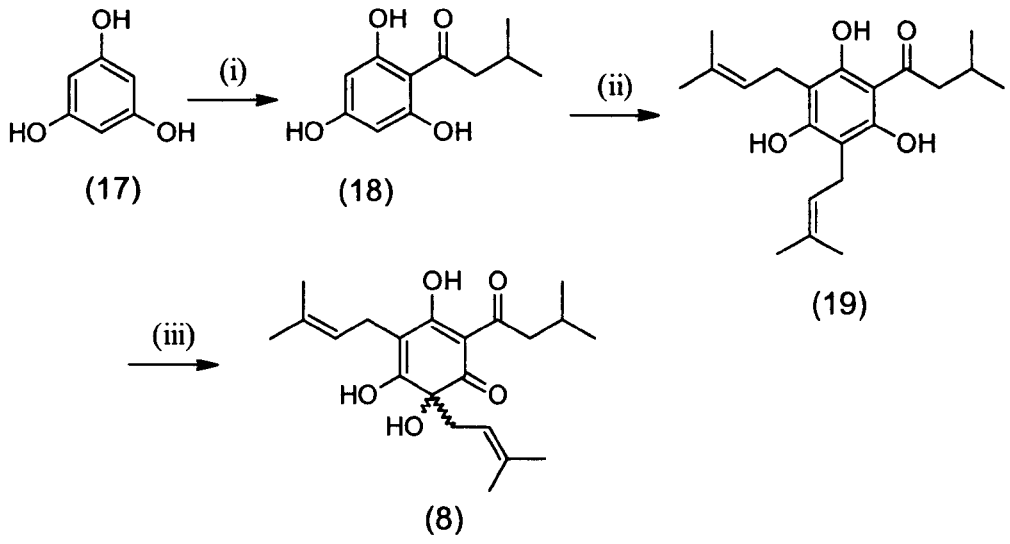
An alternative method is to form the sodium salts of the α-acids, using sodium carbonate [23] as these are sparingly soluble in water at low pH. Thus when a 0.15M solution of sodium carbonate is stirred with a benzene extract of hops, the sodium salts of the α-acids may be extracted as an aqueous solution. Acidification and extraction with hexane yields α-acids. Both of these methods have been superseded by the advent of liquid or supercritical carbon dioxide extraction of hops [24]. Liquid carbon dioxide is far more selective than any other solvent and the solvent is more

easily removed. Hop resin of this sort is used on an industrial scale in brewing and typically a hop may contain a maximum of 30 % CO_2 extractable material.

Other methods for isolating humulone (8) are cited in the literature; however evidence to show that the compound is pure is often based on UV absorption or, in some cases, counter current distribution, a multistage liquid/liquid-extraction process. The standard method used to analyse hops and hop resins produces differing retention times between humulone (8) and cohumulone (9). Pre and posthumulone are in very low concentration and therefore are removed quickly within the first recrystallisation. Humulone (8) and adhumulone (10) both have very similar structures differing only by the position of a methyl group on the acyl side chain, hence no separation is observed by HPLC. However NMR can give definitive proof that pure humulone (8) has been obtained.

1.4.1 Synthesis of (+/-) humulone (8)

(R)-(-)-humulone (8) or (4R)-1,4,5,–trihydroxy-4,6-bis(3-methyl-2-butenyl)-2-(3-methyl-1-oxobutyl)-1,5-cyclohexadien-3-one has a structure unlike any other compound found in nature although there are some similarities observed. It contains a number of functional groups, which make the synthesis challenging. Several syntheses of (+/-)-humulone (8) have been reported in the literature over the last 3 decades and during this time the overall yield has been optimised from 5-10% to around 50%. The most common starting material is phloroglucinol (17) (Scheme 4).



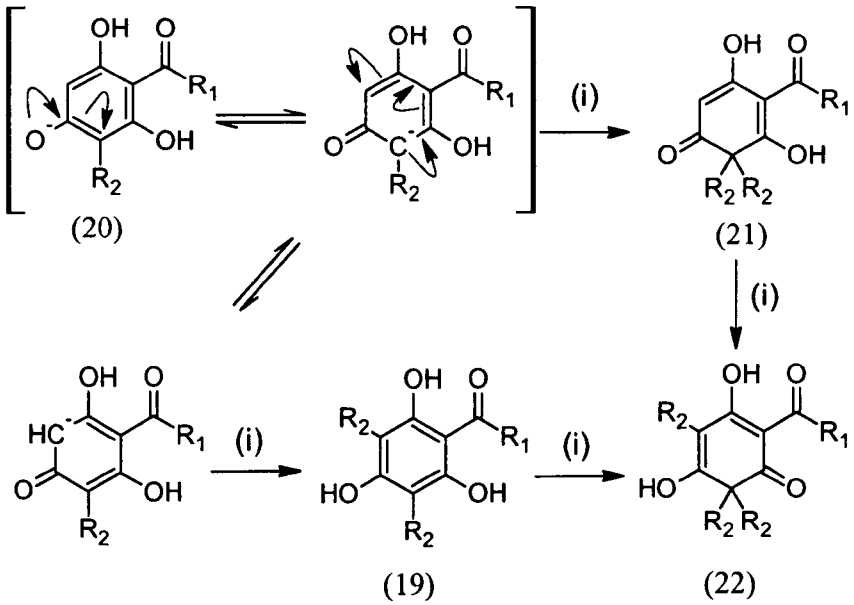
Reagents i) Isovaleryl chloride, $AlCl_3$, nitrobenzene ii) 3-methyl-2-butenyl bromide, sodium, methanol iii) $Pb(OAc)_2$, O_2

Scheme 4 Synthesis of (±) -humulone (8) from phloroglucinol (17)

Friedel-Crafts reaction of (17) with *iso*valeryl chloride provided phlorisovalerophenone (18). This step may also be carried out with isovaleronitrile using the Hoesch synthesis [25]. This initial step often provides variable yields as the solvent of choice, nitrobenzene, is sparingly volatile and must be removed by steam distillation. This was later replaced with DCM and 2 equivalents of nitromethane [17].

The *iso*prenylation reaction is the first indication of the complexity of humulone (8). *Iso*prenylation of the aromatic ring can be carried out in either basic [26] or acidic [27] media, or even using acidic or basic ion exchange resins [28]. All have their disadvantages in that neither produces particularly high yields due to the complex mixtures obtained which have to be tediously separated by column chromatography. It is the functionality contained in the molecule, which causes the synthetic difficulties. Under basic conditions an anion is formed on one or more of the phenol functions which, in the case of mono-*iso*prenylation reacts very efficiently forming 1,3,5-trihydroxy-2-(3-methyl-1-oxobutyl)-4-(3-methyl-2-butenyl) benzene (20) (Scheme 5). Due to the reactivity of the molecule the anionic

charge can then reside on the centre, which has already, been *iso*prenylated forming a 4,4-*bis*-isoprenyl compound (21) (Scheme 5). This is not selective and a significant amount of the 4,6–bis compound (19) is also formed (Scheme 5) which must be removed by chromatography. However, this rarely gives greater than 10% yield. Adding excess isoprenyl bromide simply allows for the formation of the analogous β-acid lupulone (22) [17] or tetrasubstituted lupone (23) (Figure 9) [29].

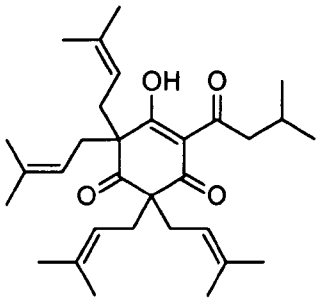


$R_1 = CH_2CH(CH_3)_2$

$R_2 = CH_2CHC(CH_3)_2$

Reagents (i) Isoprenyl bromide, base

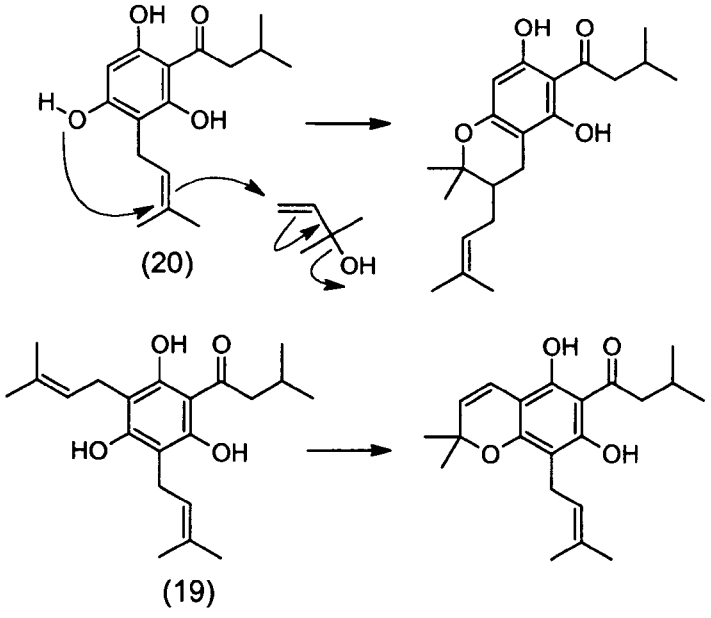
Scheme 5 Addition of second isoprenyl group to 4-isoprenylphlor-2-isovalerophenone (20)



(23)

Fig 9 Lupone

Another method for adding an isoprenyl group to the molecule is to use the corresponding alcohol in a substitution reaction and using a Lewis acid to catalyse the reaction. A commonly used reagent is 2-methyl-3-buten-2-ol and boron trifluoride, this has an advantage in that it increases the yield, however, only to about 20 %. This poor yield is due to the need for column chromatography as the strong Lewis acid also protonates the unsaturated isoprenyl groups and causes a cyclization resulting in furans and pyrans [27] (Scheme 6).

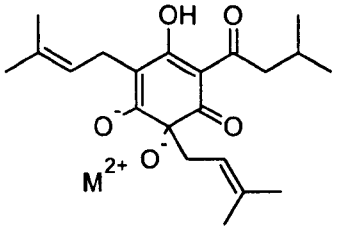


Scheme 6 Some Lewis acid catalysed cyclisations of deoxyhumulone (19) and 1,3,5 – trihydroxy-2-(3-methyl-1-oxobutyl)-4-(3-methyl-2-butenyl) benzene (20)

The use of milder Lewis acids such as zinc chloride, has increased yields to 40%, however the yields were increased to an even greater extent by 'deactivating' one of the enol functions with the use of an acetyl group, although no yields were given. The total yield from phloroglucinol (17) to (+/-)-humulone (8) was reported to be 50% which indicates that the yields are somewhat greater than 40% for this stage in the reaction [17].

There is also evidence in the literature of using phase transfer catalysis for the synthesis of humulone from phlorisovalerophenone [30].

The last stage in the reaction is the oxidation of deoxyhumulone (19) to ($^{+}/_{-}$)-humulone (8), which may be carried out by aerial oxidation. Deoxyhumulone (19) is dissolved in a methanolic solution of lead acetate as air is bubbled through. Once the molecule is oxidised it complexes with the lead ions and forms the insoluble metal humulate (24) (Figure 10), which may be removed by centrifugation, however this often results in poor yields (4-5%).

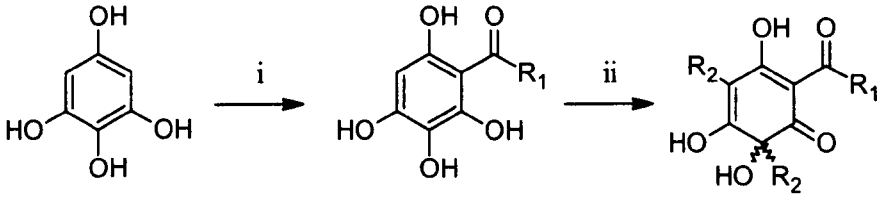


(24)

Fig 10 Formation of metal humulate (24)

More recently it has been discovered that the use of the HO^{+} synthon from peroxides and peroxy acids may be used in place of atmospheric oxygen to give yields of up to 95% [17] [31]. Humulone has also been synthesised by oxidation with enzymes isolated from hop glands [32].

An alternative method employed by a Japanese research group involved a different starting material, 1,2,3,5-tetrahydroxybenzene (25) [33] (Scheme 7).



(25) (8)

$R_1 = CH_2CH(CH_3)_2$

$R_2 = CH_2CHC(CH_3)_2$

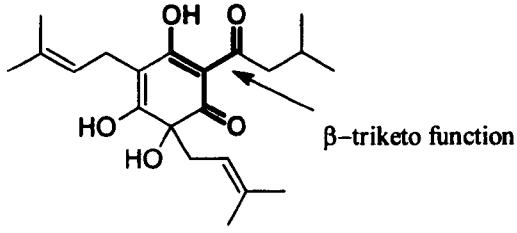
Reagents : i) Isovaleryl chloride, $AlCl_3$, nitrobenzene ii) 3-methyl-2-butenyl bromide, NaH, DMSO

Scheme 7 Synthesis of ($^{+}/_{-}$) humulone (8) from 1,3,4,5-tetrahydroxybenzene (25)

In all cases the synthesis of (+/-) humulone (8) is not stereospecific and no attempts have been made to resolve (+/-) humulone (8) into its (R)- and (S)- isomers [1]. There have been numerous papers published on the synthesis of (+/-)-humulone (8), all of which boast that the particular catalyst of choice is the best for yields and ease of use. It is the author's opinion that natural (-)-(R)-humulone (8) is the best precursor to work with as it is optically pure.

1.5.1 Chemistry of humulone (8)

One of the most interesting chemical features of humulone (8) is its ability to form complexes with metal ions. There is some confusion as to how (8) forms a complex with these metal ions. It is a general belief that when humulone (8) complexes it does so *via* the β–triketo function between (C1) and (C3) involving the isovaleroyl side chain (Fig 11).

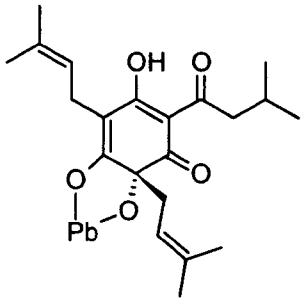


(8)

Fig 11 Proposed complexing function within the humulone (8) molecule

This is very likely to be the case as many compounds, synthetic or natural, contain the same structural moiety that binds with metal cations, such as iron (III) chloride used to visualise TLC plates of such compounds [34]. The α- and β-acids give distinctive colours upon exposure. During the isolation of the α-acids one of the methods employed involves the use of lead (II) acetate which complexes with humulone (8). It does not, however, complex with the β-acids which means that it must complex with another function in the humulone (8) molecule. It would seem logical to hypothesis that the Pb^{2+} complexes with the part of the molecule which is unique to the α-acids i.e. the tertiary alcohol, function at C4. It is known that

humulone (8) forms a 1:1 complex with lead (II) ions in the following complex (26) [35] (Fig 12).



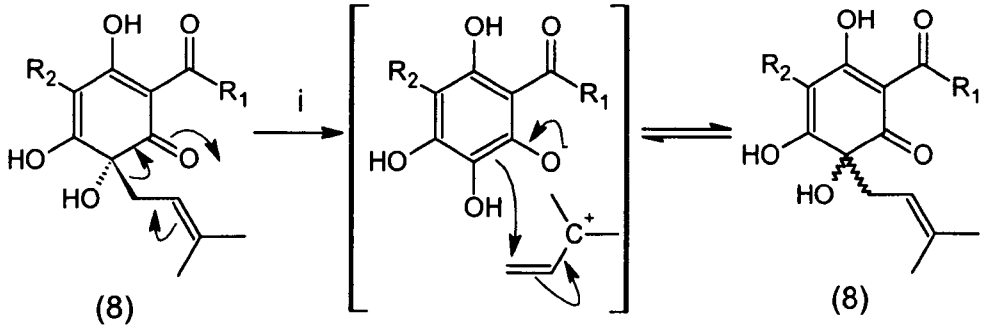
(26)

Fig 12 Lead (II) humulate (26)

These lead salts are very stable and can be heated to temperatures in excess of 100°C in air without degradation.

Humulone (8) also forms complexes with diamino compounds such as 1,2-phenylenediamine (Fig 8) used in the purification of humulone (8). This again is only observed with the α-acids and not the β-acids which would also suggest that the acidic tertiary hydroxyl function at (C4) plays a role.

Racemic (±) humulone (8) can be formed from by heating natural (-)-humulone (8) *via* a racemisation reaction [36] (Scheme 8).



$R_1 = CH_2CH(CH_3)_2$

$R_2 = CH_2CHC(CH_3)_2$

Reagents : i) Toluene, dioxane or isooctane, heat

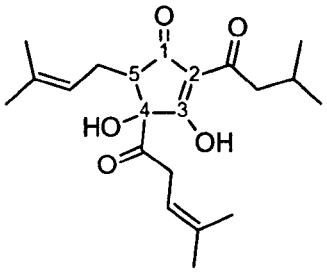
Scheme 8 Mechanism for the racemisation of R - (-)-humulone (8) to (±)-humulone (8)

Evidence in support of this mechanism is provided by the fact that it is necessary for the double bond to be present in order for the racemisation to take place. With tetrahydrohumulone (13), where the exocyclic double bonds are not present, no racemisation is observed under these conditions [37].

1.6 Derivatives of humulone (8)

1.6.1 Isohumulone (25)

One of the most important features of humulone (8) is the reaction it undergoes in the wort during brewing. Analysis of an ether extract of beer shows no α-acids [38]. It does, however show the presence of a compound not usually found in hops, malt or any of the ingredients used to brew beer. Isolation of this compound showed it to have the same molecular weight as humulone (8). It was later discovered that when humulone (8) was boiled in a solution of sodium hydroxide it undergoes a chemical transformation to afford a number of other compounds, which at the time were all termed the 'isohumulones' [39]. The separate fractions were ambiguously referred to as isohumulones A and isohumulones B as some of the earlier literature can be confusing as to what was being referred to. Subsequently it was discovered that boiling humulone (8) in sodium carbonate provides a substance identical to the major component of beer. The brewing wort is maintained at a much lower pH than a sodium carbonate solution. This compound with the same molecular mass as humulone (8), was therefore an isomerisation product and hence named isohumulone (27). This 'isomerised' product is intensely bitter and has been shown to be the principal hop compound from which beer derives its bitterness.



(27)

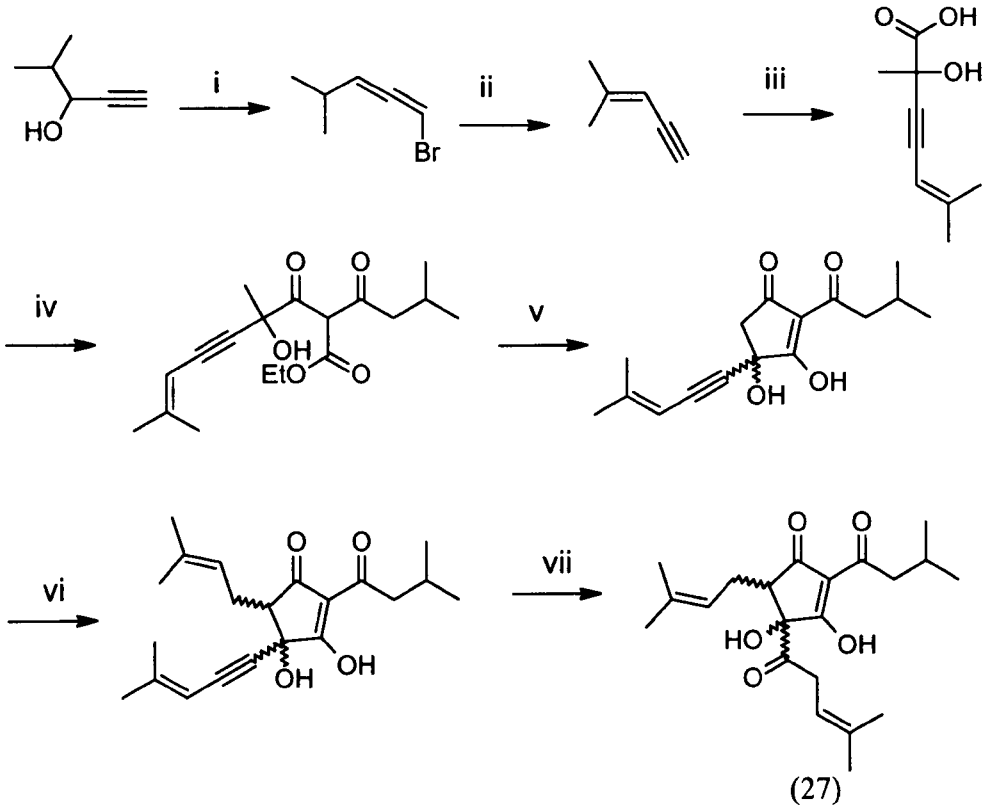
Fig 13 Isohumulone (27) [20]

Isohumulone (27) (Figure 13) contains two stereogenic centres at (C4) and (C5). A closer examination of the isomerisation products of humulone (8) revealed that there were, in fact, two compounds [40]. Both of these compounds were eventually separated and it was discovered that they both had a specific optical rotation. This provides evidence of diastereoisomers being present.

Although it has no commercial use in brewing, due to economic costs, (±)-isohumulone (27) has been synthesised [41]. Obviously simple isomerisation of synthetic (±)-humulone (8) achieves this and racemic tetra-hydrogenated isohumulone has been formed using this method from synthetic (±)-tetrahydrohumulone (13) [42]. The synthesis from lupulone was presented as a method for producing tetrahydro-isohumulone as an HPLC standard [43] [23]. A complete synthesis from acyclic starting materials has been achieved [41]. At the time of the synthesis it was still believed that it was not possible to obtain pure isohumulone A and that a small amount of isohumulone B was always inevitable. The synthesis would allow for purification at each step and no attempts were made to separate the isomers of the racemic isohumulones. Due to the nature of the synthesis both stereogenic centres were racemic hence the end product had no optical activity.

The synthesis, from 4-methylpent-1-yn-3-ol requires 7 steps (Scheme 9) and the yields are very low (<1% overall). However while there was potential to control the stereochemistry in the propargylation step it was never attempted. If this were

achieved it would give more information on the mechanism of the isomerisation reaction which has been debated many times.



(27)

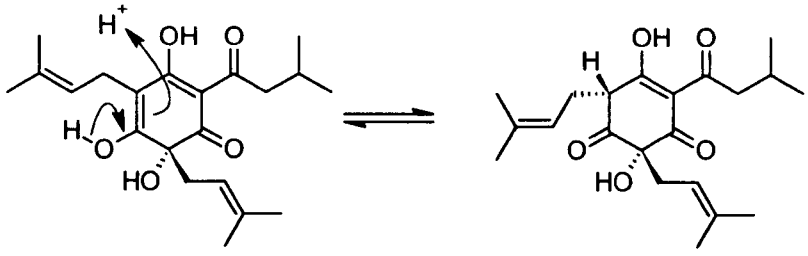
Reagents i) CuBr, NH_4Br, Cu, HBr ii) CuCN iii) THF, Mg, 1-bromopropane, ethyl pyruvate / NaOH, MeOH iv) Et_2O, $(COCl)_2$ / $Mg(OCH_3)_2$ PhH, ethyl 5-methyl-3-oxohexanoate v) t-butanol, KO-t-Bu vi) NaOEt isoprenyl bromide vii) Hg^{2+} H_2O

Scheme 9 Synthetic pathway to racemic isohumulone (27)

Isohumulone B, later named allo-isohumulone, becomes hydrated during the isomerisation procedure to form "hydrated isohumulones" [44]. These have little significance as they are formed in such small amounts, however they were considered as a nuisance by brewers at the time.

The question still remains as to which chiral centre is scrambled and which has a predetermined configuration? No attempts have been made to determine this synthetically, however it has been shown that with racemic (±)-humulone (8), racemic isohumulone (27) is provided [42]. The carbon at (C4) of humulone (8) is

transferred to the acyl side chain at (C4) on the isomerised product. As the tertiary alcohol is converted to a carbonyl group the chirality is lost. However it must be transferred to another carbon atom at some stage during the isomerisation procedure. Many papers have been published detailing the mechanisms by which humulone (8) isomerises. Probably the most widely accepted mechanism, was proposed by De Keukeleire *et al* [45, 46]. They suggested that the first step of the reaction is a keto enol tautomerisation at (C5) – (C6) (Figure 14).



(8)

Fig 14 Tautomerisation of humulone (8) into the keto form

This step is stereospecific and may be related to the conformation of the humulone (8) molecule. Verzele described the (C3) – (C4) bond as having a limited motional capacity and as a result the ring structure is slightly puckered giving the tertiary hydroxyl function a *pseudo*-axial conformation and the isoprenyl group a *pseudo*-equatorial configuration. This puckered structure [42, 34] is likely to influence the configuration at (C6) during tautomerisation [34]. The next step of the isomerisation is the ring contraction which is not stereospecific (Figure 15).

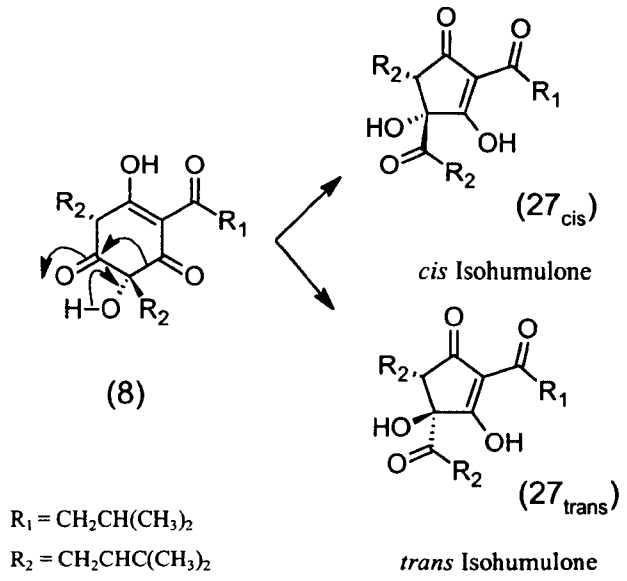


Fig 15 Ring contraction of (-)-humulone (8) to *cis*-isohumulone (27_{cis}) and *trans*-isohumulone (27_{trans})

There remains some dispute as to the mechanism of this reaction [45, 34] and many would argue that it is not the (C5) [34] carbon which has the pre-determined conformation but the carbon at (C4). If this were the case then the mechanism of formation would be different. An irrefutable piece of evidence to support the above mechanism is found when looking at one of the degradation products of the isohumulones, namely humulinic acid (28) (Figure 16).

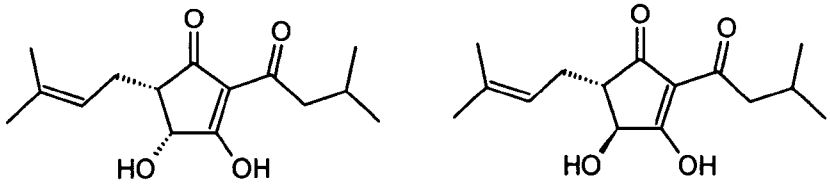
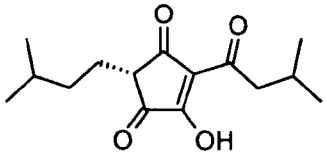


Fig 16 Isomers of humulinic acid (28)

Humulinic acid (28) is formed by the removal of the acyl group at (C4). This is achieved by refluxing either humulone (8) or isohumulone (27) in caustic solution

for 15 – 20 minutes [16] . It belongs to the fraction known as the hard resins, those fractions of hops which are insoluble in hexane. Upon careful oxidation of the secondary hydroxyl function at (C4) of hydrogenated humulinic acid (28) both *cis* and *trans* give the same optically active product (29) [45, 46] (Figure 17).



(29)

Fig 17 Oxohumulinic acid

If the carbon at (C5) had different configurations for the two isomers then the oxidation products would be different; the results show that both compounds have the same physical and chemical properties.

The isomerisation reaction of humulone (8) is a reversible reaction, if isohumulone (27) is shaken in a two-phase isooctane / aqueous buffer (pH = 5.00) for 18 hr then a 10% conversion to humulone (8) is observed [47, 28]. Although it is unclear from the publication, it seems likely that the humulone formed would be in its racemic form (8). The mechanism for this is unclear, however it is thought to proceed *via* an ionic intermediate (Figure 18).

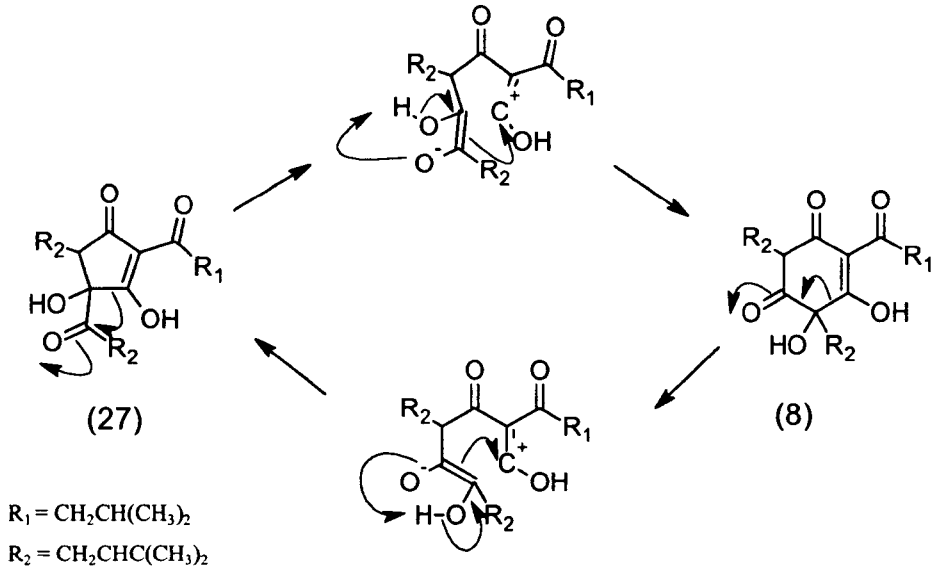


Fig 18 Interconversion of isohumulone (27) and humulone (8)

An interesting optimisation of the humulone (8) ring contraction was discovered when divalent metal cations such as Mg^{2+} or Ca^{2+} were employed as the catalyst [49]. The isomerisation rate proceeded very quickly and efficiently giving yields of approximately 95% with virtually no degradation products. Humulone (8) may also be isomerised in methanol using UV light [50,22], however this forms only *trans*-isohumulone (27_{trans}). Pure *cis*-isohumulone (27_{cis}) cannot be formed directly, however an enriched mixture may be formed using heat, i.e. when humulone (8) is boiled in methanol for several days, *cis*-isohumulone (27_{cis}) is isolated albeit in very low yield.

Interestingly enough, although humulone (8) forms a stable salt in the presence of lead (II) acetate, if the isohumulone salt of this compound is heated to 110 °C then almost complete conversion to humulone is observed.

Another method for producing pure *trans*-isohumulone (27) is still used in the industrial production of HPLC standards for hop analysis [51]. This is achieved by means of complexation, rather like the method employed for the production of pure humulone (8). A sample of isohumulones (27) (*cis/trans* mixture) are dissolved in a

small quantity of ethyl acetate and an equimolar quantity of dicyclohexylamine (DCHA) is added. The *trans*-isohumulone (27_{trans}) forms an insoluble complex and may be removed by filtration. Subsequent recrystallisation of this complex provides pure white crystals which may be employed directly as a standard. DCHA does not absorb in the same UV region as the iso-α-acids and due to the relative acidity of the industries standard HPLC mobile phase (pH ca. 5-6) the complex is decomposed as soon as the sample is injected.

The free acid may be liberated from this salt by means of acid degradation using ethyl acetate and HCl to give the pure *trans*-isohumulone (27_{trans}).

The iso-α-acids are made on an industrial scale and sold as an aqueous potassium salt as this seems to be the most stable formulation for storage and ease of use. This preparation can be added to the brew, post fermentation, to give the desired level of bitterness. The isohumulones have their disadvantages, when used in brewing, as they are not stable to light this may be a nuisance even in traditionally brewed ales. The bond to the acyl side chain at C4 is weak and may be radically cleaved by sunlight to form a 3-methylbutenyl radical [52] (Figure 19).

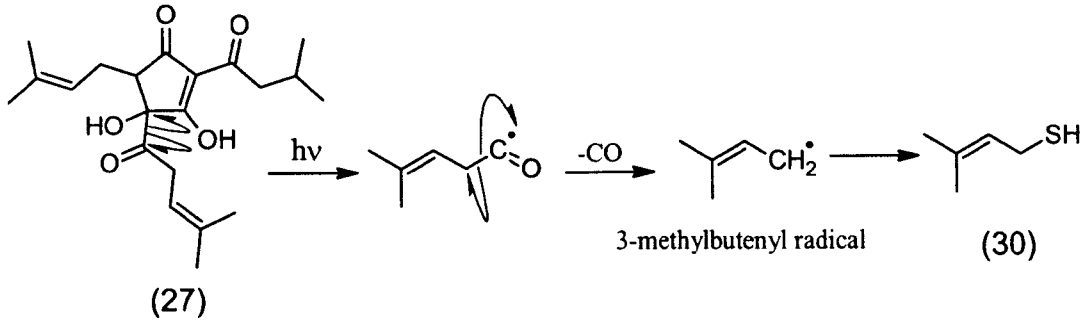


Fig 19 Formation of the 3-methyl butenyl radical from isohumulone (27)

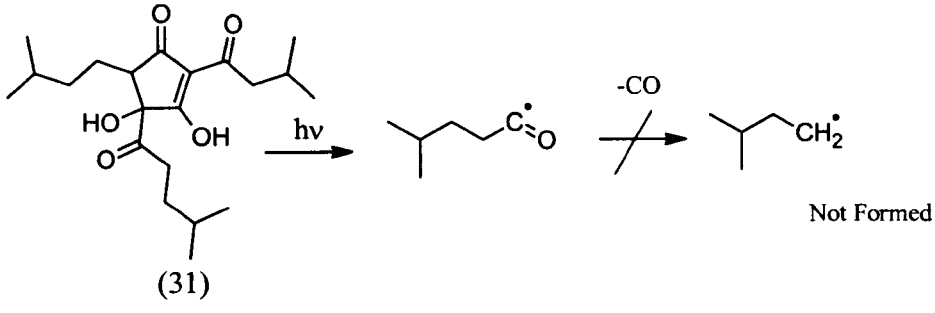
This relatively stable radical then reacts with sulphur, probably contained within proteins such as methionine or cysteine , to form a compound known as 3-methyl-2-buten-1-thiol (30) (Figure 19) or 'skunky thiol' as it is often referred to as it is the same chemical produced by North American skunks as a defence spray.

This reaction is thought to be initiated by riboflavin (vitamin B_{12}) as the photosensitiser. In beer this reaction has to be avoided, as even levels as low as 1 ppm produce a negative flavour profile in beer.

As with most photosensitive compounds packaging the product in dark glass to stop light is sufficient to inhibit this reaction. However, the consumer dictates that it is preferential for beer to be packaged in clear glass bottles. This has developed the need for 'light stable' products. Stabilisation can be achieved in one of two ways:

i) hydrogenation
ii) reduction

i) Hydrogenation over palladium produces a tetra-hydrogenated product (31) (Figure 20) whereby the exocyclic double bonds are hydrogenated. Although this does not stop the photochemical cleavage reaction it does reduce the stability of the radical generated so the thiol compound is not formed [53].



(31)

Fig 20 Tetrahydro-isohumulone (31) and the effect of UV radiation.

The tetrahydro-isohumulones have other advantages over isohumulones as they are more intensely bitter, therefore less is required. Also they give beer a greater foam stability which is important in maintaining a 'head' on the beer [54]. They also have a greater bacteriostatic effect; this will be discussed more fully later.

ii) Selective reduction of isohumulones to the ρ- isohumulones (32) (Figure 21) is achieved using sodium or potassium borohydride. The potassium reagent is used

preferentially in industry due to complexation problems associated with sodium salts. The carbonyl group is reduced to the corresponding alcohol, making the bond stronger and less susceptible to fission [55].

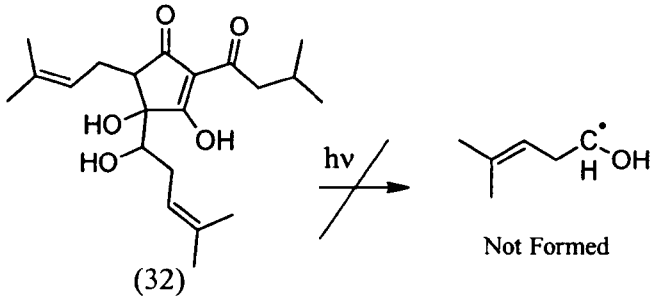
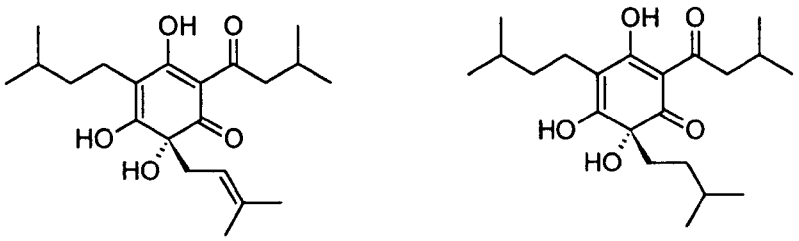


Fig 21 ρ-Isohumulone (32)

The reduction of the isohumulones introduces a new chiral centre to the molecule, which provides diastereoisomers. Although the reduced isohumulones (32) are less bitter than the isohumulones (27) or tetrahydro-isohumulones (31) they have the advantage in that production costs are considerably less than the hydrogenated product. It should be noted that in addition to these products a material which is both hydrogenated and reduced with borohydride is available, known as the hexahydro-isohumulones.

1.6.2 Reduction of humulone (8)

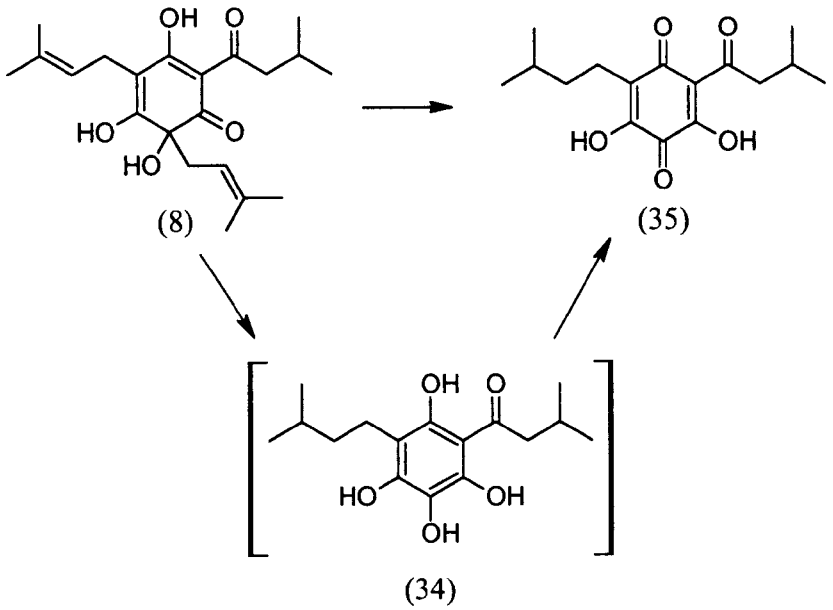
This is not a well-explored area as although humulone (8) contains a number of functional groups, reduction of these is not always possible. For example it remains inert to mild reduction conditions of sodium borohydride, although strong reducing agents such as the aluminium hydrides have not been tested. All of the literature methods of reductions use hydrogen gas, hydrogenation over platinum (IV) oxide to give primarily dihydro (33) and eventually tetrahydrohumulone (13) (Figure 22) [56].



(33) (13)

Fig 22 Dihydrohumulone (33) and tetrahydrohumulone (13)

Shaking a methanolic solution of humulone (8) with hydrochloric acid and hydrogen (Pd-Cat), gives humulohydroquinone (34) which is rapidly oxidised to humuloquinone (35) (Figure 23) during work up [57].



(8) (35) (34)

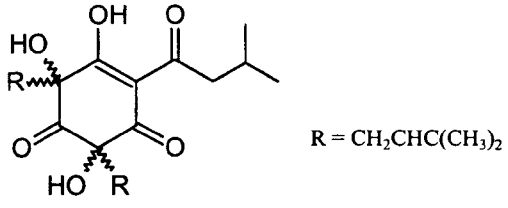
Fig 23 Humulohydroquinone (34) and humuloquinone (35)

1.6.3 Oxidation of Humulone

The oxidation of humulone (8) is of great interest to brewing chemists. During prolonged storage of hops at ambient temperatures in air the α-acid content can be reduced by up to 70 %, however beer brewed with such hops still retains 80% of its bitter character [58]. This led chemists to hypothesise that the oxidation products are bitter. There are hundreds of oxidation products of humulone (8), often formed as

complex mixtures, which must be tediously separated using counter current separation. However there are many compounds, which are of great interest and can be isolated in reasonable to good yields.

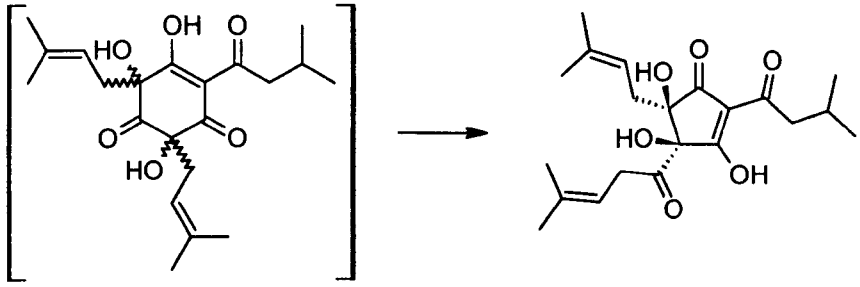
Oxidation of (-)- humulone (8) with cumenylhydroperoxide gives an hydroxylated, optically inactive product, which has been named humulinone (36) (Figure 24) [59]. This compound was first thought to be found naturally in hops [60], however it was later discovered to be an artefact of extraction [61].



(36)

Fig 24 Proposed structure for humulinone (36)

This is not the universally accepted structure for humulinone; it has been argued that the above product undergoes a spontaneous ring contraction to give the following five membered ring product (37) (Figure 25) [62].

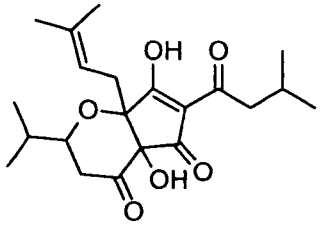


(36) (37)

cis-racemate

Fig 25 5-Membered ring structure of humulinone (37)

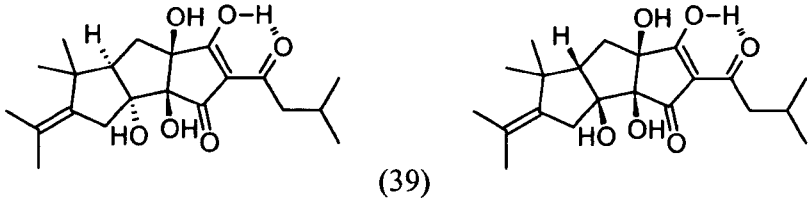
The argument remains open as another group of researchers seem to have isomerised humulinone (37) into isohumulinone (38) (Figure 26) [63]. However the structure of this differs still from the molecules proposed earlier [64].



(38)

Fig 26 isohumulinone (38)

This structure for isohumulinone was later rejected due to its lack of reactivity towards sodium borohydride [65]. It was hypothesised that the structure was more likely to exist as the following tricyclic compound (39) (Figure 27).

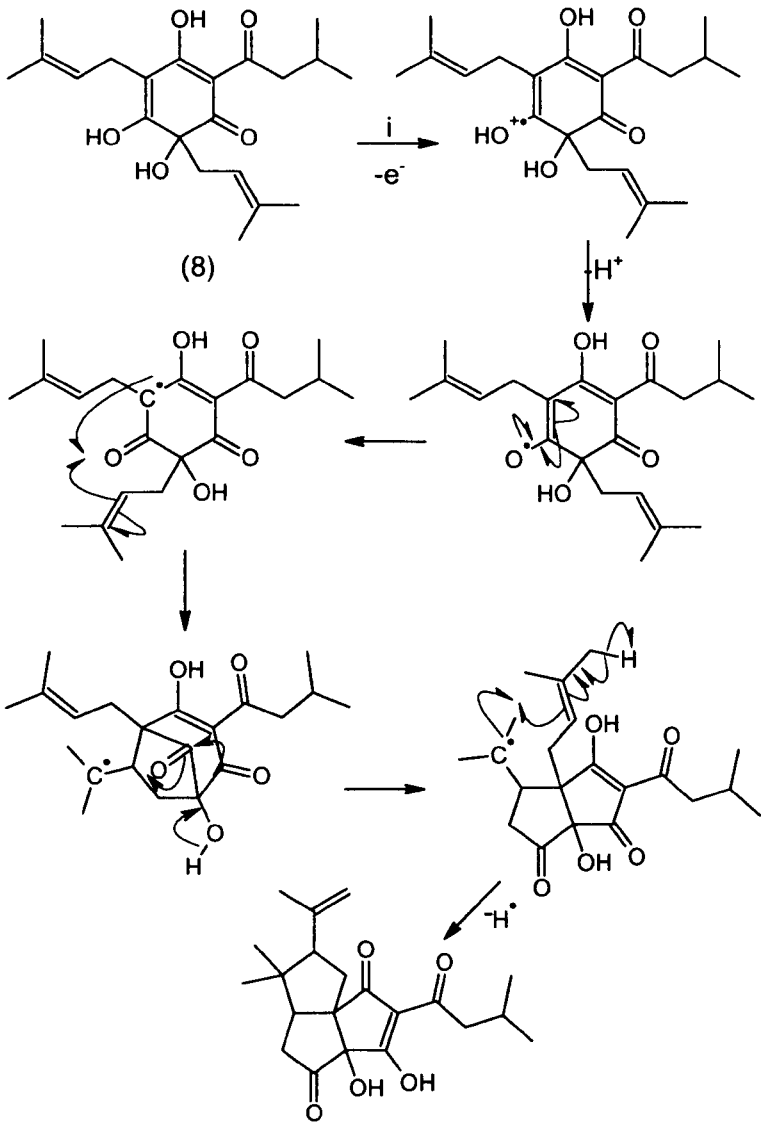


(39)

Fig 27 Diastereoisomers of isohumulinone (39)

Prolonged heating or treatment with acid yields degradation products, which are tricky to separate.

Free radical oxidation of humulone (8) yields a compound named TCD or tricyclodehydro-isohumulone (40) (Scheme 10). This is a solid derivative formed by the reaction of lead (IV) acetate in acetic acid at low temperatures [66]. The product of the reaction is an acetic acid solvate of TCD, which must be purified by sublimation or by shaking overnight in water [67]. Advances in free radical chemistry may allow for better reaction conditions, hence a product, which is easier to purify.



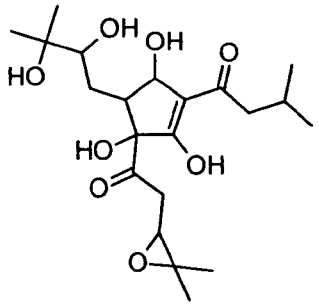
Reagents i) $Pb(OAc)_4$, AcOH

Scheme 10 Formation of TCD (40)

Many structures have been proposed for TCD. However, structural analysis of the product supports the above structure (40).

Other methods for the oxidation of humulone (8) are the use of peroxy acids, with different acids giving very different products. It is likely that the pH of the resultant reaction mixture has a large influence on the product distribution. Reaction of

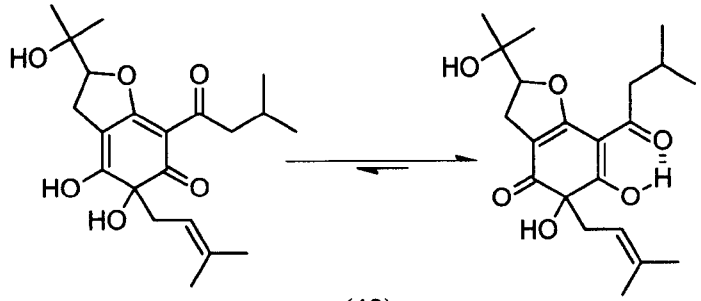
humulone (8) with monoperphthalic acid, in diethyl ether, followed by column chromatography yields the following compound (41) (Figure 28) [68].



(41)

Fig 28 Monoperphthalic acid oxidation product (41) of humulone (8)

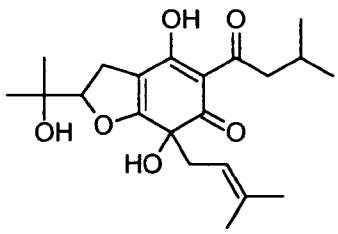
In contrast to this, oxidation of humulone (8) using *meta*-chloroperbenzoic acid gives the following product (42) (Figure 29) [58, 69].



(42)

Fig 29 Proposed Structure for mCPBA oxidation product (42) of humulone (8)

There is some scepticism as to the determination of this structure and it has been argued that although, in principal, the molecule is correct, the β–triketo-function remains intact. It is not clear as to how it was deduced that this function is unaffected. Possibly the deshielding of the hydrogen bonded proton would be less pronounced and thus the resonance by NMR spectroscopy would be less downfield. This being the case the more generally accepted structure for the product is as follows (43) (Figure 30).



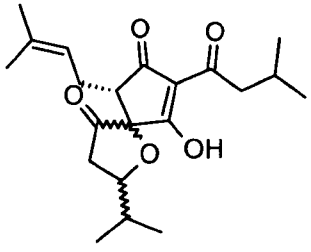
(43)

Fig 30 Preferred structure for mCPBA oxidation product (43) of humulone (8)

The two loudest voices in this argument, concerning structure, carried out the reaction in different solvents, which may have some effect on the outcome.

1.6.4 Rearrangement Reactions of Humulone

The isohumulones (27) are the most important reaction bi-products during brewing. They readily undergo the loss of an acyl side chain to form humulinic acids (28). However, if the isomerisation reaction is carried out in a non-aqueous medium, such as methanol, the products formed are known as the spiro-isohumulones (44) (Figure 31). Due to the loss of the double bond, new chiral centres are formed. The result is the formation of four diastereomers. As with the isomerisation to isohumulone the centre at C5 is not racemic [70].



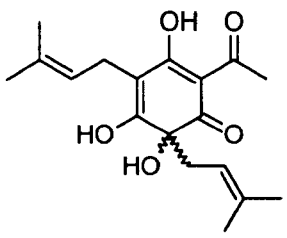
(44)

Fig 31 Spiroisohumulone (44)

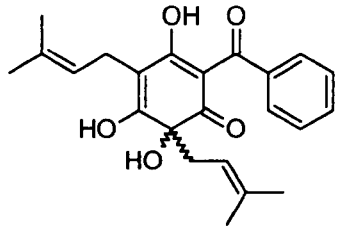
1.6.5 Synthetic Derivatives of Humulone (8)

Little work on the derivatisation of humulone (8) has been documented. Any derivatisation work that has been carried out has been to aid the elucidation of the

structure of many of the compounds discussed previously. The derivatives studied have been synthesised from organic materials not derived from hops. Such materials are direct analogues of humulone (8) where the side chains have been replaced, for example, the acyl side chain has been replaced by acetyl (45) or benzoyl (46) groups (Figure 32) [71].



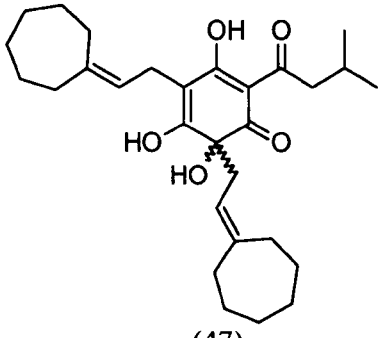
(45)



(46)

Fig 32 Acetyl humulone derivative (45) and benzoyl humulone derivative (46)

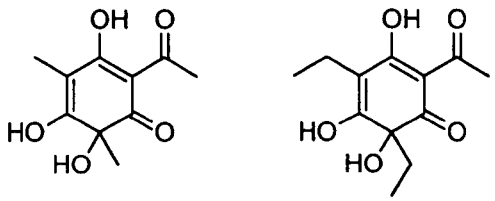
The side chains have also been replaced with other groups to form a more stable compound (47) (Figure 33) to aid analysis [58].



(47)

Fig 33 Side chain analogue (47) of humulone (8)

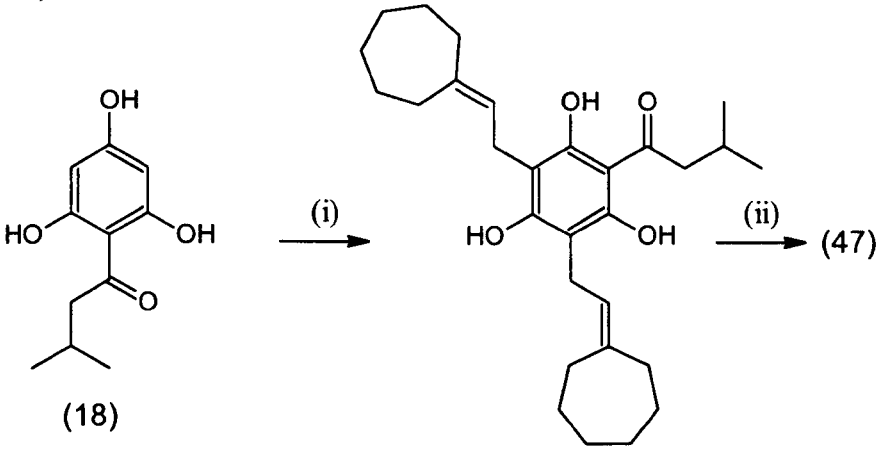
To assist in the structure determination of humulone (8) simpler model compounds were synthesised with much smaller side chains such as methyl (48) or ethyl (49) groups (Figure 34) [72]. The results of these were unsatisfactory, as the model compounds apparently did not undergo the important ring contraction reaction of humulone (8) [73]. However, earlier reports indicated that these compounds did indeed isomerise into compounds analogous to the iso-α- acids [74].



(48) (49)

Fig 34 Model compounds of humulone (8)

An important feature in the synthesis of humulone derivatives is the presence of the double bonds in the exocyclic chains. Compound (45), for instance, can be synthesised from commercially available 2',4',6'-trihydroxy acetophenone directly and compound (46) may be synthesised in the same way *via* a Friedel Crafts acylation of phloroglucinol (17) with benzoyl chloride. Compound (47) was synthesised from phlorisovalerophenone (18) using the corresponding unsaturated alcohol and an acid catalyst, followed by oxidation to give compound (47) (Scheme 11).



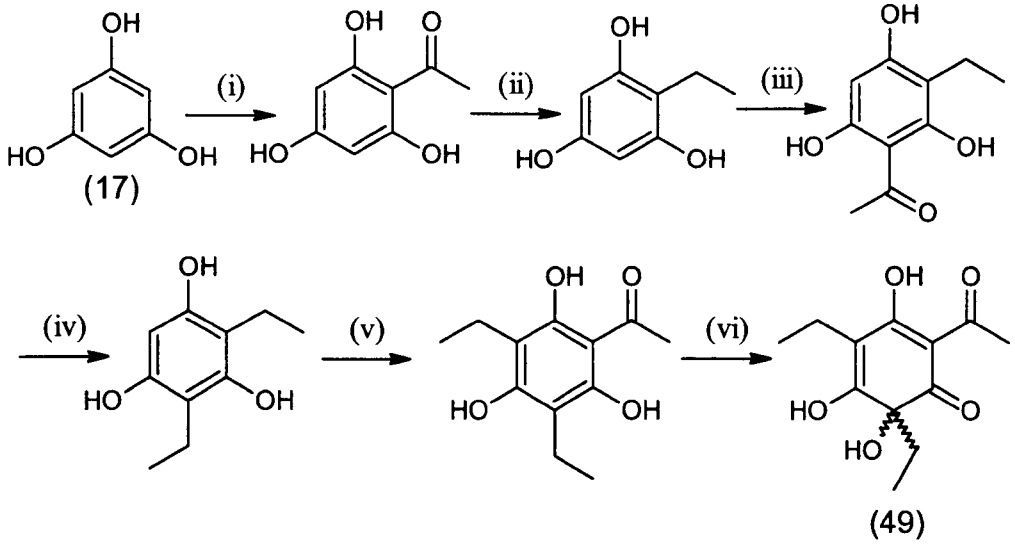
(18)

Reagents i) H^+, 2-cycloheptylideneethanol, ii) $Pb(OAc)_2$, O_2

Scheme 11 Synthesis of compound (47)

Compounds (48) and (49) cannot be synthesised from 2',4',6'-trihydroxy acetophenone using alkyl halides as the alkylating agent as this leads to formation of ethers. It seems that it is necessary for the double bond to be present in order for the electrophile to react at the carbon centre in contrast to the oxygen moiety. Production of compounds (48) and (49) were achievable *via* the method outlined in

a patent by Guinness and Son Ltd [25] whereby phloroglucinol (17) was acylated to the corresponding formyl or acetyl derivative which was then reduced by Clemmensen reduction to from the appropriate alkane this was repeated and finally the compound was acylated to give the deoxy derivative of the model compound which was then oxidised to give the respective compound (48) or (49) (Scheme 12):



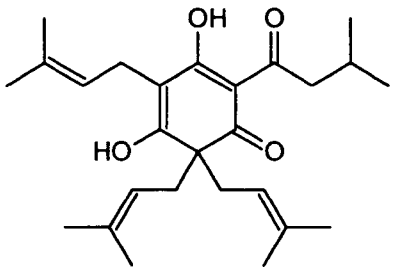
Reagents i) AcCl, $AlCl_3$, ii) Zn/Hg HCl, iii) AcCl, $AlCl_3$, iv) Zn/Hg, HCl, v) AcCl, $AlCl_3$, vi) O_2
Scheme 12

If other alkyl derivatives are required the yields can be poor due to the increased number of synthetic steps. These synthetic derivatives can now be synthesised using the method discussed above whereby 1,2,3,5-benzenetetrol is employed as the starting material.

1.7 Beta Acids

1.7.1 Structure

Although the β-acids are not, as such, derivatives of the α-acids they are structurally analogous (Figure 35) and do have similar chemical properties.

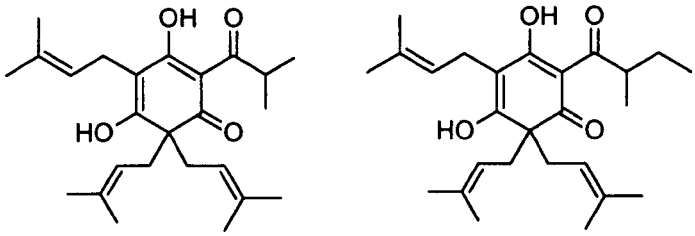


(22)

Fig 35 Structure of β-acid lupulone (22)

1.7.2 Isolation

The isolation of the β-acids is a little less difficult than the isolation of the α–acids. Indeed even allowing a liquid CO_2 extract of hops to stand for a few days will precipitate β-acids, although the resinous material is too thick to filter. After extraction of the α-acids, by whatever method, a mixture of β-acids, oils, fats and waxes remain. Using a strong alkaline solution will give a solution of the β-acids and subsequent acidification and hexane extraction yields, after removal of the solvent, a crude β-mix as a red brown oil. This can then be redissolved in a small quantity of hot hexane and allowed to cool. Upon cooling the β-acids are precipitated as white needles. The β-acids (Figure 36) differ from the α-acids in more than one way, the known β-acids are as follows:



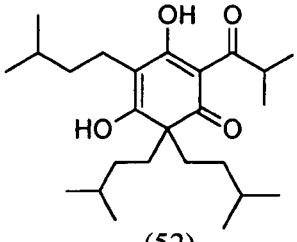
(50) (51)

Fig 36 Structures of colupulone (50) and adlupulone (51)

So far no pre or post lupulones have been detected in hops. However the ratios of the β-acids differ, with colupulone (50) found in the highest abundance (certain

European varieties will give higher amounts of lupulone but they are not common). After numerous re-crystallisations from hexane or isooctane (2,2,4-trimethylpentane) pure colupulone (50) is isolated. A mixture of the β-acids are much more soluble in petrochemicals than when pure. Pure colupulone is very insoluble in light petroleum (1g in 100 ml at rt) [75]. Once the β-acids have been isolated, they degrade very rapidly in air so they must be stored at low temperatures in the absence of light and oxygen. A sample of β–acids converts very rapidly (ca 1- 2 weeks) from white needles to a brown resin if left unprotected [76].

Hydrogenated β-acids (hexahydro-β-acids, HHBA) are commercially available as an off white powder which is more stable in air [76] (52) (Figure 37).



(52)

Fig 37 hexahydro-colupulone (52)

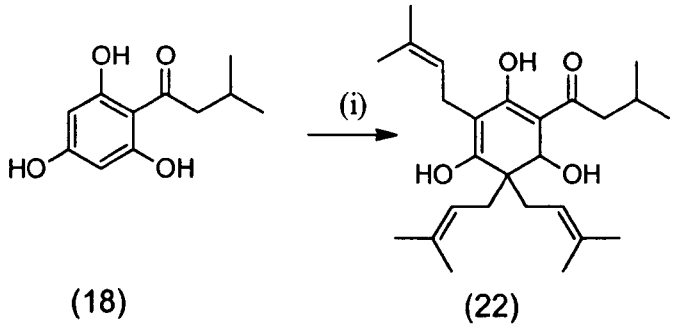
1.7.3 Chemistry of the β-acids

The chemistry of the β-acids, with respect to brewing, has received more attention than the α-acids due to the oxidation and consequent rearrangement products of the β-acids imparting bitterness to the brew [9]. Indeed the number of different compounds isolated from a brewing simulation of lupulone is immense.

Noteworthy is the fact that, unlike the α-acids, the β-acids exist in more than one tautomeric form [77].

1.7.4 Synthesis of β–acids

Many of the methods outlined for the synthesis of humulone can be used in the synthesis of lupulone. Indeed in many of the described synthesis lupulone is an unwanted by-product and must be removed by chromatography. In an innovative optimisation of the synthesis of lupulone, liquid ammonia was used as the solvent and base to produce lupulone in 64% yield from phlorisovalerophenone (18) [78] (Scheme 13).



(18) (22)

i) $NH_3(l)$, $(CH_3)_2CCH_2Br$,

Scheme 13 Synthesis of β-acids

1.8.1 Natural humulone analogues

There are a number of compounds found in nature that contain the humulone (8) nucleus (Figure 38).

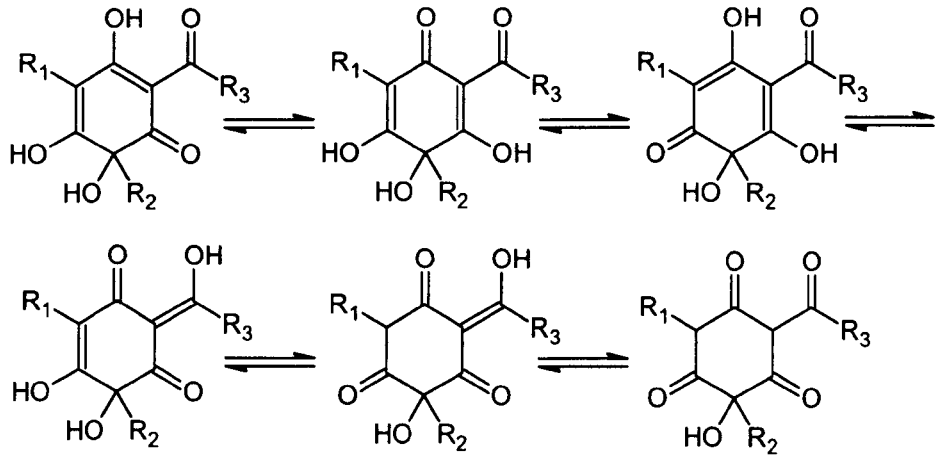
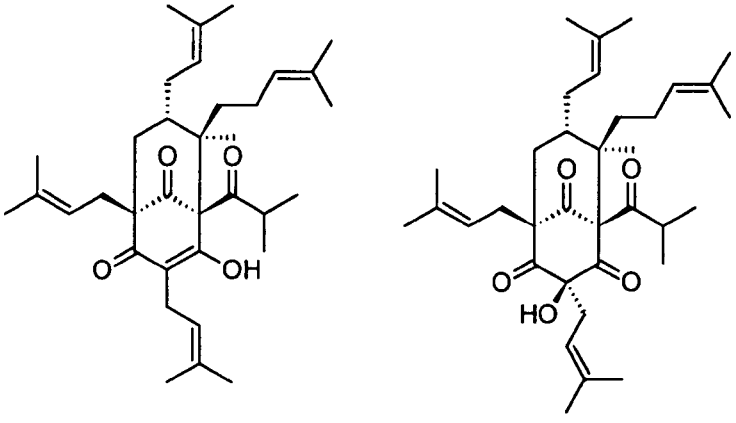


Fig 38 Tautomeric forms of the humulone (8) nucleus

The analogues of humulone (8) detailed below comprise a non-comprehensive list of all of the analogues of humulone (8), indeed the humulone (8) nucleus is found in over 300 known compounds in the Beilstein Index alone.

The biological activity and reactivity of these compounds has not been as extensively researched as humulone (8). However, some activity has been reported and where relevant the reactivity has been applied to the humulone (8) moiety.

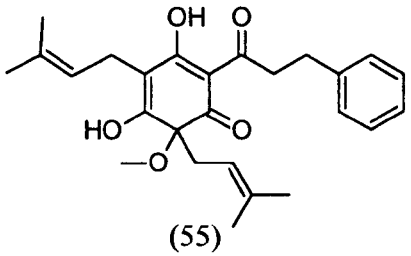
The plant *Hypericum perforatum* is known for its anti-depressant activity [79] which is attributed to a compound known as hyperforin (53) [80]. While this compound is more structurally similar to deoxyhumulone (19), the oxidation product of this, 8-α-hydroxyhyperfoin (54) will undoubtedly be present in any herbal preparation as an artefact. It is also known to be active against serotonin reuptake (Figure 39) [79]. Other oxidation products of this compound are similar to the oxidation products of the hop acids [81].



(53) (54)

Fig 39 Hyperforin (53) and 8-α-hydroxy hyperforin (54) respectively

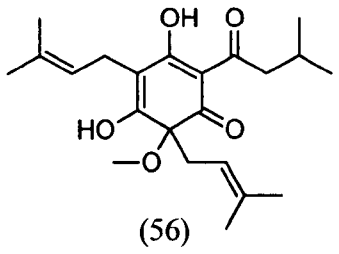
Helihumulone (55) has been isolated from the botanicals of the genus *Helichrysum* [82] and in *Metalasia cymbiforia Harv* [83]. Some derivatisation work has been undertaken with this molecule [84] (Figure 40).



(55)

Fig 40 Helihumulone (55)

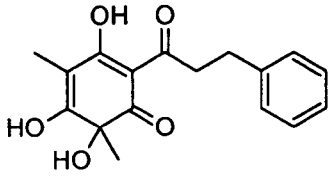
A methylated analogue of humulone (56) (Figure 41) has been isolated from a number of *Helicrysum* species including *scabrum, rosum, revolutum, petiolare, felinum* and *dregeanum* [82].



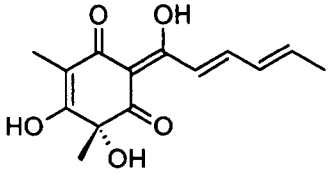
(56)

Fig 41 Methylated derivative of humulone (56) from *helicrysum*

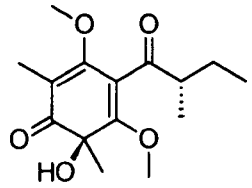
Ceratiolin (57) (Figure 42) has been isolated from the leaves of the plant *Ceratiola ericoides* [85] although little work has been conducted on this compound, some chemistry has been described [86].



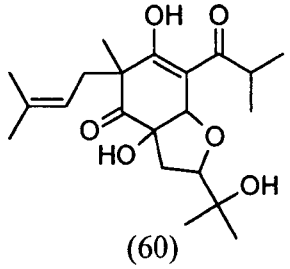
(57)



(58)



(59)

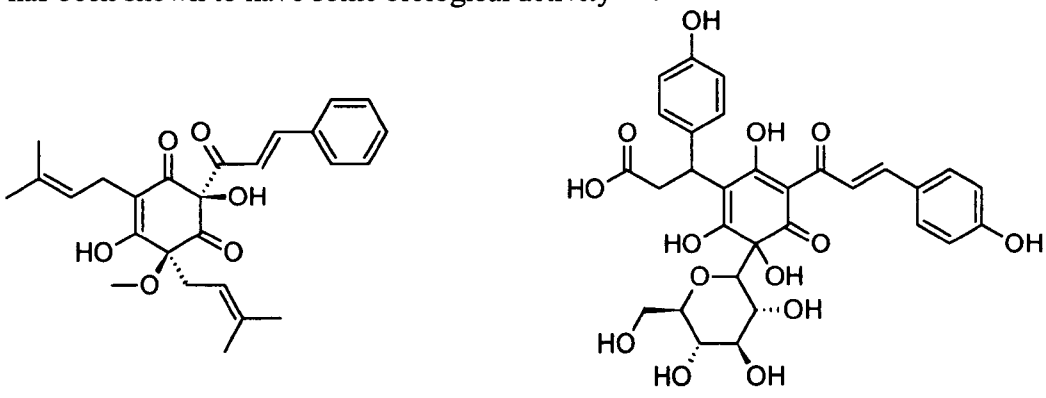


(60)

Fig 42 Ceratiolin (57), oxosorbicillinol (58), wasabidienone B_1 (59), Furanoid humulone analogue (60)

The compound, oxosorbicillinol (58) (Figure 42), has been isolated from the bacterium *Thricoderma sp.* USF-2690 and has some accompanying bioactivity data [87]. Wasabidienone B_1 (59) (Figure 42) was isolated from *Aspergillus viridi-nutans* and has some antibiotic activity [88]. *Hypericum papuanum* is found to contain a furanoid humulone analogue (60) (Figure 42) [89].

A compound found in the bark of the tree *Cedrelopsis microfoliata* (61) (Figure 43) has been shown to have some biological activity [90].



(61) (62)

Fig 43 (2R,6S)-2,5-Dihydroxy-6-methoxy-4,6-bis-(3-methyl-but-2-enyl)-2-[(E)-(3-phenyl-acryloyl)]-cyclohex-4-ene-1,3-dione (61) and *Safflomin* C (62)

A compound isolated from the petals of Safflower called *Safflomin C* (62) (Figure 43) has also been studied [91, 92, 93].

Chapter 2

Biology of Hop and Beer Bitter Acids

2.0 Biology of Hop and Beer Bitter Acids

2.1 Biosynthesis of Hop Bitter Acids

Contained within the hop cone are glands known as the lupin glands and during the maturation period of hop growth, the α-and β-acids are synthesised in these glands (Figure 44). Little work has been carried out as to the exact pathway, however some radio-labelled experiments have been conducted. The data collected involved measuring the radiation given off from a variety of degradation products [15]. Although this provides some understanding into the biosynthetic pathways, more precise radio labelling experiments using NMR techniques would give a greater understanding of these processes.

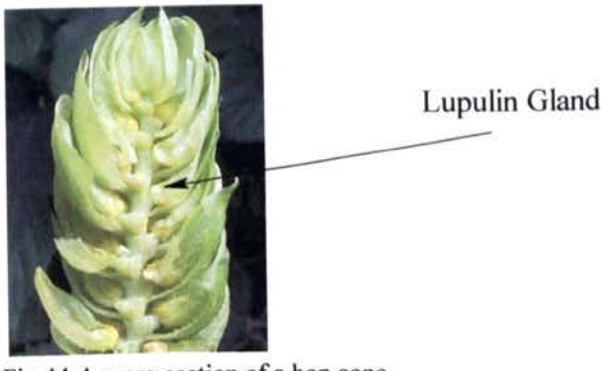
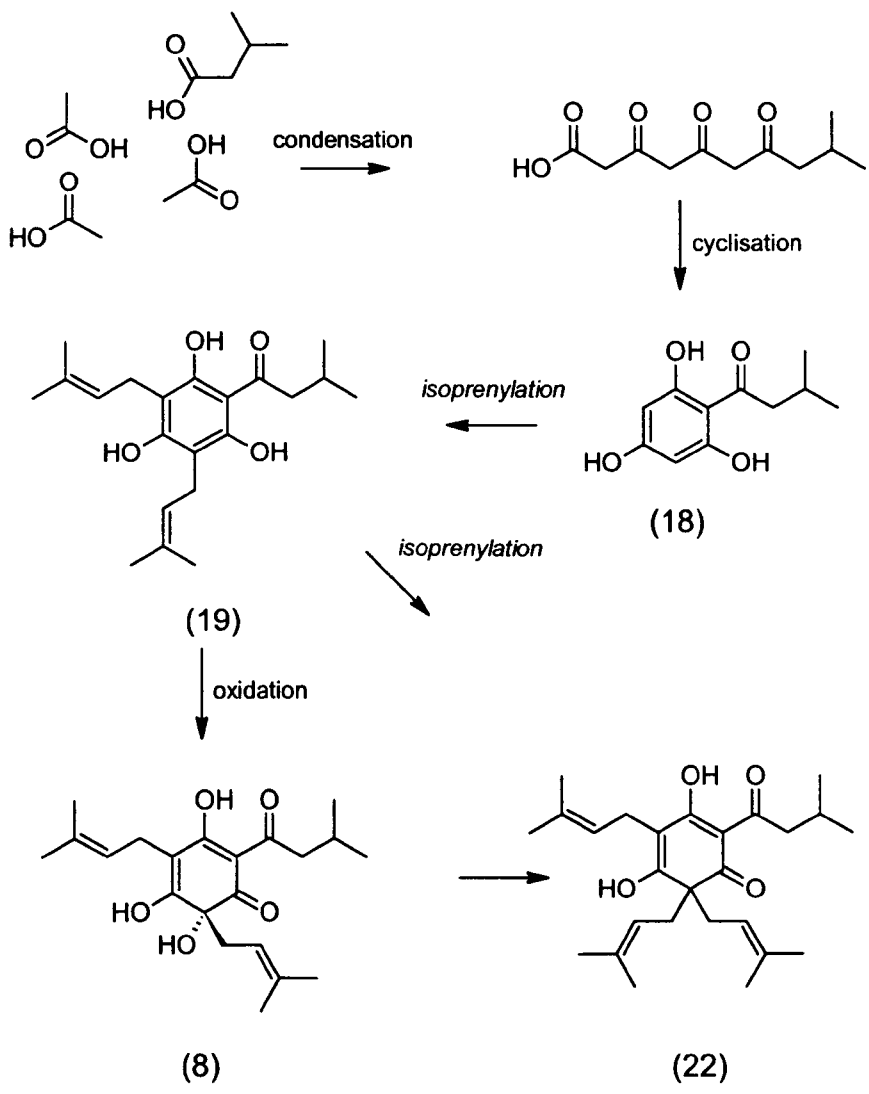


Fig 44 A cross-section of a hop cone

The biosynthesis of humulone and lupulone is shown in Scheme 14:



Scheme 14 Biosynthesis [94] of humulone (8) and lupulone (14)

Although this is the currently accepted biosynthetic pathway it has also been suggested that humulone and the other α-acids may be biosynthesied from the β-acids via an oxidation processes [94].

2.2.1 Biological Activities of the Hop Acids and their Known Derivatives

Hops have been known to have an anti-bacterial effect on certain organisms, mainly the gram-positive bacteria. Experimental data cited in the literature has shown that

there is little difference in activity between the analogous α-acids. Many of the micro-organisms tested are lactic acid bacteria *Lactobacillus* [95] on which humulone (8) seems to have a marked effect. Between 1 and 10 ppm of humulone (8) was sufficient to restrict the growth of *L. plantarum* to 1% of a control [96]. Many other experiments with bacteria have also shown promising results with the lactic acid forming bacteria. However, a report published in 1958 [97] demonstrated that humulone (8) has a bacteriostatic [Greek-*statikos*- causing to stand or stopping] effect on such bacteria. This means that the growth of the bacteria is inhibited over a period of time but the bacteria are not killed. After the 'lag phase' when reproduction ceases the bacteria will begin to form again. The length of the lag phase is directly proportional to the concentration of humulone (8). Indeed the published data for the inhibition of *Lactobacillus casei* shows that 8 μg per ml of humulone (8) gave slightly over 40 hours of lag phase. However, after this lag phase the newly formed bacteria were still affected by humulone (8), indicating no mutating or tolerance to the compound. This has been attributed to some alteration of the humulone (8) molecule itself. In order to understand this it would be necessary to look at the mode of action by which humulone (8) effects these bacteria.

An interesting report on the use of hop derivatives as anti-microbials was published in 1993 [98] on *Lactobacillus brevis* in which the authors were curious as to why hop acids inhibit bacterial growth. One of the conclusions reached in their report was that the activity was dependant upon pH, if the pH was low then the activity was relatively higher compared to trials carried out at a higher pH. Indeed humulone (8) had an MIC (Minimum Inhibitory Concentration) of 32 μM, this equates to approximately 12 ppm at pH 3.6. However at pH 5.9 the MIC is 2512 μM, approximately 910 ppm. Much of the rest of the trial was carried out on *trans*-isohumulone (27_{trans}). The reason for this was that the trial was on beer stability and humulone (8) is not present in beer. However, as both compounds showed activity in previous trials it is fair to predict that they would exert similar properties in others.

It was also discovered that the activity of *trans*-isohumulone (27_{trans}) is affected by the presence of cations. If monovalent cations were present then the anti-microbial activity was increased, however, if divalent cations were introduced then the activity was decreased. For isohumulone at a concentration of 200 mM of potassium ions then the MIC was approximately 15.8 μM, however, if the same concentration of manganese (II) ions was present then the MIC was 63μM. The question was whether the isohumulones (27) were bacteriostatic or bactericidal [Latin-*cida*-to kill]. The results showed that when concentrations lower than the MIC (<40μM) were used the growth rate of the organisms was reduced. When the MIC (=40μM) was applied the growth was immediately stopped. The halt in the growth was not death, if the organisms were suspended in fresh media, growth restarted after a 7 hour lag phase. The normal lag phase for these organisms is 1 hour. Death of the organism was observed when concentrations higher than the MIC were employed (>40μM). The death rates for 80 and 120 μM were very similar. This evidence shows that the isohumulone was active as a bacteriostatic and a bactericide depending on concentration.

This research team discovered that *trans*-isohumulone (27_{trans}) has a marked effect on the following physiological parameters:

i) It reduces the cellular ATP content of non-growing cells metabolising Glucose;
ii) It does not cause general disruption of cell permeability;
iii) It inhibits the uptake of [^{14}C]-L-leucine from preloaded cells;
iv) It promotes the slow efflux of [^{14}C]-L-leucine from preloaded cells;
v) It dissipates the trans membrane pH gradient of cell completely but has less effect on the membrane potential;
vi) It does not inhibit the activity of the proton-translocating ATPase;

Using these findings it was hypothesised that isohumulone (27) acts as an ionophore, effectively facilitating the transport of ions across the cell membrane as

opposed to disrupting cell permeability. The compounds actively 'carry' ions across the membrane. This was confirmed by an experiment whereby the organism (*L. brevis*) was suspended in an unbuffered medium, whereupon the addition of HCl caused the extra cellular pH to drop dramatically. After a short time (ca. 40 seconds) the extracellular pH has increased, albeit only a small amount. After the addition of *trans*-isohumulone (27_{trans}) the pH was observed to increase dramatically.

The proposed mechanism by which isohumulone (27) affects microorganisms is that it allows for the passage of H^+ ions through the otherwise impermeable cell membrane. Under normal working conditions the bacteria will use an H^+ pump to create a pH gradient which allows for the transport of essential nutrients through the cell membrane by diffusion. Isohumulone (27) allows for protons to enter the cell and Mn^{2+} to leave the cell. This effectively removes the pH gradient, hence no nutrients may enter the cell which causes cell death or a cessation of cell activity.

Hop extracts, those containing mixtures of alpha, beta, deoxy alpha etc. show activity against a whole range of organisms such as *Clostridium botulinum*, *Clostridium dificile*, *Helicobacter pylori* [99] and *Listeria monocytogenes* [100]. Interestingly enough *H. pylori* are an example of gram negative bacteria known to be affected by hop acids. There is evidence to suggest that if hop resin and a sodium hexametaphosphate (1:10) mixture was added to mashed potato it was capable of restricting the growth of *E. coli* [101].

A by product of the hop industry is 'base extract' which contains mostly, hop oil, beta acids and fats / waxes, This has been under trial in the sugar industry to replace formaldehyde [102, 103]. Currently 400 tonnes of β-acids are sold per annum to the sugar industry as a 10% aqueous solution known as Betastab 10™.

Many naturally occurring molecules contain a β-triketo function and all of these will exert a biological activity against certain strains of bacteria [104, 81].

The bacteriostatic activity of hops was a point of great interest in the mid 20th century. Although much of the now common place spectroscopic techniques were unavailable for some of the compounds they were still tested against various strains of bacteria. One such trial tested the activities of lupulone (22), humulone (8), isohumulone (27) and humulinone (37) against *Mycobacterium phlei, B. coli, Staph. aureus, Lactobacillus brevis,* and *Lactobacillus plantarum* (Table 1) [105].

Activities (Dilutions W/V)

	M. phlei	*B. coli*	*Staph. aureus*	*L. brevis*	*L.plantarum*
Lupulone (22)	1:100,000	Inactive	1:400,000	Inactive	1:40,000
Humulone (8)	1:10,000	Inactive	1:50,000	1:20,000	1:100,000
Isohumulone (27)	1:10,000	Inactive	Inactive	1:20,000	1:200,000
Humulinone (37)	1:10,000	Inactive	Inactive	1:10,000	1:20,000

Table 1Activities of hop components against microorganisms

This data confirms, in part, the structure activity relationship between polarity and efficacy. Lupulone (22) has a significantly greater activity against *S. aureus* and *M. phlei* than analogous humulone (8). Lupulone (22) is active at 10 ppm, whereas humulone (8) is only active at 100 ppm against *S. aureus*. However, also noteworthy is the apparent inactivity of lupulone (22) against *L. plantarum* whereas isohumulone (27) is active at only 5 ppm, this is in complete contrast to previous theories.

2.2.2 Bacterial Resistance to Hop Acids.

While it is common for gram positive bacteria to be resistant to the effects of the hops acids, there are a few cases of gram negative bacteria that are also resistant to the effects of hop acids.

Beer is a relatively hostile environment for bacteria, it has a low pH, ca 3.8-4.7 , it has a low oxygen content and it can contain as much as 10 % ethanol by volume [106]. Even with these conditions it is still possible to grow bacteria in a medium that match these conditions.

The reason most bacteria can tolerate these conditions but they cannot survive in beer is the presence of the hop bitter acids, namely the iso-α-acids.

This has led biologists to examine the bacteria that are resistant to the hop acids to develop an assay for fast determination. At the time this work was being conducted there did not seem to be any simple explanation for this resistance. This suggested that the only way was to test if a bacteria is resistant to hop acids was to test hop acids on the bacteria.

The most common place to discover bacteria which are resistant to hop acids is "spoiled" beer. The common cause of beer spoilage (60 – 70% of occurrences) is from the lactic acid bacteria (LAB) *Lactobacilli* [107] and *Pediococuss* [106, 108] . Other beer spoilage bacteria are anaerobic gram-negative bacteria, typically *Pectinatus cerevisiiphilus* and *Megasphaera cerevisiae,* although there are around twelve known gram-positive bacteria that are known to spoil beer [108]. Interestingly the frequency of beer spoilage by these organisms has increased as a result of modern breweries improving their methods for reducing the oxygen content in the product in order to reduce spoilage caused by acetic acid bacteria [108].

This apparent resistance to hop acids is considered more than a nuisance to brewers as spoiled beer is unusable as it may become turbid and cause unpleasant sensory changes such as smell. Thus it was necessary for brewing scientist to discover what was causing this phenomenon. As the use of hops in beer can be dated back 3000 years it is unsurprising that some bacteria have developed a resistance to this type of antibiotic.

Many bacteria, and indeed other cells such as cancer and fungal cells that show a resistance to drug action have been investigated. There are functions common to all drug resistant cells however a major mechanism for drug resistance is protein catalysed extrusion of cytotoxic drugs from the cell [109]. These come in two categories; proton driven antiporters and ATP-binding cassette (ABC) transporters. Often these proteins are substrate specific but many are also able to transport multiple drugs from the cells.

In the case of LABs these transporters seem to be specific to hop acids, the multi drug transporters found in LAB are HorA, an ABC transporter [110] and HorC, a proton driven antiporter. There have been many genetic studies to determine the presence of these proteins by DNA sequencing [111, 112].

Figure 45 shows a representation of an hop acid sensitive cell [113];

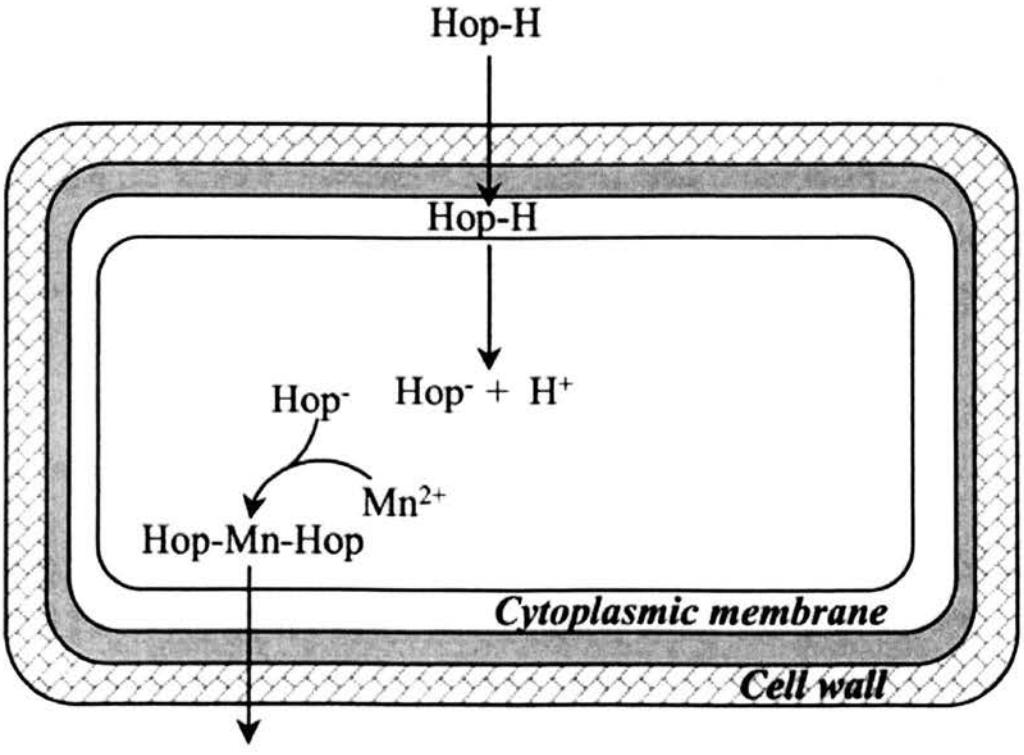


Fig 45 A hop sensitive cell [113]

Fully associated hop acid (Hop-H) enters the cell through the cell wall and remains in the associated form in the cytoplasmic membrane. Once in the cell the relatively higher pH dissociated the acid into Hop^- and H^+ and two acid molecules bind with divalent manganese cations and allow passive diffusion through the cell. This action not only removes the metal cations but also disrupts the pH gradient across the cell membrane.

Figure 46 show a representation of a Hop Acid resistant cell [113];

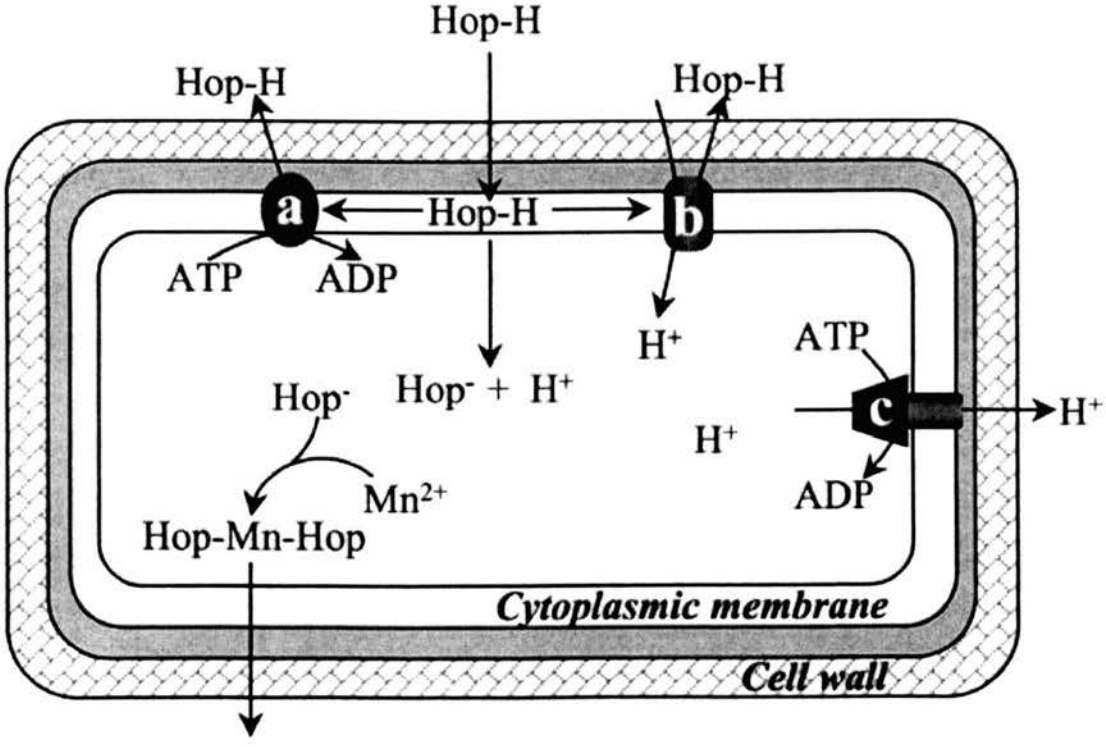


Fig. 46 Hop Resistant Cell [113]

In the Hop resistant cell, the hop acids exerts the same mechanism, however, HorA (a) and HorC (b) are able to excrete the majority of the hops acids from the cell before it passes out of the cytoplasmic membrane and into the cell. The pH gradient is maintained and increased H^+-ATPase activity. There is still the issue of the removal of divalent manganese which the cell utilises to produce ATP. There has been reports of hop resistant cells using the metabolism of pyruvate, malate, citrate and argenine as energy source [107].

Chapter 3

Results and Discussion

3.0 Results and Discussion

3.1 Aims

The aims of this project are as follows:

- To develop an improved method for the isolation of large quantities of humulone (8).

- To obtain pure crystalline derivatives of humulone (8) in order to obtain single crystal X-ray diffraction data.

- To spectroscopically characterise humulone (8) and lupulone (22) using advanced spectroscopy.

- To develop a method for resolving racemic (±) humulone (8).

- Synthesise humulone derivatives in order to provide an in depth chemical study and for biological screening.

- To biologically evaluate a library of the above compounds.

3.2 Isolation of humulone (8)

An alternative "preparative" technique for the isolation of humulone was published that used centrifugal partition chromatography [114], though this is not suitable for isolating large quantities of humulone. The current literature methods for extracting and isolating significant amounts of humulone (8) are time consuming and involve the use of highly toxic lead (II) salts. The salts form a finely divided solid which may be removed by repeated centrifugation and washing with benzene followed by

methanol. Despite repeating each washing α-acids are still difficult to obtain. The current method for the isolation of humulone (8) from hops requires seven major steps as seen in (Scheme 3, Chapter 1).

1) Extraction of hops.

The preferred solvent for extracting the desired compounds from hops for this purpose is to use methanol, this produces a green extract containing the α- and β-acids. This extract also contains a number of chalcones, xanthohumol (4), desmethylxanthohumol (5), prenylated flavonoids isoxanthohumol (6), 8-prenylnaringenin (7) and polyphenols (hard resin) and significant amounts of volatile oil and miscellaneous fats and waxes.

Upon cooling and / or the addition of ca. 10% water to the methanol the fats and waxes are precipitated and must be removed by centrifugation to give a material suitable for the next step of the purification. The hard resin can be removed by passing the extract through a column of PVPP [115].

The soft resins (α-and β- acids) are commercially isolated from hops via carbon dioxide extraction under either liquid (60 bar, 10°C) or supercritical (300 bar, 50°C) conditions. These selective conditions provide an extract free from polyphenols and waxes, hence the purity of the α-acids is much greater. As the CO_2 extract is commercially available it makes a perfect starting material for the separation of α-acids.

2) Isolation of α-acids

The hop extract is treated with a methanolic solution of lead (II) acetate. The subsequent precipitate of the lead salts of the α-acids can then be isolated by centrifugation. After acidification with 10% sulphuric acid in methanol the free

acids must be separated from the finely divided insoluble lead (II) sulphate by further centrifugation followed by addition of water and extraction using hexane.

In an alternative method, a hexane solution of the above extract can be treated with 0.15 M sodium carbonate to form sparingly water soluble sodium salts. Due to this apparent insolubility of these salts in water vast quantities of the solution are required if large amounts of α-acids are required (ca. 5 L for 100g of α-acids).

The purified α-acids must then be complexed with 1,2-phenylenediamine, in hot benzene, to give a pure mixture of α-acids.

During the course of this research it was discovered that a solution of commercially available CO_2 extract in heptane, dried over sodium sulphate, could be treated with an ethyl acetate solution of 1,2-phenylenediamine. The subsequent precipitate could then be separated by simple vacuum filtration to afford a crude complex containing mostly α-acids.

3) Separation of humulone from the α-acids

Re-crystallisation of the 1,2-phenylenediamine complex from benzene (7 – 10 times) gives a pure humulone complex (16), however due to the relative toxicity of benzene, toluene may be used. We have found that during the course of this research a 10% solution of water in methanol or pure acetonitrile is adequate to provide pure humulone (8).

4) Liberation of humulone from the diamine salt:

Previous reports state that the humulone (8) can be freed from the complex (16) by shaking an ethereal solution with 10% HCl(aq). This forms the water soluble diamine dihydrochloride as a by product. Removal of the ether gives humulone (8)

as a yellow crystalline solid. This method is far from ideal as the diamine salt of humulone (16) is also soluble in ether and many washes are required to ensure complete degradation of the complex.

As a part of this research we discovered that if the diamino complex (16) was dissolved in 4 volumes of acetic acid then the diamine diacetate could be removed by the addition of water and the humulone (8) could be extracted by washing with pentane. We also showed that humulone (8) may be further purified by re-crystallisation from acetonitrile to provide a pale yellow crystalline solid mp 80 °C.

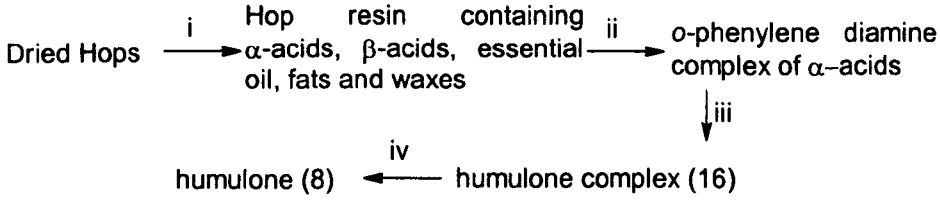
3.2.1 Development of a Novel Isolation Technique

In contrast to the traditional methods for isolating humulone (8) we have established an effective alternative procedure that provides access to kg quantities of humulone (Scheme 16). The main advantages of this method over the existing method include:

i) No need for separation of the α-acids from hop resin which reduces the number of individual steps;

ii) The use of less toxic materials makes this procedure more suitable for industrial production;

iii) Reduction of solvent volumes that allows more humulone (8) to be isolated per unit volume, hence reducing the cost of industrial production which is valued at volume per hour;



Reagents i) CO_2 60 bar, 10°C ii) heptane / o-phenylenediamine, EtOAc 45°C iii) 6 – 8 recrystalisations from methanol / water (9:1) iv) acetic acid / water, pentane.

Scheme 16 Novel isolation of humulone (8)

Using this protocol it was possible to access 1kg of spectroscopically pure humulone. This involved a 5 litre jacketed reaction vessel, from a CO_2 extract of hops, in 25% recovery from the total humulone content. The waste stream from this isolation would be suitable for use as a starting material for the production of iso-α-acids for use in brewing.

3.3 Structure determination of the hop acids.

In order to obtain a thorough understanding of the chemistry and biological activity of humulone and its derivatives it is important to understand which tautomer / configuration predominates under the conditions of the investigation. Although this information is available in the literature [116], it has been collected using techniques that have now largely been superseded by modern spectroscopy and have, at times, been proven incorrect.

3.3.1 NMR Spectroscopy

NMR spectroscopy provides a useful method for determining the structure of humulone and its derivatives. In order to understand the chemistry of humulone (8) more fully we need to clarify the ambiguous assignment of the key structural features, for example the hydroxyl groups and the ring carbon atoms of both humulone (8) and lupulone (22) using NMR techniques.

During the 1930s the structure of humulone (8) was determined using techniques such as elemental analysis and IR spectroscopy. These techniques have now largely been superseded by NMR (nuclear magnetic resonance) spectroscopy which provides direct structural detail. The lack of NMR facilities during the 1930s, for structural elucidation, meant that it was necessary to chemically degrade a molecule to volatile compounds and then use techniques such as gas chromatography (GC) in order to identify the degradation products.

This unfortunately led to the wrong identification of humulone (8) and lupulone (22) as a result of an hydrogenolysis reaction [57]. Under normal conditions alkenes are thought to be hydrogenated by the following mechanism that involves the adsorption of the alkene onto a metal surface (Figure 47)

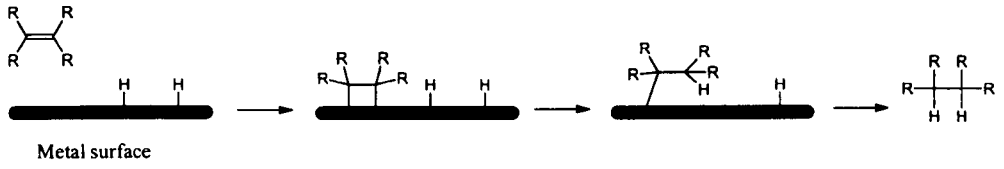


Fig 47 Hydrogenation of alkenes on palladium metal

Under conditions of hydrogenolysis carbon – oxygen bonds are broken. This is observed in the cleavage of benzyl ethers (Figure 48).

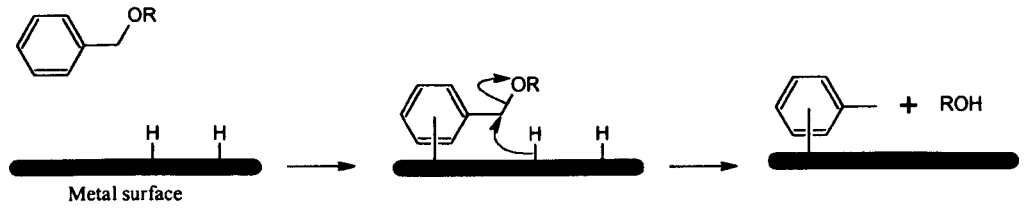


Fig 48 Hydrogenolysis of benzyl ethers on palladium metal

At the time that the structure of the hop acids was being elucidated ether cleavage reactions were the only known hydrogenolysis reactions and as a result the following mechanism was suggested for the hydrogenolysis of humulone (Figure 49):

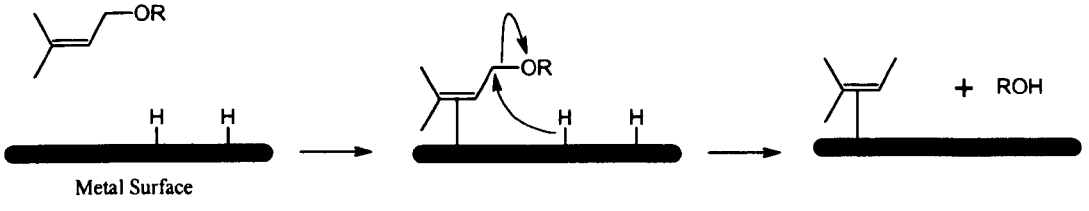
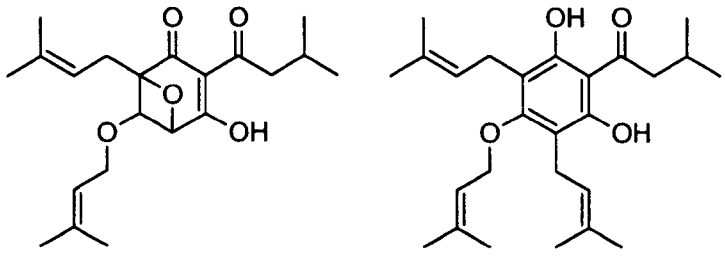


Fig 49 proposed mechanism for hydrogenolysis of isoprenyl ethers

This led to the following structures for humulone (63) and lupulone (64) being proposed [117, 16] (Figure 50). These structures were consistent with the empirical formulae $C_{21}H_{30}O_5$ and $C_{26}H_{38}O_4$ respectively.



(63) (64)

Fig 50 Harris' proposed structures of humulone (63) and lupulone (64) respectively

The arguments for these proposed structures were supported by the proposed mechanisms from their synthesis and the isomerisation of humulone (8) to isohumulone (27). Modern NMR spectroscopy, on the other hand, would have ruled these compounds out as possible structures.

The now accepted mechanism for the hydrogenolysis of the C-C bonds in humulone (8) and lupulone (22) is as follows (Figure 51):

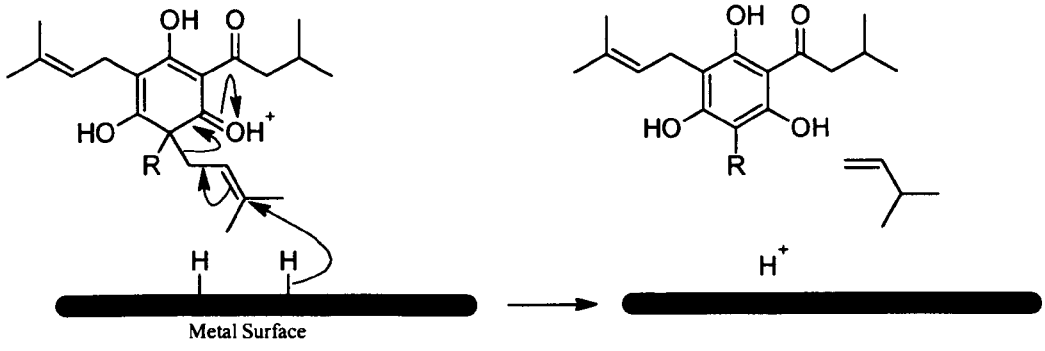


Fig 51 Proposed mechanism for hydrogenolysis of hop acids

3.3.1.1 Solvent Dependency and NMR Spectroscopy of humulone (8) and, by analogy, related compounds

The choice of solvent used in NMR spectroscopy often has little effect upon the chemical shifts of non-heteroatom bonded protons (± 0.1ppm). There are a few examples where solvent does have an effect, however these are only really significant when benzene-d_6 is employed as the solvent. Shifts of up to a 1.5 ppm have been observed using benzene compared to other solvents [118]. This similarity

in chemical shift from one solvent to the next is also observed in ^{13}C NMR spectroscopy.

In contrast to this it is observed that the chemical shift of protons attached to heteroatoms such as oxygen can be greatly influenced by the solvent.

Table 1 shows the ^{1}H NMR chemical shift (δ) in ppm for HOD (monodeuterated water) in the following solvents:

Solvent	δ (ppm)	Solvent	δ (ppm)
Acetic Acid*	11.59	Ethanol*	5.26
Acetone	2.85	HMPA	-
Acetonitrile	2.16	Methanol*	4.84
Benzene	0.5	DCM	1.52
Carbon Tetrachloride	-	Nitromethane	-
Chloroform	1.54	Pyridine	4.96
Cyclohexane	-	THF	2.23
Ether	-	Toluene	4.96
DMF	3.48	TFA*	11.5
DMSO	3.32	Water	4.82
Dioxane	2.43		

* indicates deuterium transfer

Table 2 Chemical Shift of HOD in a variety of solvents[118]

This phenomenon is also observed with the hydroxyl proton in ethanol and methanol. It comes as no surprise then that chemical shifts (δ) for the three hydroxyl protons present in humulone also appear to be solvent dependant.

Previously reported experimental data cannot be reproduced easily as the use of the solvent CCl_4 is no longer permitted, however there are some data obtained from HPLC-NMR using acetonitrile / water, that may be used [119, 120]. Figure 52 shows

the ^{1}H NMR for humulone in $CDCl_3$ and shows one obvious feature, this is the sharp downfield signal at δ 18.93 ppm, which has been attributed to the strongly hydrogen bonded proton at (C1). [77]. The other two OH signals are observed at δ 7.49 and δ 4.55 ppm. These represent the resonances for the enol function (C5) and the signal for the tertiary hydroxyl signal (C4) respectively. We have been able to discriminate between the two hydroxyl signals at δ 7.49 and δ 4.55ppm by comparison with the ^{1}H NMR spectrum of lupulone (22).

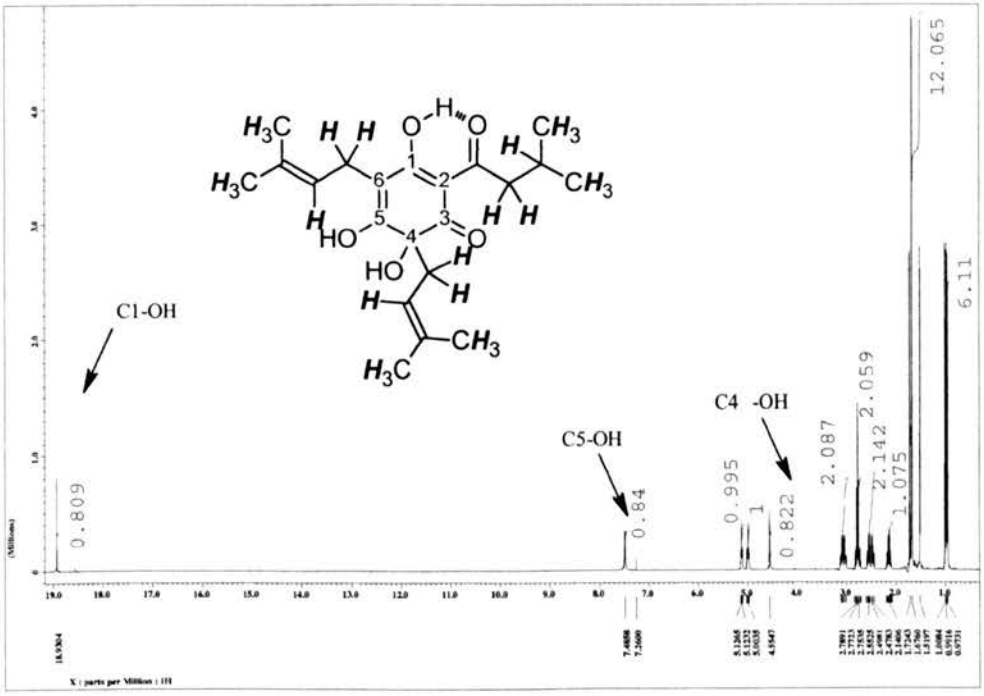


Fig 52 ^{1}H NMR Spectra of humulone (8) in $CDCl_3$

In contrast to humulone (8), lupulone (22) does not contain an hydroxyl group at (C4) however the (C5) enol function is observed at δ 7.1 ppm. Therefore by analogy the C5 hydroxyl in humulone (8) may be associated with the resonance at δ 7.49 ppm and the C4 hydroxyl is associated with the resonance at δ 4.55 ppm.

Interpretation of the remaining resonances in Figure 52 is easily achievable, considering the simplicity of the humulone (8) molecule. There are effectively 11 chemical environments nearly all of which appear as separate resonances. We were

able to assign these signals by analysing the spectra of two analogous compounds, deoxyhumulone (19) and lupulone (22):

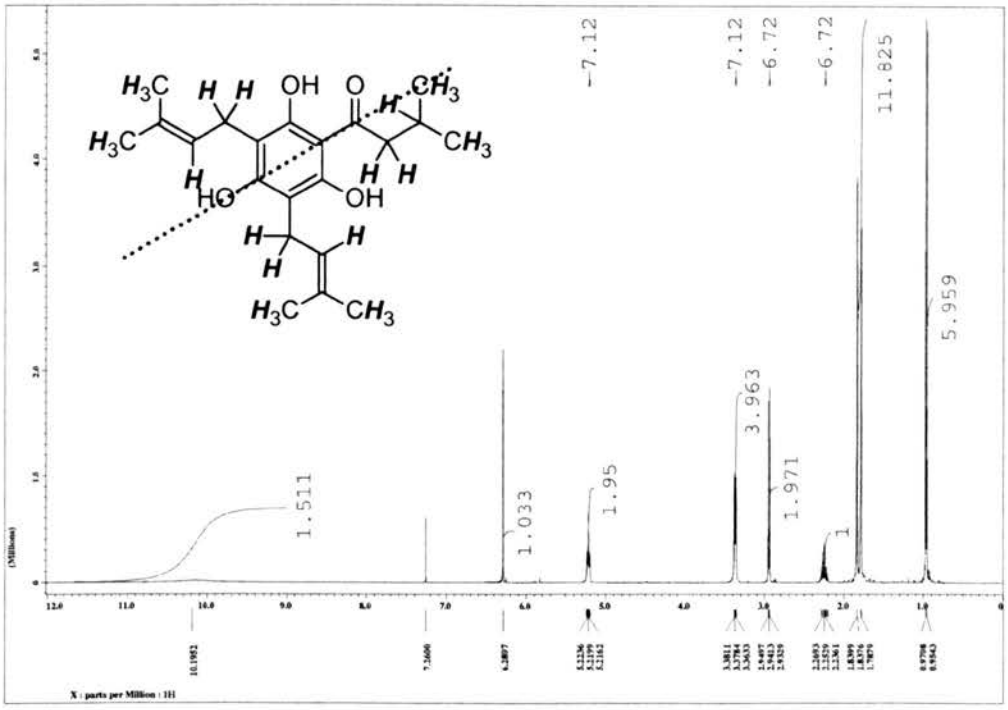
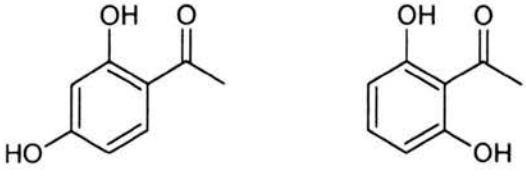


Fig 53 ^{1}H NMR spectra of deoxyhumulone (19)

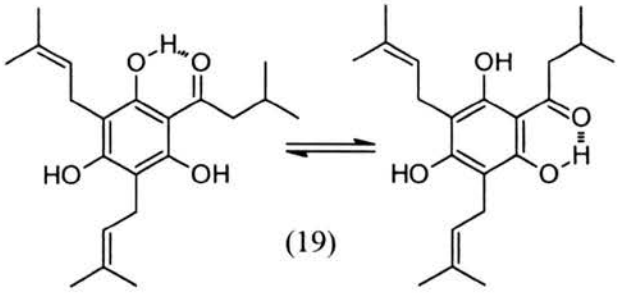
Deoxyhumulone (19) is a symmetrical molecule (Figure 53), illustrated by the dotted line, and two hydroxyl protons *ortho* to the carbonyl appear as one broad deshielded resonance at δ 10.2 ppm. The hydroxyl group *para* to the carbonyl is observed at δ 6.29 ppm. Interestingly the two hydroxyl protons for 4-acetyl resorcinol (65) resonate at δ 9.64 and δ 12.85 ppm, whereas for 2-acetyl resorcinol (66) the hydroxyl protons resonate synchronously at δ 11.45 ppm with an integral of two protons (Figure 54).



(65) (66)

Fig 54 Structures of 4-Acetyl resorcinol (65) and 2-acetyl resorcinol (66) respectively

This suggests that the hydrogen bridge between the hydroxyl at (C1) and the carbonyl of the acyl side chain of deoxyhumulone (19) is reversible at room temperature, and free rotation about the bond at (C2) is not restricted and thus provides a symmetrical molecule with the resonance at δ 10.2 ppm (Figure 55). There are no protons attached directly to the aromatic ring therefore interpreting the spectrum for the rest of the molecule can be broken down into two distinct moieties: the isovaleryl side chain and the isoprenyl side chains (Figure 56).



(19)

Fig 55 Rotational isomers of deoxyhumulone (19)

The expanded view of these resonances is illustrated in Figure 56:

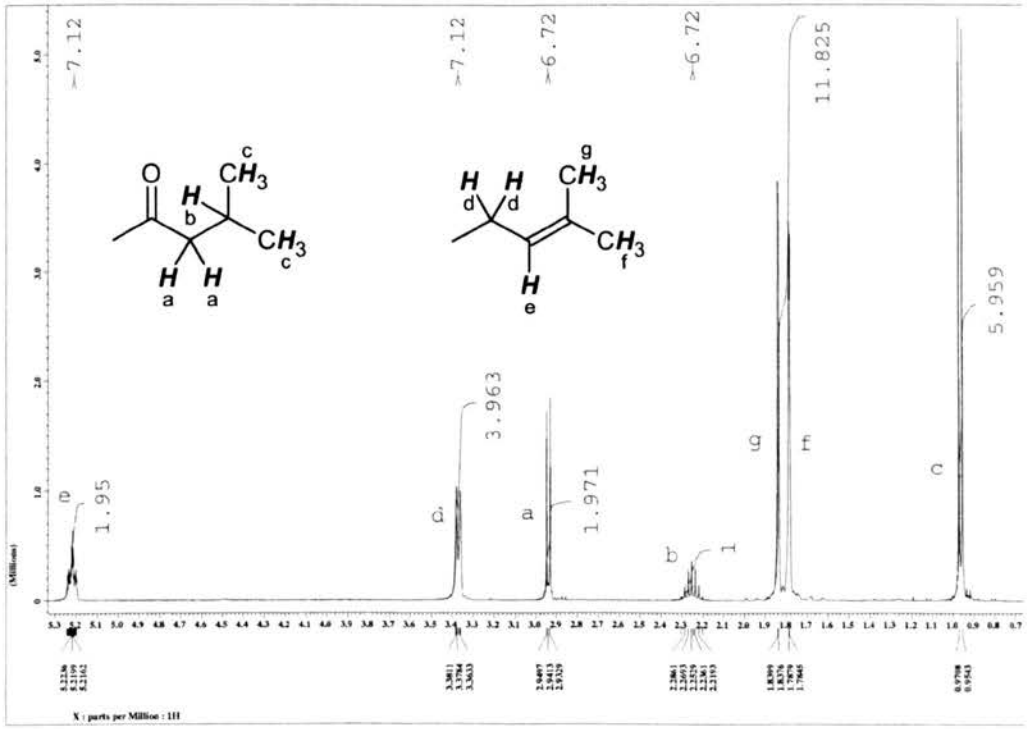


Fig 56 Expanded view of the ^{1}H NMR spectra of deoxyhumulone (19)

From the proton couplings (Hz) shown at the top of the spectra one can see that protons (e) and (d) share that same coupling constant, 7.12 Hz implying that these protons are coupling with one another, likewise protons (a) and (b) also share the coupling constant 6.72 Hz.

The methyl carbon atoms bearing protons (f) and (g) are vinylic thus one would not expect there to be any observed coupling. Closer examination (Figure 57) shows that protons (g) at δ 1.8 ppm is a doublet J = 0.94 Hz and (f) appears as a broad multiplet with one coupling J = 1.34 Hz being clearly defined. The weak coupling of these two sets of protons comes as a result of long range coupling (f) appears to couple with (e) due to the similarity in the J value (J = 1.34 Hz) and (g) appears to couple with (d) this is clarified by the similarities in the coupling constant (J = 0.94 Hz)

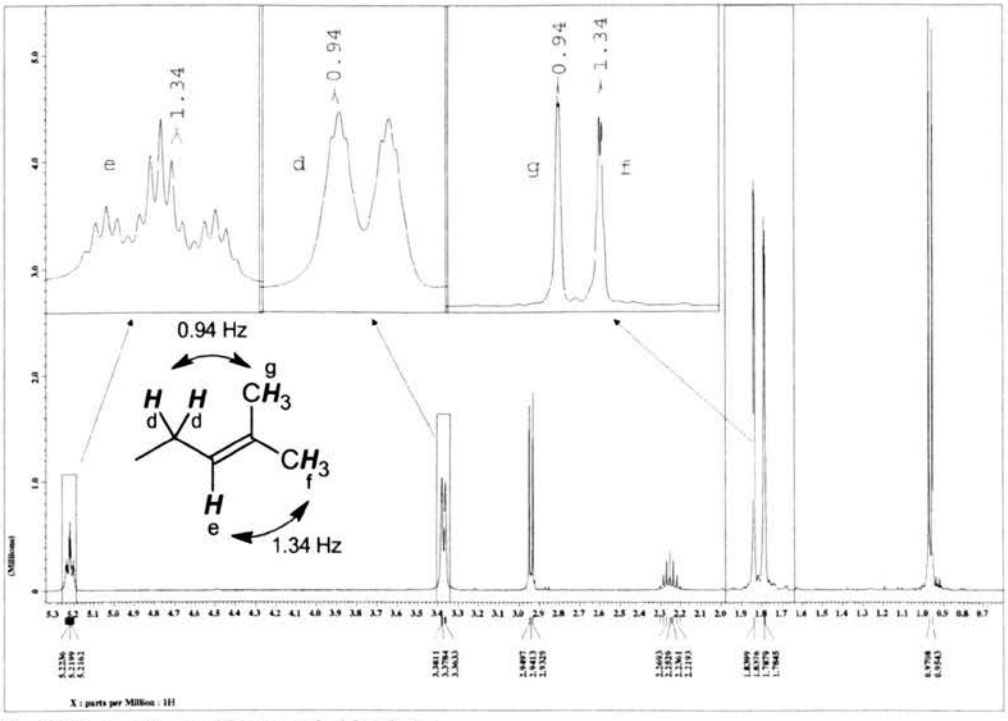


Fig 57 Expansions of isoprenyl side chain

However we also know that in scalar coupling the interaction is not a 'through space' effect. Indeed the phenomenon of coupling is a result of electron interactions

through the bonds. If we study the COSY plot of deoxyhumulone we can observe some of the long range coupling (Figure 58).

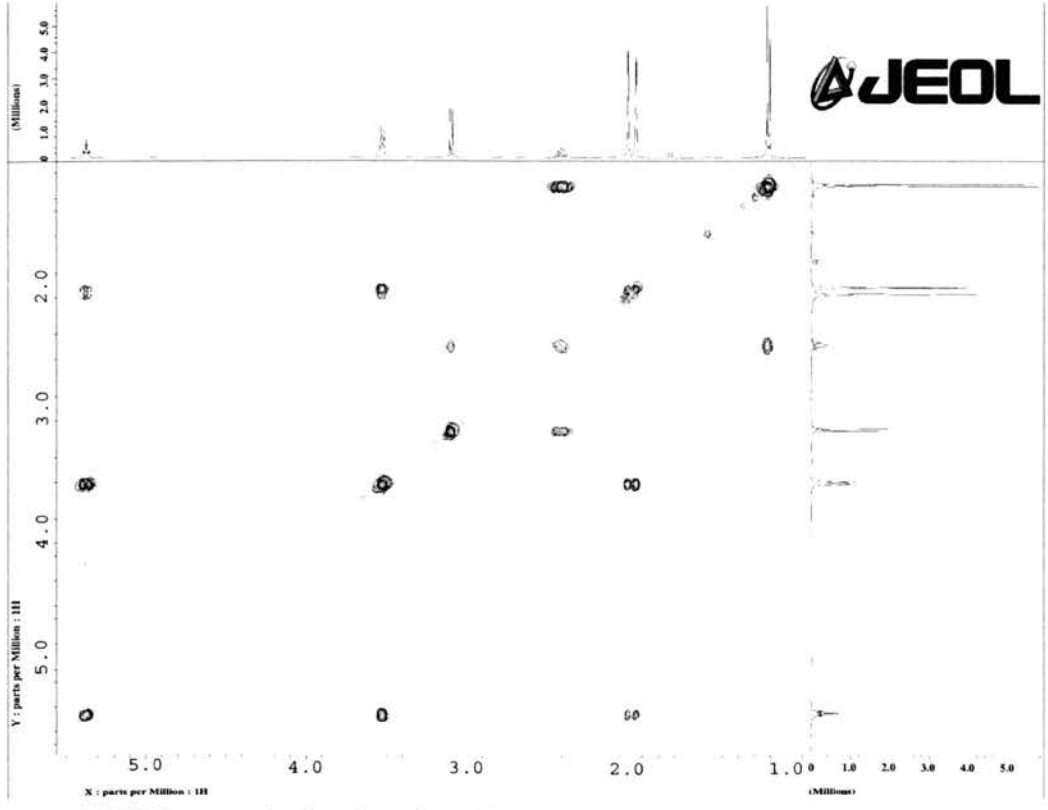


Fig. 58 COSY Spectrum for deoxyhumulone (19)

Most significantly we can see that the geminal dimethyl groups are indeed coupling with the allylic proton and the methylene group.

The COSY plot is showing that both of the geminal dimethyl groups are coupling with both the allylic proton and the methylene protons (Figure 59). This is in contrast to the information acquired from the coupling constants alone, which indicates that each of the geminal dimethyl groups are coupling with only one of the allylic or methylene groups (Figure 57).

This means that we cannot rely on proton-proton coupling to assign these resonances.

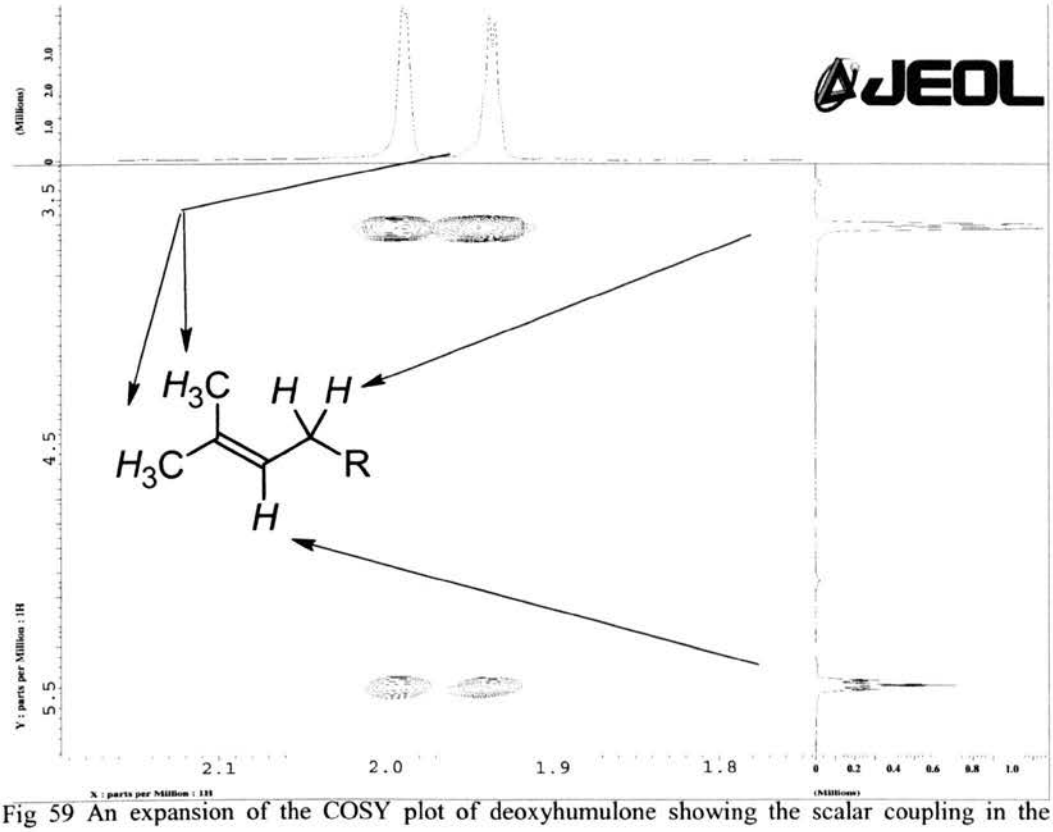


Fig 59 An expansion of the COSY plot of deoxyhumulone showing the scalar coupling in the dimethylallyl side chains

We therefore cannot rely on proton-proton correlations to determine the chemical shift of these protons.

In order to assign the resonances to the specific protons on the dimethylallyl groups it is necessary to use proton-carbon correlation spectroscopy HETCOR as assignment of the carbon resonances is made more simple by the distinct chemical shift differences between the terminal carbons *cis* to the olefinic hydrogen and those in the *trans* position.

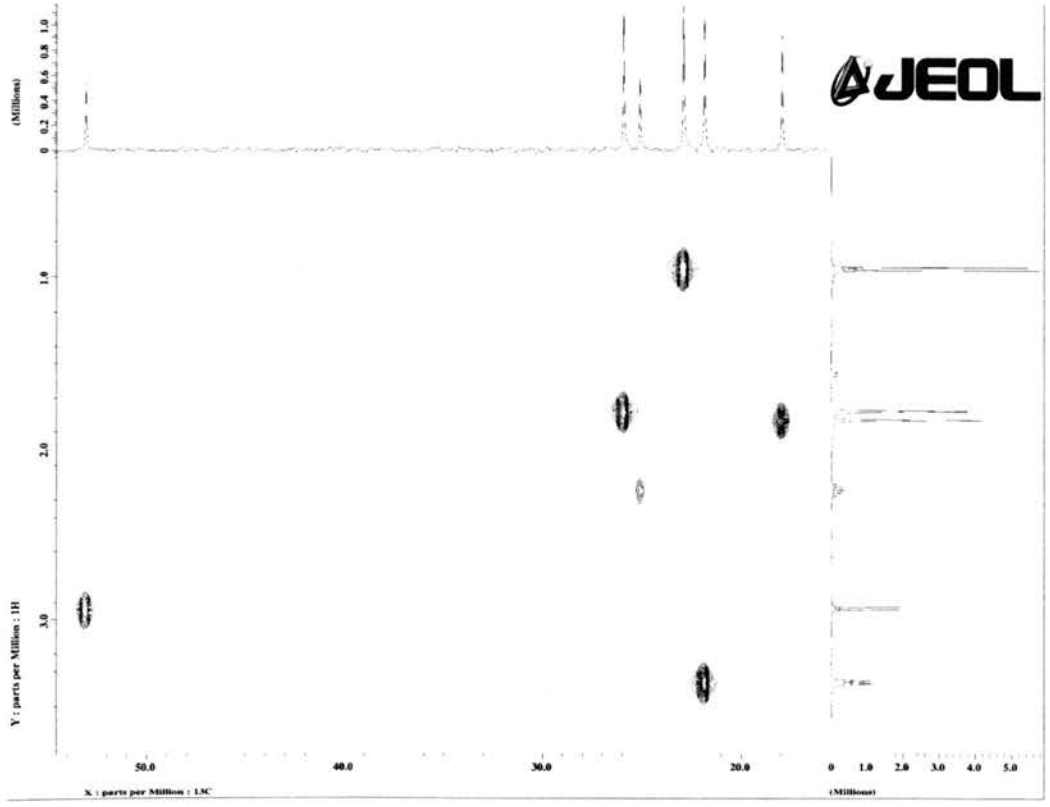


Fig 60 Expansion of the 1H 13C 2D HETCOR spectra showing the resonances in the isoprenyl group of deoxyhumulone (19)

We know from previously collected ^{13}C data that in an allylic system, methyl groups *trans* to an allylic hydrogen have a chemical shift of around δ18 ppm and those *cis* to the allylic proton are more deshielded and resonate at δ25 ppm [121]. Therefore if we study the above correlation and compare it to the ^{1}H spectrum of deoxyhumulone (Figure 57) we can see that of the two geminal methyl proton resonances, the more downfield resonance δ 1.79 ppm corresponds to the methyl *trans* to the allylic proton and the upfield resonance δ 1.84 ppm represents the methyl group *cis* to the allylic proton.

Comparison with humulone shows distinct similarities, however the introduction of the hydroxyl function at (C4) has removed the symmetry and put the two isoprenyl groups into significantly different environments. The introduction of a chiral centre

has also introduced diastereotopic protons; this has complicated the splitting patterns on the spectra significantly (Figure 61).

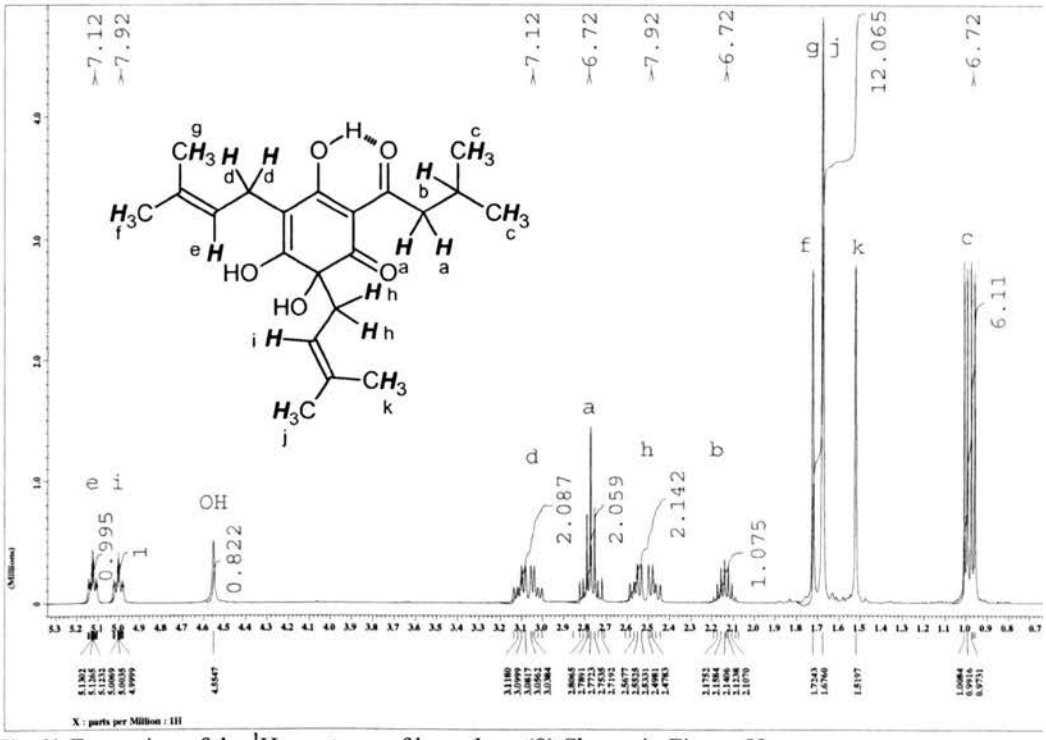


Fig 61 Expansion of the ^{1}H spectrum of humulone (8) Shown in Figure 52

Goese [122] and his co-workers conducted a series of NMR experiments on natural and ^{13}C and ^{2}H enriched biosynthesised humulone. They concluded from their carbon-hydrogen correlation experiments that, the above assignments are correct for one of the isoprenyl units, the unit at C4 has a more down field resonance for the methyl group *cis* (j) to the allylic proton and a more upfield resonance for the *trans* methyl group (k).

Goese made a great deal of assumptions based on the incomplete works carried out by Verzele and De Keukeleire thus his results still remain ambiguous. De Keukeleire made assumptions based on the similarities between the structures of humulone and lupulone.

During the course of our research we have conducted HETCOR experiments on humulone.

Our data is in agreement with Goese, in as much as one of the isoprenyl groups is indeed in a very different chemical environment to the other but it is not sufficient to distinguish which is the group at C4 and which is the group at C6.

3.3.1.2 NMR Studies of Lupulone (22)

If we now consider the proton NMR of analogous lupulone (22) (Figure 62) it is clear that two resonances are present for the hydrogen bonded proton at C-1 at chemical shift δ 19.23 and 18.39 ppm when the experiment was run in $CDCl_3$. This difference is very evident when comparing the ^1H NMR of lupulone (22) with humulone (8) (Figure 63).

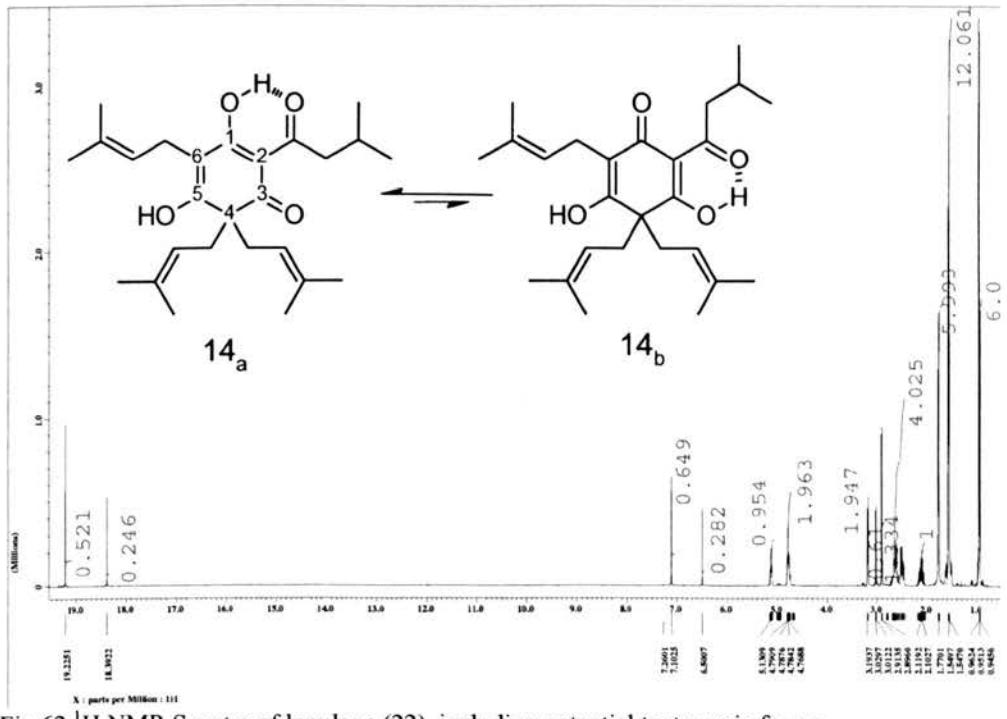


Fig 62 ^1H NMR Spectra of lupulone (22), including potential tautomeric forms

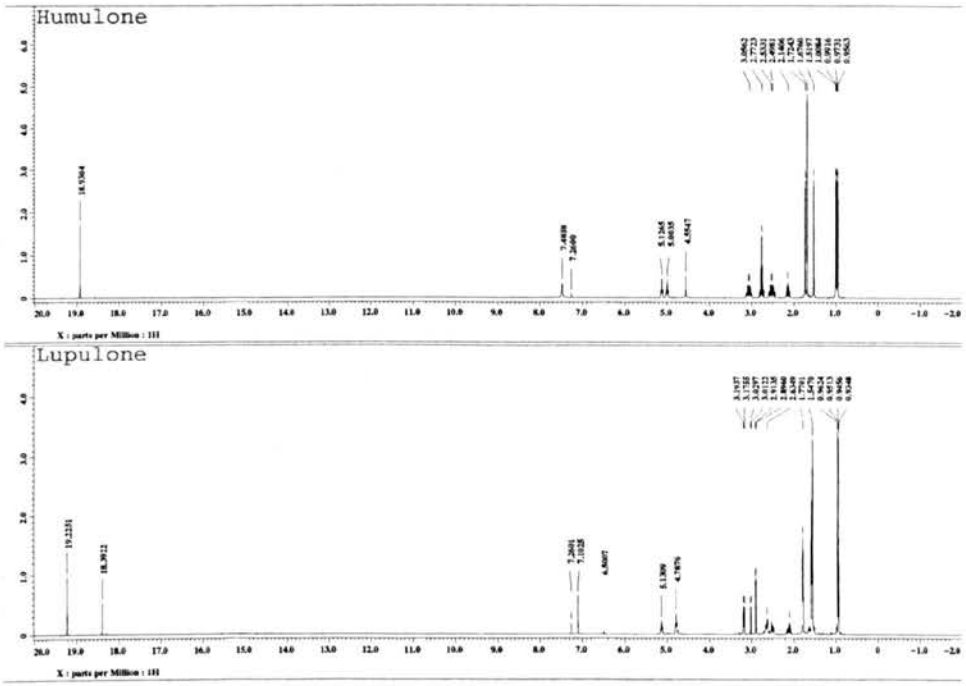


Figure 63 Comparison of the ^{1}H NMR spectra of humulone and lupulone Peak identification has been moved to the top of the figure for clarity

The presence of these 2 resonances for the C-1 hydroxyl of lupulone supports the suggestion proposed by Verzele that lupulone exists in two tautomeric forms. The (C-5) enolic OH signals are also present as two resonances at δ 7.10 and δ 6.50ppm, however not all resonances are affected this dramatically although all resonances show some small degree of duplication.

A more detailed study of the ^{1}H NMR for lupulone (22) (Figure 64) reveals that the hydroxyl protons at δ 7.10 and δ 6.50 ppm show a 7:3 ratio of the integrals for tautomers (14_a) and (14_b). In order to gain a better understand of the tautomeric nature of lupulone it was decided to perform the NMR experiments in a variety of solvents on the basis that the tautomerisation may be solvent dependant. Instead of focusing upon the hydroxyl protons, which undergo deuterium transfer in protic solvents, we studied the two resonances at δ 2.9ppm and δ 3.0ppm which integrate

for 1.33 and 0.61 protons each respectively. These resonances are attributed to the methylene protons H_a in the isovaleryl side chain (Figure 64).

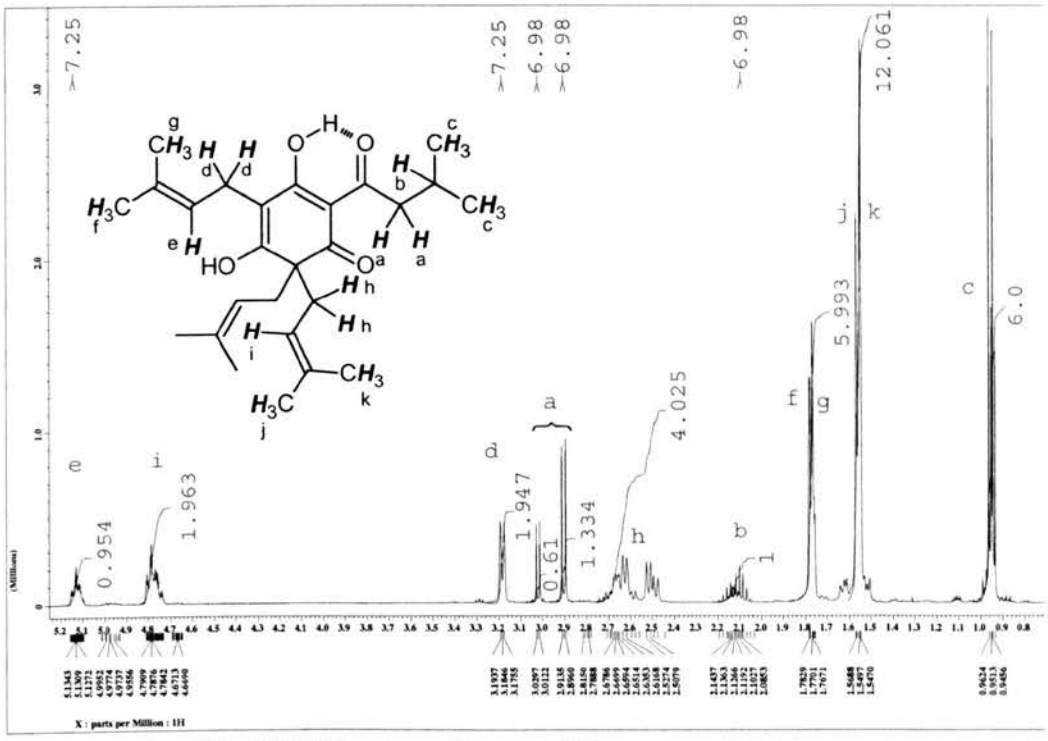


Fig 64 Expansion of the ^1H NMR spectra of lupulone (22)

Analysis of the integrals for H_a in a range of solvents is shown (Table 3).

Solvent	Major Tautomer	Minor Tautomer
Cyclohexane	78	22
Acetonitrile	85	15
Chloroform[a]	68	32
Methanol	100	0
Acetic Acid	100	0
Pyridine	100	0
DMSO	100	0
NaOD / D_20	100	0
Notes a) a similar result was observed at 40 °C		

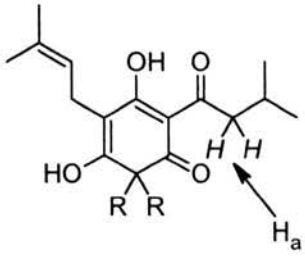


Table 3 Ratio of tautomeric forms of lupulone in a variety of solvents

From these data we can see that in five of the solvents the presence of a second tautomer was not observed. The effect is very marked with polar / protic solvents. Acetonitrile appears to provide an enhanced proportion of the main tautomer compared to chloroform and cyclohexane.

Figure 66 shows part of the ^{1}H NMR spectrum for lupulone (22) in cyclohexane. We can clearly see that the two protons in the isoprenyl side chain at δ 3.19 ppm appear as a broad doublet and seem to be unperturbed by the change in conformation (Figure 65).

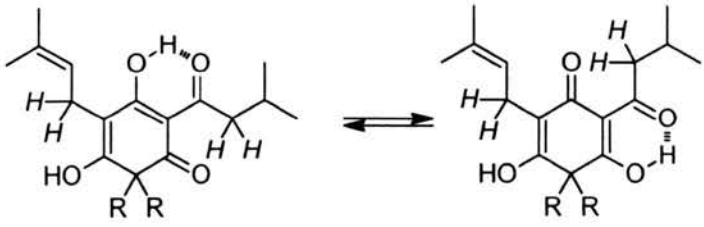


Fig 65 Conformational changes of lupulone (22)

The protons in the acyl side chain, however, appear as a pair of doublets at δ 2.87 ppm and δ 3.00 ppm integrating for 1.56 and 0.44 protons respectively.

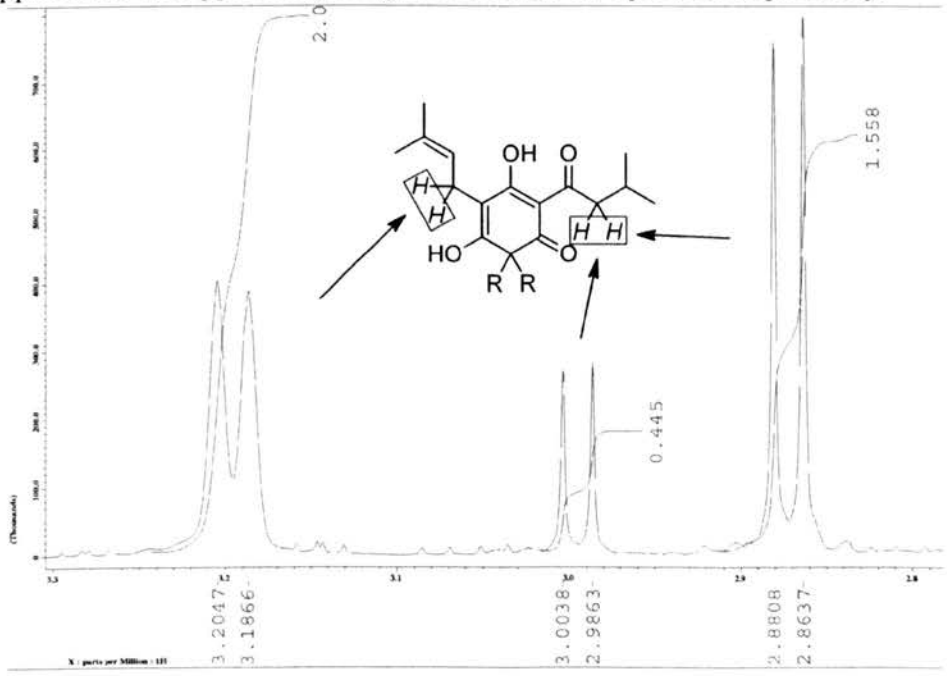


Fig 66 Expansion of the ^{1}H NMR spectra of lupulone in cyclohexane

If we now look at the same resonances for lupulone (22) in pyridine (Figure 67) the resonance at δ 3.00 ppm is lost. These have been replaced with one resonance at δ 3.17 ppm integrating for two protons. This suggests that in pyridine, lupulone (22) exists as a single tautomer.

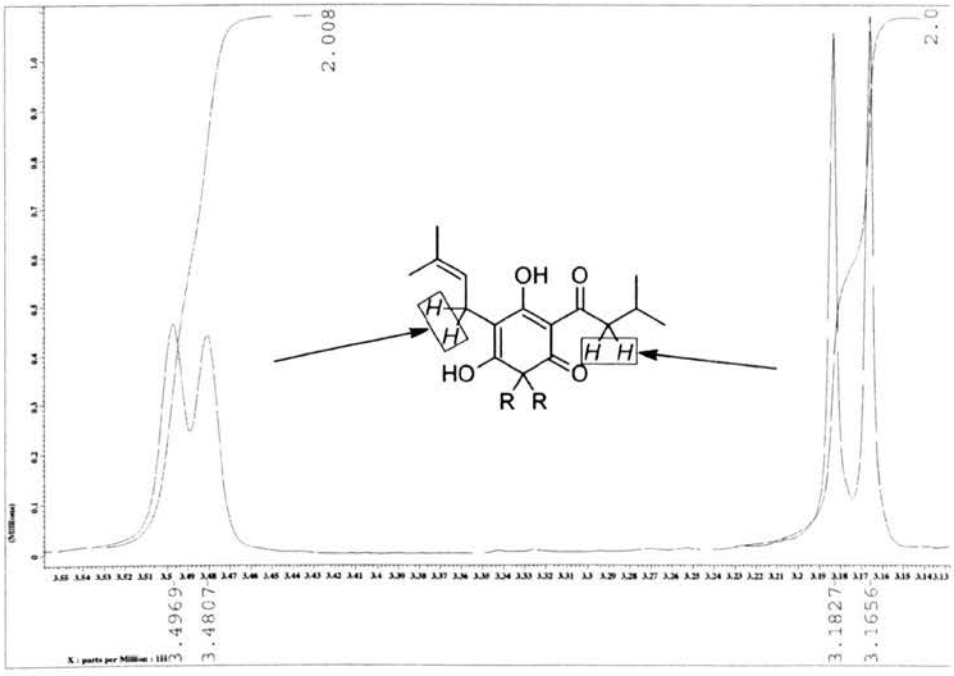


Fig 67 Expansion of the ^{1}H NMR spectra of lupulone lupulone in pyridine

Assignment of the isoprenyl protons is made easier by the fact that the signals for the isoprenyl groups at (C4) all integrate for double those of the isoprenyl group at (C6). This observation of all the signals in the (C4) isoprenyl groups integrating for two (Figure 68) allows us to distinguish one from the other in the humulone molecule giving rise to the assignments above.

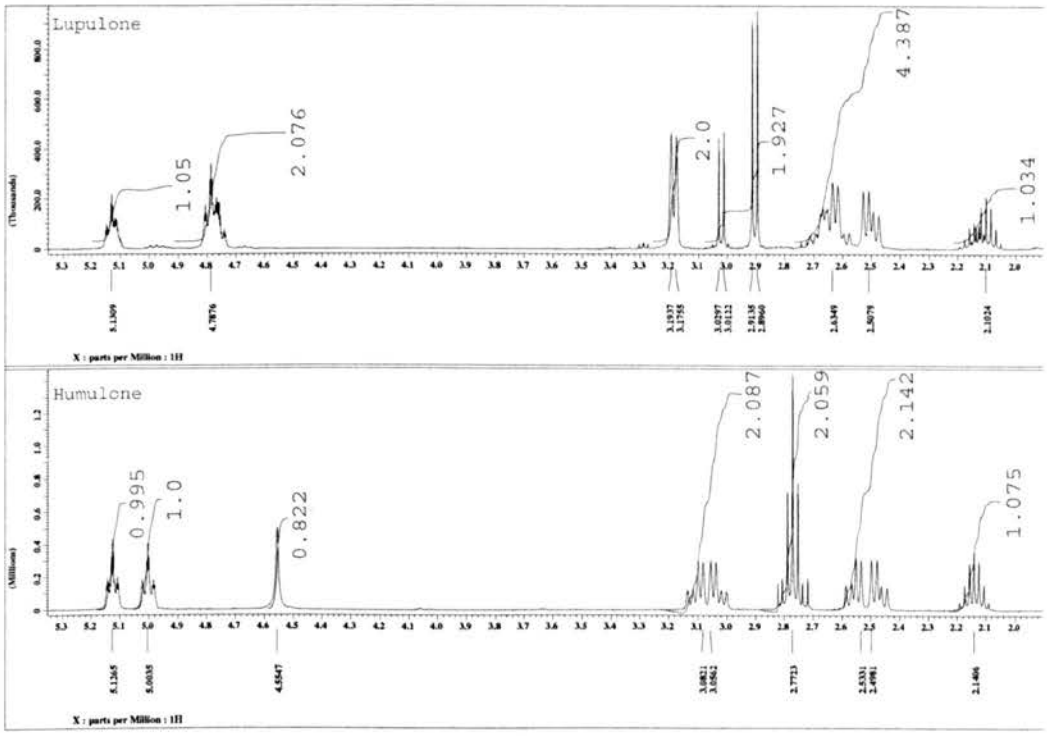


Fig 68 Comparison of the expanded 1H NMR spectra for lupulone (22) (top) and humulone (8) (bottom)

However we have conducted HETCOR experiments on lupulone (Figure 69) and discovered that the anomalous chemical shifts of the dimethylallyl groups of humulone are not observed in lupulone. Indeed the same pattern of the *cis* and *trans* methyl groups and their relative chemical shifts is observed for the group at C6 and the groups at C4.

Fig 69 A 2D HETCOR plot of lupulone in CD_3OD (Top) and an expansion (bottom) clearly showing the relationship between the protons and carbons in the terminal dimethylallyl groups

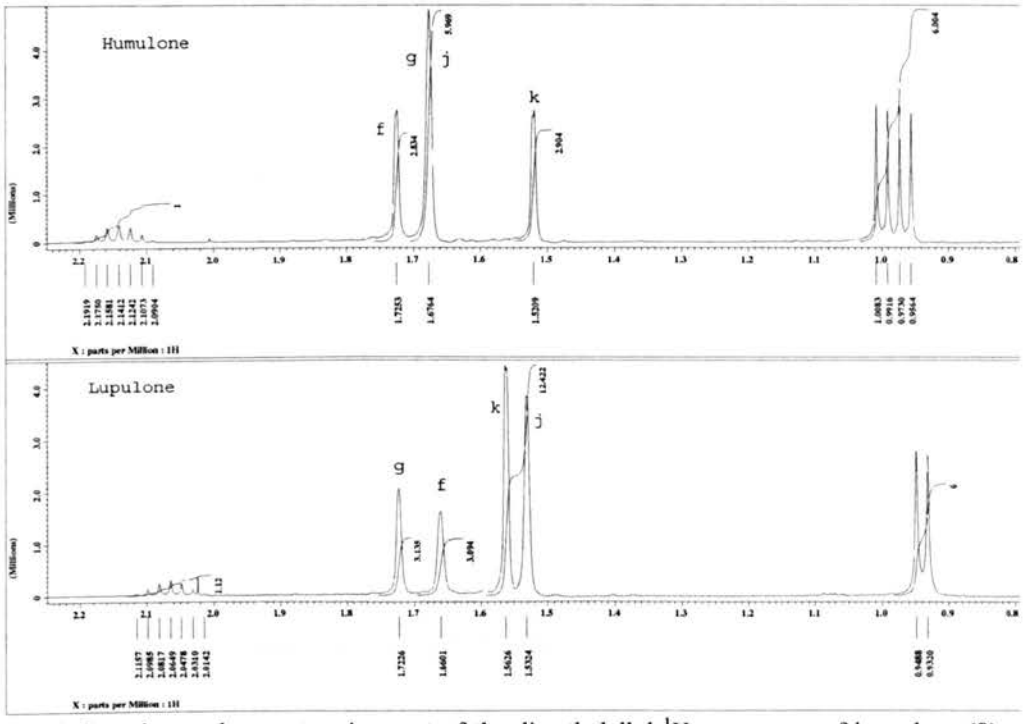


Fig 70 Complete and correct assignment of the dimethylallyl ^{1}H resonances of humulone (8) and lupulone (22). Notice the large change in δ for protons (j) and (k) in both humulone (8) and lupulone (22).

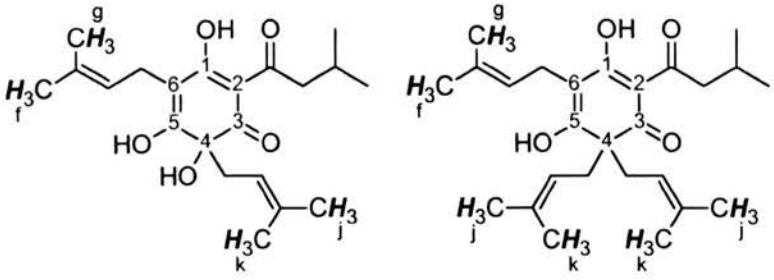


Fig 71 Complete assignment of the geminal methyl groups of humulone (8)

From this data we can say with certainty that the assignments of dimethylallyl groups of lupulone are correct due to the integration of those at C4 being double those at C6. However we have seen that the presence of the tertiary hydroxyl function at C4 has had a dramatic impact on the chemical shifts of these methyl protons. If we consider that these protons are 6 bonds away from the oxygen then it is fair to conclude that making assumptions of the ^{1}H chemical shifts of the α- and β- acids based on comparisons with each other is not appropriate. In order to fully

assign both the α- and β- acids fully we need to produce further spectral data for each compound.

While a complete assignment has not been achieved, this data has clarified the chemical shifts of the hydroxyl resonances of humulone (8) (Figure 72).

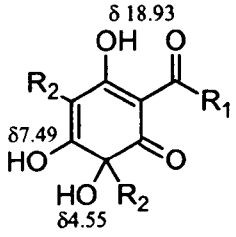


Fig 72 Assignment of the ^{1}H δ values of the hydroxyl protons for humulone (8)

3.3.1.3 ^{13}C NMR Spectroscopy of Humulone (8) and related compounds.

The ^{13}C NMR spectra of humulone (8) and lupulone (22) are also of great interest, mainly because until now the carbon atoms have been incompletely assigned. Borremans was the first person to attempt a complete assignment of the ^{13}C NMR spectra for humulone in 1975 [77]. However, he was unable to complete his analysis and in particular he was unable to confidently assign he ring carbons C-1, C-3 and C-5 with the resonances at δ167.94, 191.05 and 195.39ppm (Figure 73).

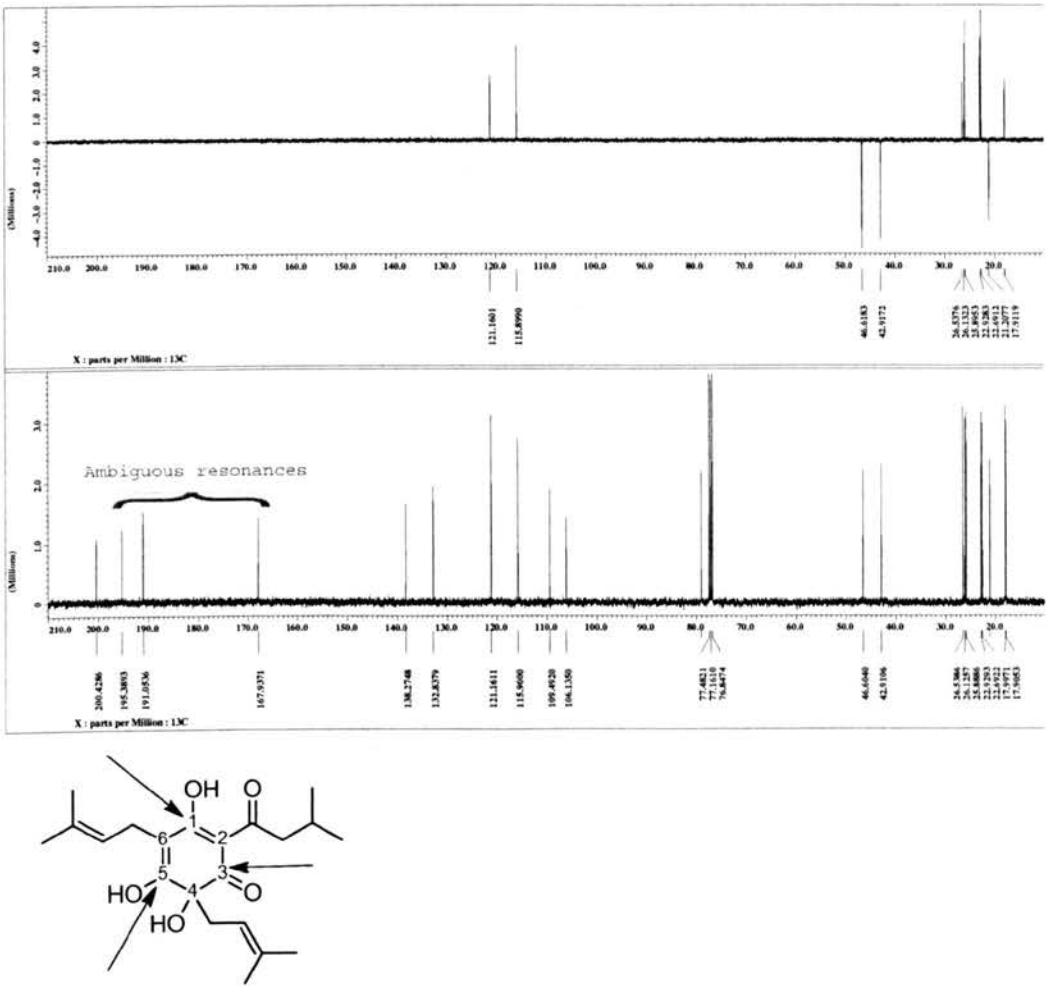


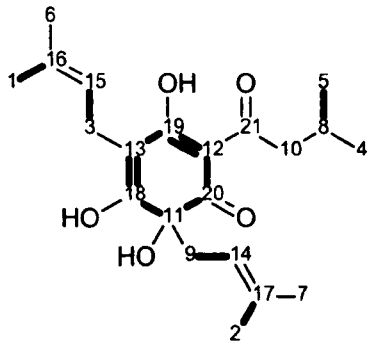
Fig 73 ^{13}C and DEPT 135 NMR spectra of humulone, the ambiguous carbon atoms are indicated in the structure by arrows

Using INADEQUATE NMR spectroscopy it is possible to suppress the signals from the 1% of molecules that contain a single ^{13}C nucleus in order to observe the 0.01% of molecules that contain two J-coupled ^{13}C spins [123]. This in turn gives us the J-values for each resonance. By matching these J-values it is possible to determine which carbon atoms are adjacent to one another. This single technique allows us to completely map the carbon backbone of a molecule. However this technique is restricted only to very simple molecules due to the very small differences in J-values observed. This problem with more complex molecules is circumvented by the use of 2D INADEQUATE spectroscopy. This is by no means

a simple technique as it requires very high sample concentrations (600 – 700mg / ml) and several days for data acquisition.

Goese *et al* [122] studied the effects of ^{13}C enriched glucose on the biosynthesis of humulone (8) and consequently were able to conduct INADEQUATE experiments on the ^{13}C enriched compound. The following results were obtained (Table 4):

Carbon	^{13}C Enrichment (%)	Carbon	^{13}C Enrichment (%)
1	8.3	12	7.7
2	7.8	13	9.1
3	8.7	14	1.4
4	6.7	15	1.5
5	6.4	16	2.3
6	2.1	17	2.0
7	2.1	18	2.7
8	1.3	19	2.3
9	8.3	20	2.3
10	1.6	21	7.0
11	8.7		



Thicker bonds indicate observed C-C coupling.

Table 4 Results from 2D INADEQUATE obtained by Goese et al

This information, although very useful in looking at biosynthesis, is incomplete as without the carbon-carbon coupling data for the exocyclic carbon atoms it is not possible to say with certainty that this is the correct tautomer as assumptions must be made as to which carbon is bonded to the acyl group and which is bonded to the isoprenyl group. We have conducted the first complete 2D INADEQUATE spectrum for natural humulone (8) and are now in a position to provide the

complete assignment of all the carbon atoms, including those which previously were ambiguously assigned (Figure 74).

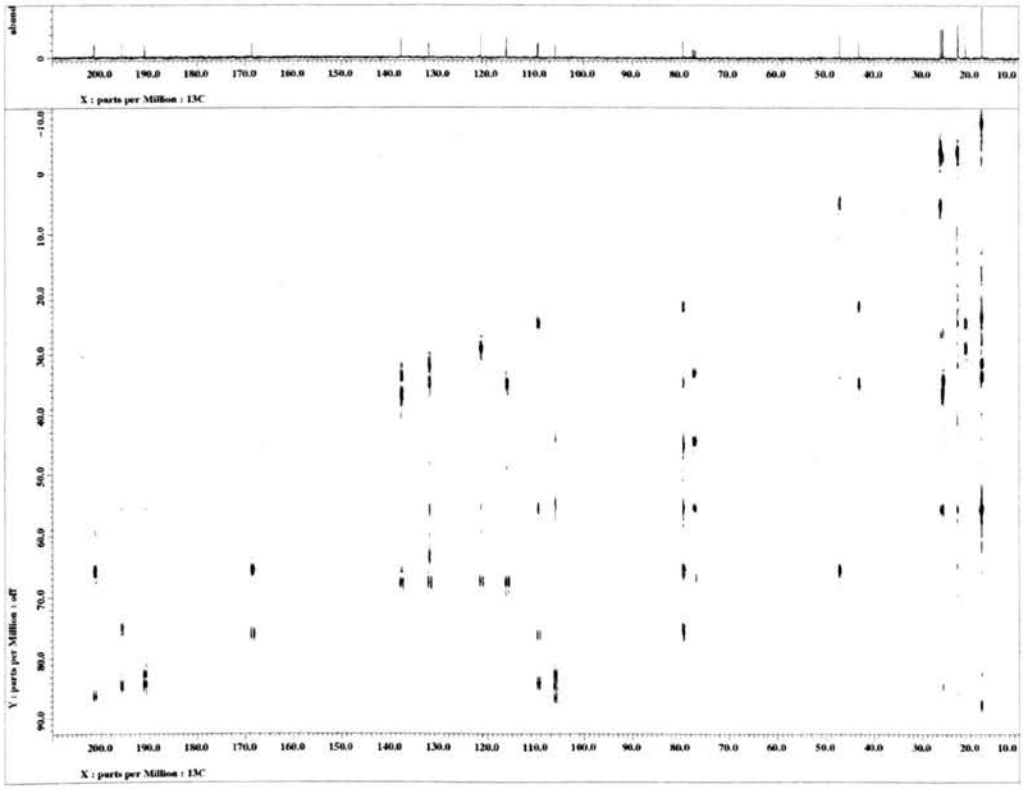


Fig 74 2D INADEQUATE spectra for Humulone (8)

This assignment has been made by examining the connectivity as follows (Figure 75).

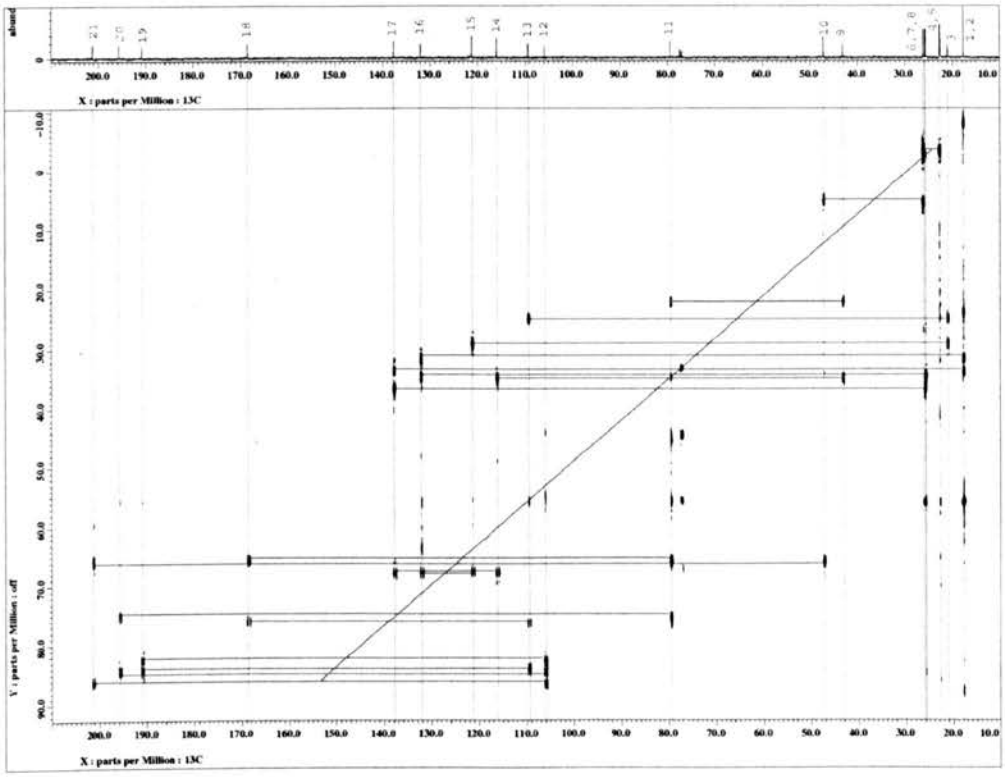


Fig 75 2D INADEQUATE NMR of humulone (8), the diagonal line illustrated above is an imaginary line which passes through the mid point of each C-C connection

From these data we can say with certainty that the following carbon resonances are coupling with one another (Table 5).

Resonance	δ(ppm)	INADEQUATE
1	17.91	16
2	18	17
3	21.21	13, 15
4	22.69	8
5	22.93	8
6	25.89	16
7	26.13	17
8	26.54	4, 5, 10
9	42.91	11, 14
10	46.6	8, 21
11	79.06	9, 18, 20
12	106.14	19, 20, 21
13	109.49	3, 18, 19
14	115.9	9, 17
15	121.16	3, 16
16	132.84	1, 6, 15
17	138.27	2, 7, 14
18	167.94	11, 13
19	191.94	12, 13
20	195.39	11, 12
21	200.43	10, 12

Table 5 ^{13}C data for humulone including INADEQUATE connectivity

From this data we can establish the carbon-carbon connectivity throughout the humulone (8) molecule and hence fully assign the carbon resonances, assuming that the tautomeric form proposed by Verzele is correct. This will be discussed more fully later. If we now compare these data to the data collected from the carbon-hydrogen correlation, we can now unequivocally assign all of the protons without having to make assumptions based on ambiguous data.

In order to complete the picture we must now compare the results of NMR studies of lupulone. In chloroform lupulone (22) exists in two tautomeric forms, this is evident by two resonances for each carbon, one existing in much lower abundance (Figure 76). In methanol there is only one resonance for each carbon atom suggesting the presence of only one tautomer (Figure 77).

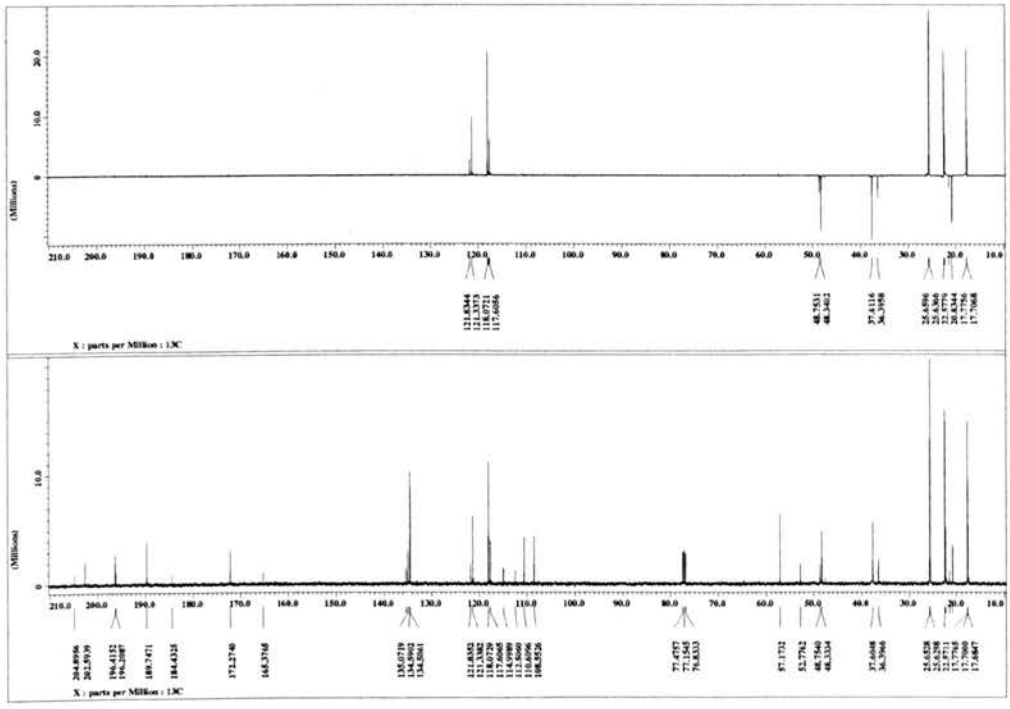


Fig 76 ^{13}C and DEPT 135 NMR Spectra of lupulone (22) in chloroform

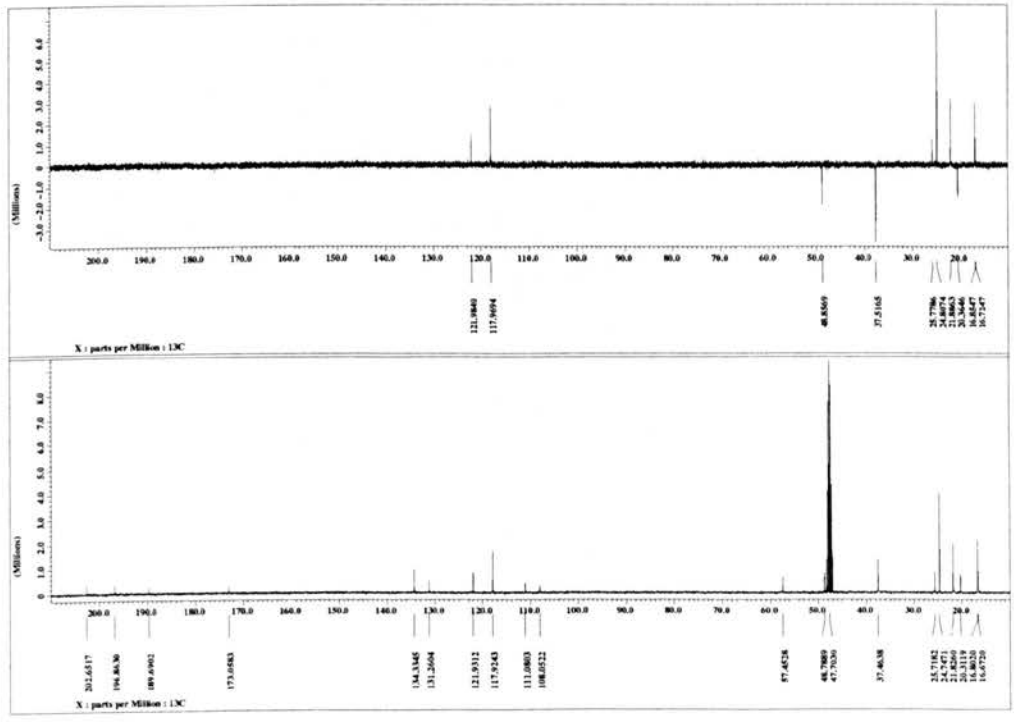


Fig 77 ^{13}C and DEPT 135 NMR Spectra of lupulone (22) in methanol

In addition to these data we were able to acquire an INADEQUATE NMR spectrum of lupulone (22) (Figure 78). The signals are not as intense as those

observed for humulone (8) due to the much lower relative concentration of the major isomer.

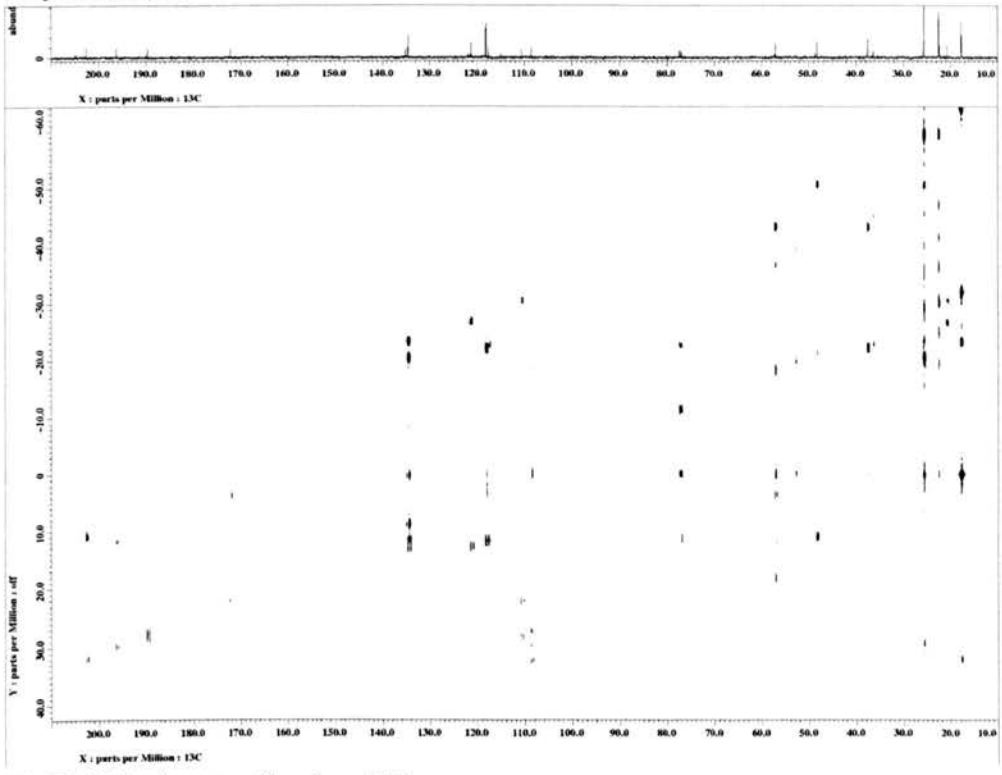


Fig 78 2D Inadequate of lupulone (22)

The signal intensity can be increased by changing the sensitivity (Figure 79), which increases the overall noise, however this allows for the downfield signals to be more easily identified (Table 6). We still have to assume at this stage that the tautomeric forms of humulone (8) and lupulone (22) suggested by Verzele are correct, we will come on to the assignment of tautomeric forms later.

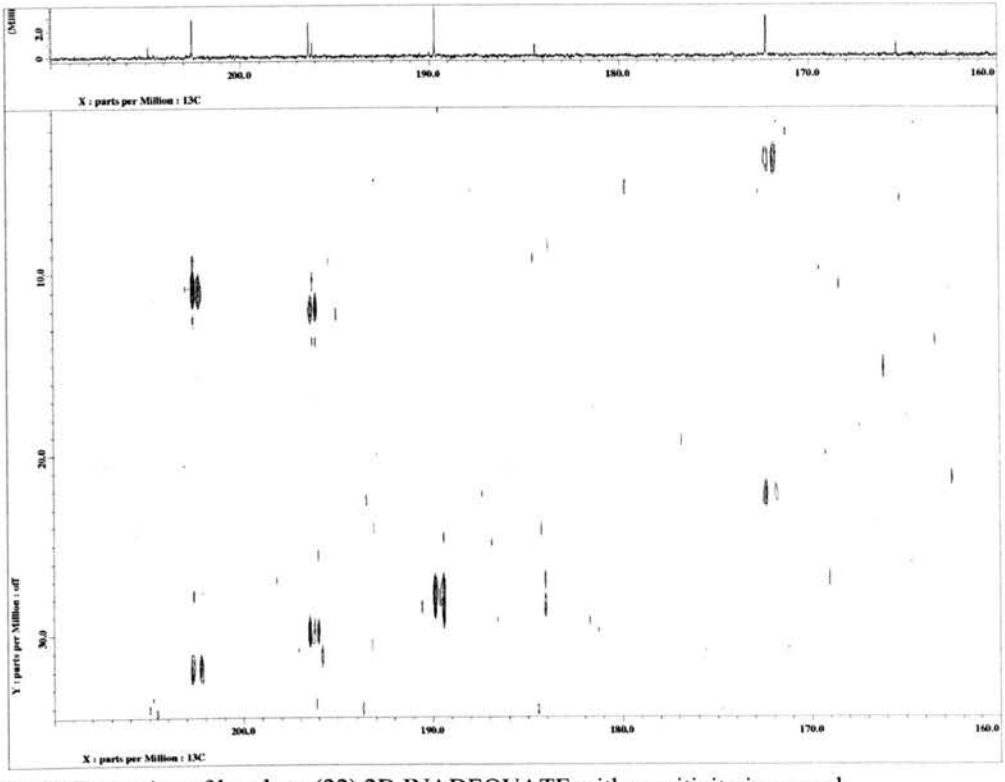


Fig 79 Expansion of lupulone (22) 2D INADEQUATE with sensitivity increased

Carbon	Chemical Shift	INADEQUATE
1	17.71	16
2	17.78	15
3	20.84	14, 12
4	25.75	5, 9
5	22.58	4
6	25.63	15
7	25.66	16
8	37.61	10, 13
9	48.34	20
10	57.18	8, 17, 19
11	108.56	18, 19, 20
12	110.62	3, 17, 18
13	118.08	8, 15
14	121.34	3, 16
15	134.51	2, 7
16	134.6	1, 6
17	172.28	10, 12
18	189.75	11, 12
19	196.42	10, 11
20	202.6	9, 11

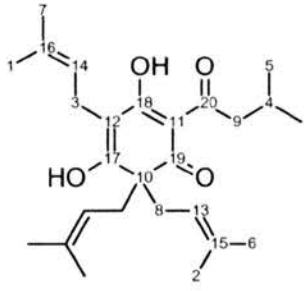


Table 6 ^{13}C data for humulone including INADEQUATE connectivity lupulone (22)

From these data coupled with the INADEQUATE spectrum of humulone (8) we can suggest that, as the INADEQUATE pattern for the rings are almost identical, that humulone (8) and lupulone (22) do indeed exist in the same tautomeric forms.

This is of great importance when studying the chemistry of these compounds. Without a definitive structural elucidation of the starting material, it would be impossible to accurately predict the structure of the product.

Below is the complete assignment for humulone (Table 7) based upon the comparison of the 2D INADEQUATE data in Table 5 and Table 6.

Carbon	Chemical Shift (ppm)	Carbon	Chemical Shift (ppm)
1	17.90	12	106.13
2	18.00	13	109.49
3	21.21	14	115.90
4	22.69	15	121.16
5	22.93	16	132.84
6	25.89	17	138.27
7	26.12	18	167.94
8	26.54	19	191.05
9	42.91	20	195.39
10	46.60	21	200.43
11	79.06		

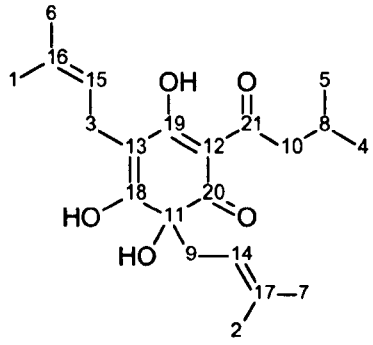


Table 7 Complete ^{13}C data for humulone (8)

The ^{13}C NMR spectra of deoxyhumulone (19) (Figure 80) does not require such experiments, due to the aromatic and symmetric nature of the molecule the ^{13}C NMR can be fully assigned.

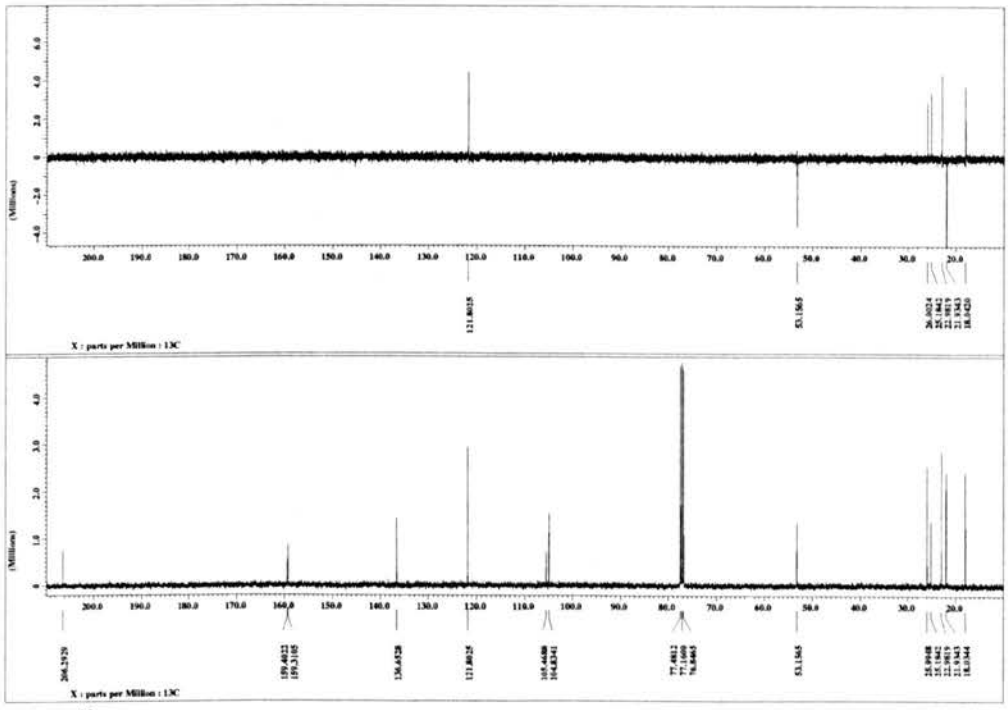


Fig 80 ^{13}C and DEPT 135 data for deoxyhumulone in $CDCl_3$

Carbon	Chemical Shift (ppm)
1	18.03
2	21.93
3	22.98
4	25.18
5	25.99
6	53.16
7	104.83
8	105.47
9	121.80
10	136.65
11	159.31
12	159.40
13	206.29

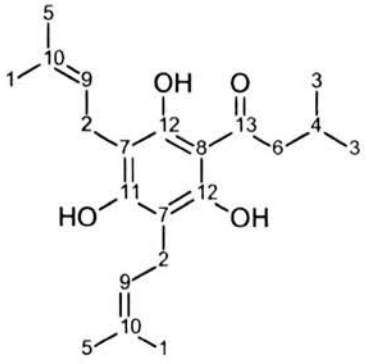


Table 8 Complete assignment of the ^{13}C NMR of deoxyhumulone (19)

We can see by comparison of the NMR data of deoxyhumulone (19) with humulone (8) and lupulone (22) (Table 9) that the ^{13}C chemical shift of the isovaleryl side chain are in significantly different chemical environments, again this

suggests that we should use comparative data tentatively and that the new data we have collected is of great importance due to lack of ambiguity.

R =	Deoxyhumulone	Humulone	Lupulone
C1	δ206.29	δ 200.43	δ 202.60
C2	δ 53.16	δ 46.60	δ 48.34
C3	δ 25.18	δ 26.54	δ 25.75
C4	δ 22.98	δ 22.69	δ 22.58
C5	δ 22.98	δ 22.93	δ 22.58

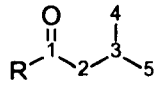


Table 9 ^{13}C chemical shifts of HA isovaleryl side chain

It is of interest to see that the chemical shifts of the ring carbons of the aromatic precursor, deoxyhumulone (19), humulone (8) and lupulone (22) are significantly different showing the aromatic character has been completely disrupted.

From our NMR studies of humulone, lupulone and deoxyhumulone (19) we have achieved the following:

- Confidently assigned all ^{1}H chemical shifts for humulone (8), lupulone (22) and deoxyhumulone (19);
- Recorded the first 2D carbon-hydrogen (HETCOR) and 2D carbon-carbon (INADEQUATE) correlations for lupulone;
- Recorded the first complete 2D INADEQUATE spectra for humulone;
- Confidently assigned all ^{13}C chemical shifts* for humulone (8) and lupulone (22) including those which, until now, have remained ambiguous;
- Demonstrated that slight changes to the 6-membered ring of these compounds has a significant effect on atomic shielding and hence chemical shift;

* Based on the assumption that Verzele and De Keukeleire's proposed tautomeric forms are correct [19,20] this will be discussed in the next section.

3.3.2 X-ray Diffraction Sample Preparation

All of the historic data, collected in the literature, in order to determine the absolute structure and absolute configuration of (-)-humulone (8) is dubious as it involved the use of old and unsuitable techniques. These will be discussed later in this chapter. As a result it was decided that X-ray crystallography may give irrefutable proof of the structure of the hop bitter acids.

Single crystal X-ray diffraction is a powerful technique for the determination of structure and conformational studies of organic and inorganic compounds. The usefulness of this technique for the determination of the absolute configuration of an unknown chiral centre is slightly restricted due to the following criteria:

Crystal morphology, it is necessary for a crystal to be the correct size in order for single crystal X-ray diffraction to be possible. Crystals must be in the order of 0.1 mm in length on at least 2 sides of the crystal;

If a known chiral centre is present in the molecule then all other chiral centres will be determined. Thus a chiral auxiliary must be used or a chiral molecule which co-crystallises with the unknown molecule;

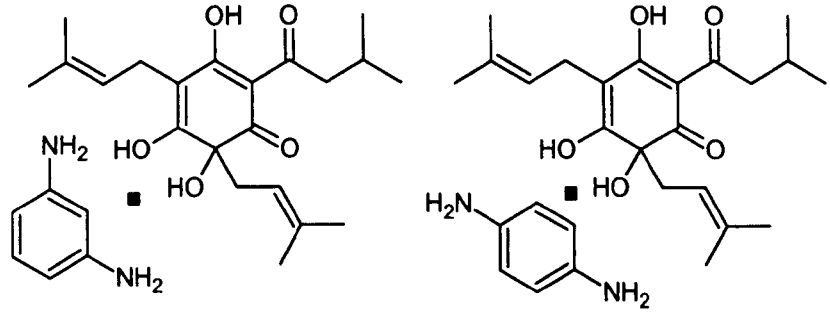
If there are no known chiral centres then it may be necessary to use a phenomenon known as "Anomalous Dispersion" [124].

Normal X-rays are reflected by the atoms within a crystal, these X-rays are 180^{o} out of phase with the incident beam and can only determine absolute configuration if a known chiral centre is present. Anomalous dispersion occurs when the incident X-rays are absorbed by the atoms and re-emitted in all directions. These scattered X-rays can be used to determine the configuration of the molecule as they are independent of the incident beam.

In order for anomalous dispersion to be useful it is necessary for there to be "heavy atoms" present in the crystal. In crystallographic terms a heavy atom is defined as any atom heavier than oxygen or more commonly defined as being silicon or heavier.

In the absence of heavy atoms it is possible to use copper as the source of X-ray radiation as Cu Kα radiation is more readily absorbed by "light atoms" [125] than the more commonly used Mo Kα although inherent difficulties with using copper as the radiation source means that in many laboratories this is not used routinely.

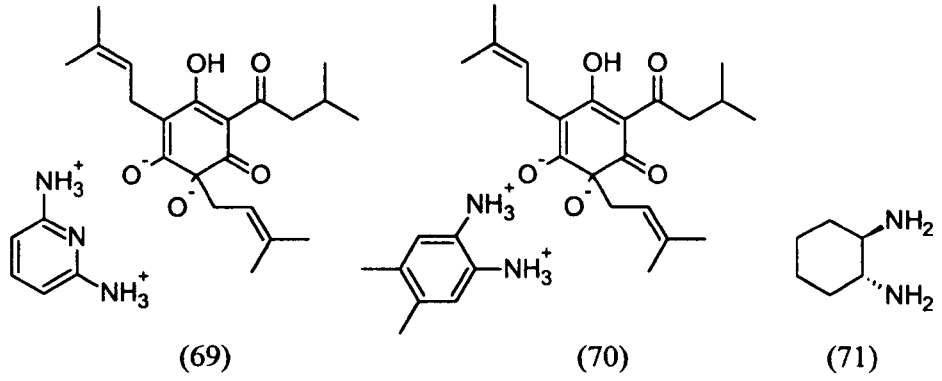
Many attempts were made to form a suitable crystal from natural humulone for tautomeric studies. Recrystallisation of humulone (8) from acetonitrile furnishes adequately sized granular crystals in the order of 0.1 mm in each dimension however none were suitable for a single crystal X-ray as no unit cell could be determined. In order to attempt to form a suitable crystalline derivative of humulone (8) for X-ray analysis a number of diamine salts were synthesised. The phenylenediamine salt (16) used in the above isolation procedure was crystallised as yellow needles. Despite numerous attempts to isolate these in a form which would be suitable for XRD, none have had a suitable morphology. Consequently 1,3- (67) and 1,4-phenylenediamine salts (68) (Figure 81) were also investigated as suitable candidates. Neither of these produced a crystalline material, neither did diaminomaleonitrile.



(67) (68)

Fig 81 1,3-Phenylene diamine complex (67) and 1,4-phenylene diamine complex (68)

Exposure of humulone with 2,6-diaminopyridine produced a complex (69) (Figure 82) of green cuboidal crystals about 1mm in size however these crumbled quickly into an amorphous powder on contact with the atmosphere. 4,5-Dimethyl-1,2-diamino benzene complex (70) (Figure 82) also produced crystals of similar morphology to that of the 1,2-phenylenediamine complex (16).

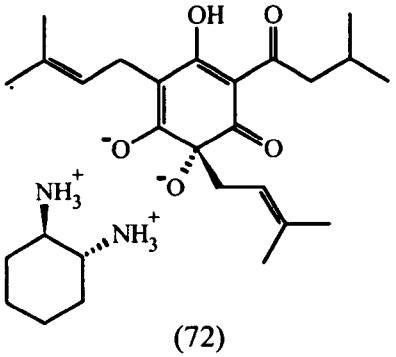


(69) (70) (71)

Fig 82 2,6-Diaminopyridine complex (69), 4,5-dimethyl-1,2-diaminobenzene complex (70) and 1R-2R-(-)-diaminocyclohexane (71)

The observation that humulone forms crystalline derivatives with aromatic diamines led us to investigate chiral diamine compounds to introduce a known chiral centre. Firstly it was decided to use commercially available diamines, the obvious choice was *trans*-1,2-diaminocyclohexane (71) (Figure 82). This is analogous to 1,2-phenylenediamine so it was hoped that this would form a crystalline derivative. In order to determine the correct isomer a solution of natural R – (+) humulone (8) in hexane was mixed with a 2 fold excess of racemic 1,2-diaminocyclohexane (71) in hexane. The resultant precipitate was collected by filtration and re-crystallised from ethyl acetate 3 times. Subsequent decomposition of the complex, using dilute hydrochloric acid in ether, was followed by an optical rotation measurement on the aqueous phase. This showed it to have a negative rotation which is in agreement with the optical rotation of 1R,2R-(-)-diaminocyclohexane (71_R) in HCl solution, thus it was concluded that R-(+)-humulone (8) preferentially complexes with 1R,2R-(-)1,2-diaminocyclohexane (71_R) (Figure 82).

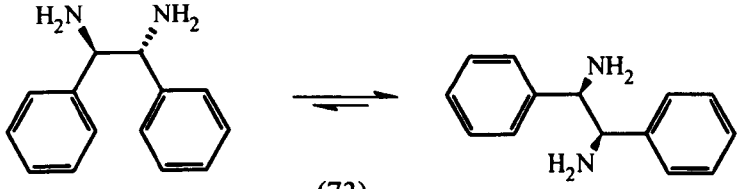
The appropriate crystalline derivative of humulone was then synthesised from natural humulone and 1R,2R-diaminocyclohexane (71_R) (Figure 83) however, the crystals were similar in their morphology to that of the phenylenediamine complex (16) and deemed unsuitable for X-ray analysis.



(72)

Fig 83 Chiral humulone complex of natural humulone and (1R, 2R)-diaminocyclohexane (72)

A number of other chiral diamine compounds were tested during the course of this research. One such chiral diamine tested was 1,2-diphenylethane-1,2-diamine (73) (Figure 84). The reason for this particular choice is that the material is commercially available and readily prepared by a literature procedure from ammonium acetate and benzaldehyde. Separation of the isomers by complexation with tartaric acid gives the appropriate isomer. If the crystals of the diphenyl derivative were not suitable, a whole range of other diamines could be formed from substituted benzaldehydes.



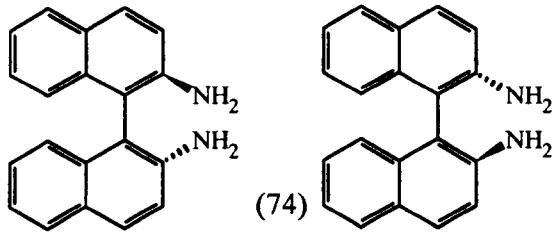
(73)

Fig 84 (1*R*,2*R*)-1,2-diphenylethane-1,2-diamine (73) and its most likely conformation.

Attempted complexation with humulone (8) provided no crystals. This seemed to be anomalous with previous findings as the above compound appears to fulfil the criteria necessary for crystal formation. It was then hypothesised that the free

rotation of the bond between the amine bearing carbons and the bulky phenyl groups may result in a conformer that is not suitable for humulone (8) binding.

The second commercially available chiral diamine is 2,2'-diamine-1,1'-binaphthalene (74). The (R)-(+) and (S)-(-) isomers were tested, however no crystals were formed (Figure 85).



(74)

Fig 85 R-(+)-2,2'-diamine-1,1'-binaphthalene and S-(-)-2,2'-diamino-1,1'-binaphthalene respectively (74)

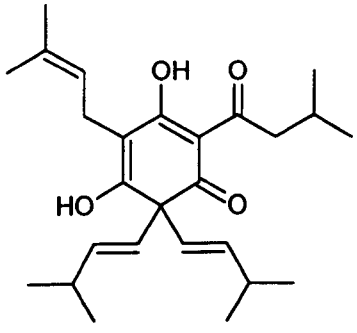
Pure (R) – (+) – humulone (8) is a solid at room temperature (mp. 80 ^{0}C) thus it was decided to examine the crystal properties of this material. Previously humulone (8) was isolated as an amorphous solid by evaporation of pentane. Crystallisation from aqueous acetic acid has been described [126] however the ratio of acetic acid to water is not given and this has proven to be very difficult to perform without significant degradation of humulone (8). It was discovered, through the course of this research, that acetonitrile could be used for recrystallisation of humulone (8) to form very pale yellow crystals. Acetonitrile has the advantage over the acetic acid / water mixture in that the evaporation of acetonitrile is considerably faster than the evaporation of acetic acid in water. This means that the degradation of humulone is minimalised whilst the solvent is removed by slow evaporation. Using this method it has been possible to produce crystals of lupulone and colupulone that are suitable for X-ray analysis, however lupulone (22) can also be re-crystallised from glacial acetic acid. This process requires seeding if good quality crystals are desired.

3.3.2.1 Preparation of β-acids and derivatives

The β-acids are analogous to the α-acids, differing only in the presence of an alkyl side chain in the place of the tertiary hydroxyl of (C4) making the β-acids more stable as they do not undergo the isomerisation reaction associated with the α-acids. This makes understanding the chemistry of these compounds an important foundation to the understanding of the α-acid chemistry.

3.3.2.2 Lupulone (22)

Since its discovery in 1863 lupulone has been assigned a number of structures. The first, and incorrect, structure proposed by Wieland [127] and Wollmer [128] (75) in 1925 was similar to the presently accepted structure but the exocyclic double bonds were misplaced (Figure 86).



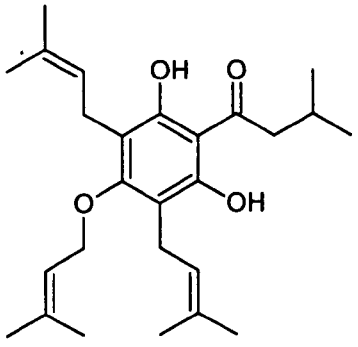
(75)

Fig 86 Incorrect proposed structure of lupulone (75)

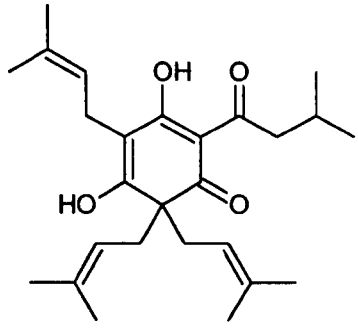
This structure was contradicted by Verzele and his research group [129] by the observation that upon ozonolysis of lupulone three moles of acetone are observed derived from the alkenyl side chains.

Other suggested structures were proposed by Howard and Harris [16]. They observed that lupulone loses an isoprenyl group upon hydrogenolysis, however at the time this type of hydrogenolysis had only ever been observed with C-O

cleavage and never with C-C cleavage. Thus the structure (64) below was presented (Figure 87) however ^{1}H and ^{13}C NMR has ruled this proposed structure.



(64)



(22)

Fig 87 Harris' proposed structure for lupulone (64) and the currently accepted structure (22)

The currently accepted Verzele structure [129] for lupulone (22) is shown (Figure 89). Although this structure is accepted there is still much discrepancy as to the tautomeric forms of lupulone (22). Indeed much of the older literature describes lupulone as existing in the tricarbonyl tautomeric form (Figure 88) [130].

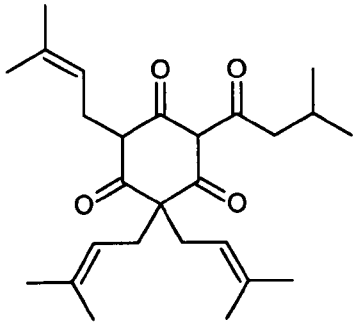
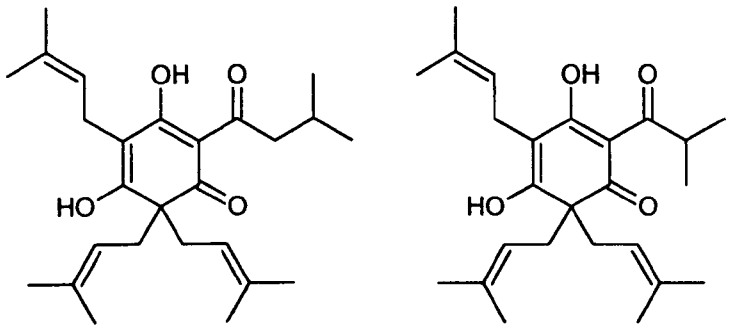


Fig 88 Commonly used tricarbonyl form of lupulone (22)

NMR spectroscopy however, shows that there are 2 resonances representing hydroxyl protons which contradict the tricarbonyl model. Arguments put forward by Verzele et al [1] rationalise the structure of lupulone (22), by comparison with that of humulone (8), thus the following structure of lupulone (22) and colupulone (50) are currently accepted (Figure 89).



(22) (50)

Fig 89 Lupulone (22) and colupulone (50) respectively

NMR spectroscopy of these compounds is of high importance when we consider the proposed structure for lupulone and colupulone. When the original NMR spectroscopy was carried out [77] it was conducted on colupulone due to its naturally high abundance, ease of isolation by recrystallisation and its apparently greater stability. It was hypothesised that colupulone exists in two different tautomeric forms (discussed more fully later). Looking at the structure of colupulone (50) it is clear that it could, in fact, exist in many possible forms. The ^{1}H NMR spectrum shows that there are two hydroxyl functions present, this limits the number of possible tautomeric forms to three (Figure 90).

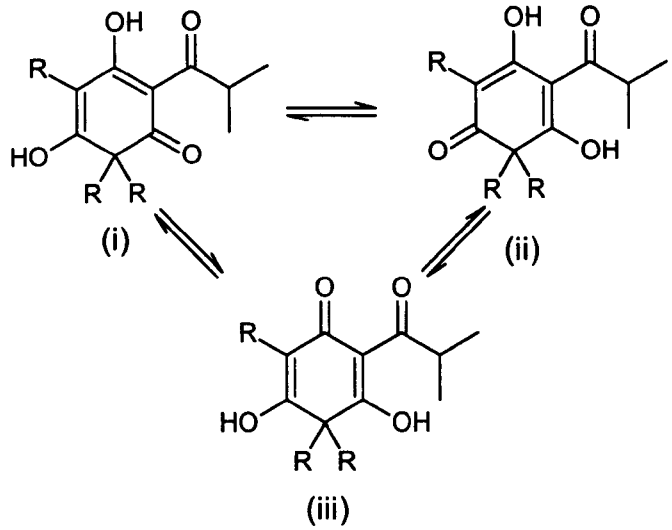
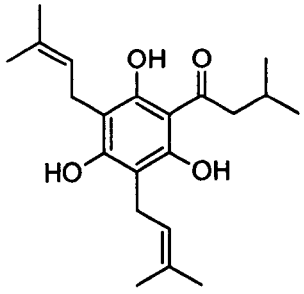


Fig 90 Potential tautomeric forms of colupulone (50)

The analogy with the α-acids was used to argue that tautomeric form (ii) is not present as the hydroxyl resonances would be present as one signal integrating for both protons. This was discussed in (Figure 54) for 4-acetyl resorcinol (65) shows 2 resonances for the two hydroxyl protons, however, 2-acetyl resorcinol (66) shows just one signal.

This phenomenon is also observed with deoxyhumulone (19) (Figure 91) which has three hydroxyl groups but the ^{1}H NMR spectrum shows two resonances at δ6.29 ppm (1H) and δ10.21 ppm (2H) for the hydroxyl protons one of which integrates for 2 protons.

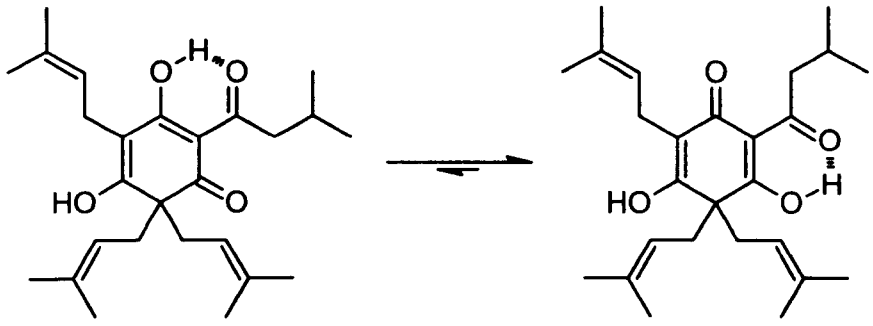


(19)

Fig 91 Deoxyhumulone (19)

It is noteworthy however that the isomers of acetyl resorcinol and deoxyhumulone (19) are all substituted aromatic compounds, whereas lupulone (22) is not aromatic and cannot be expected to behave in the same way. It was suggested by Verzele [46] that lupulone (22) should adopt the same form as humulone, thus the major form of lupulone is suggested as (22_a) (Figure 92). The widely accepted form of the minor tautomer is structure (22_b).

We have conducted computer modelling (using Quantum CACHE, MM3 CONFLEX extensive search) of the tautomeric forms of lupulone (22) and found Verzele's hypothesis to be supported in terms of the minimum energy (Figure 92) thus tautomer (22_a) is 23.11 kcal/mol more stable than the minor tautomer (22_b).



22a 22b

49.73 kcal/mol 72.84 kcal/mol

Fig 92 Tautomeric forms of lupulone (22) and their respective energies.

Careful recrystallisation of lupulone (22), from acetic acid, yielded crystals suitable for X-ray diffraction studies. The reason for choosing acetic acid was the apparent formation of just one tautomer in D_4 Acetic acid as an NMR solvent (discussed later). On analysis of the computer modelling data we can say with some certainty that the major isomer of lupulone and the isomer found in acetic acid is (22_a) from single crystal X-ray diffraction studies the following structure was observed (Figure 93).

Analysis of the modelling data suggests that the major tautomeric form of lupulone (22) is that illustrated as (22a). Data for the X-ray diffraction studies supports this structure (Figure 93).

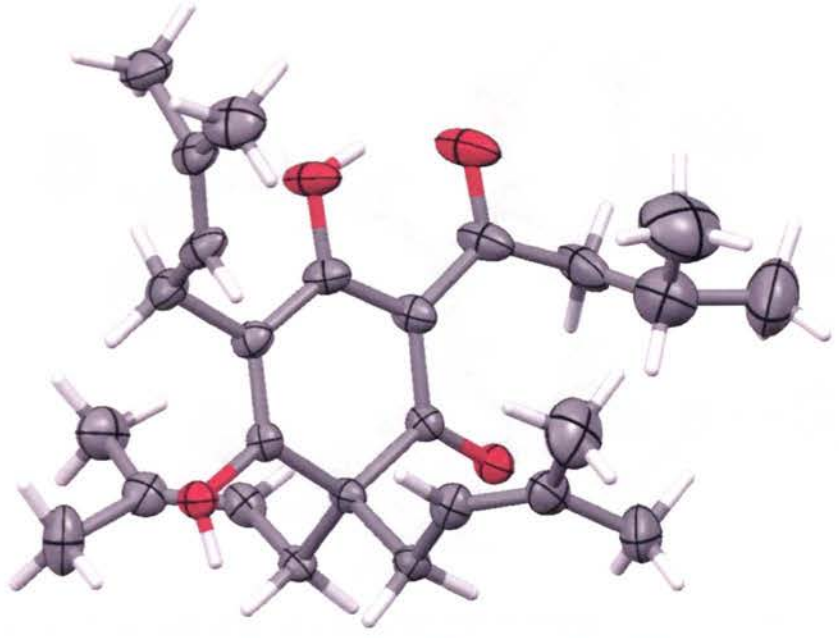


Fig 93 X-ray structure of lupulone (22) represented as 50% displacement ellipsoids

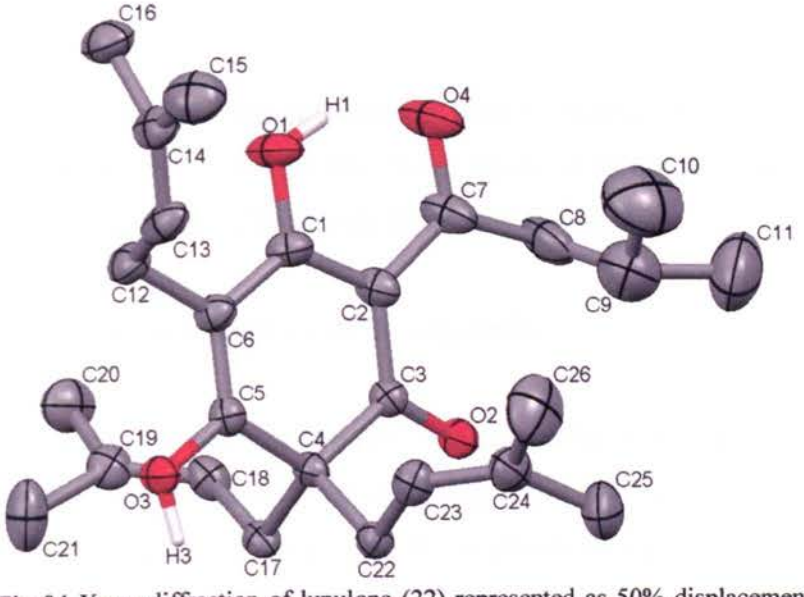


Fig 94 X-ray diffraction of lupulone (22) represented as 50% displacement ellipsoids. Hydrogens have been omitted for clarity with the exception of the hydroxyl hydrogens.

These give strong evidence that lupulone exists as tautomer (i) (Figure 90) in acidic solution.

This data was further clarified by the collection of single crystal X-ray data for colupulone (50) recrystallised in a similar manner to lupulone (22) (Figure 95).

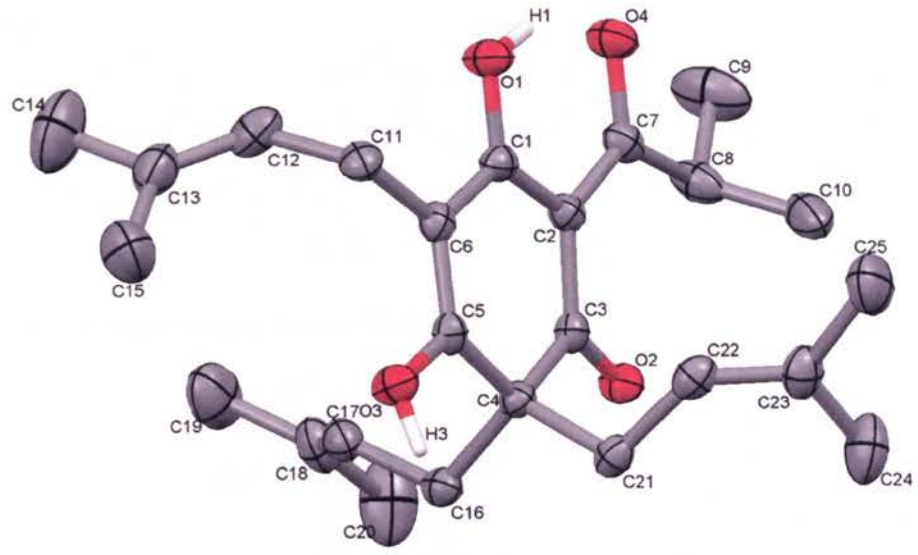


Fig 95 X-ray diffraction of colupulone (50) represented as 50% displacement ellipsoids; the hydrogens have been omitted from the bottom figure for clarity with the exception of the hydroxyl protons.

If we now refer this information back to humulone (8) we may propose that humulone also exists in this form, hence confirming what Verzele *et al* have already suggested with their perhydrogenation studies.

3.3.2.3 Crystal structure of the hop acids

Lupulone (22) contains two enol groups and two ketone moieties. These functional groups are capable of forming hydrogen bonds. The proton that forms part of the keto-enol group hydrogen-bonds intramolecularly. This is supported by the downfield chemical shift observed by ^{1}H NMR spectroscopy at δ 19.23 ppm. Hydrogen bonding is also evident in the X-ray structure above.

From X-ray diffraction studies it also appears that the other hydroxyl function also hydrogen bonds with a carbonyl group. These functional groups are spatially too far apart for intramolecular hydrogen bonding. This suggests that a lupulone molecule forms hydrogen bonds to another lupulone molecule in close proximity. This

phenomenon allows lupulone to form a solid at room temperature (Figures 96a and 96b).

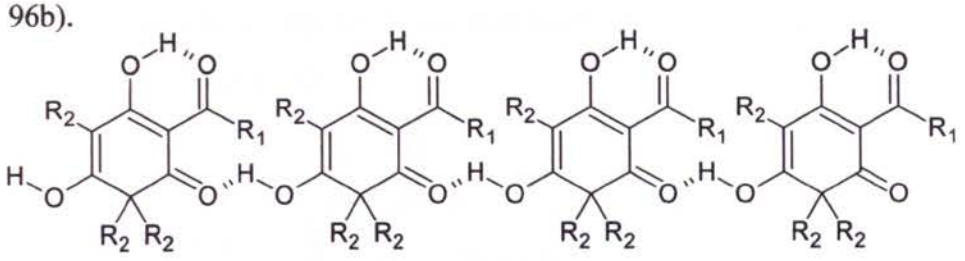


Fig 96a Hydrogen bonding between the β-acids

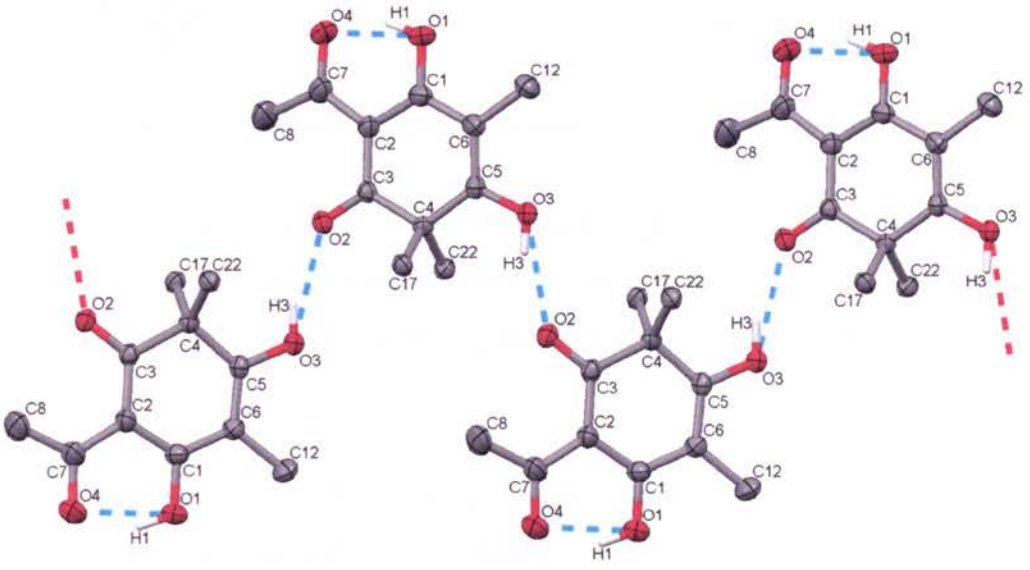


Fig 96b. Single crystal X-ray showing hydrogen bonding of lupulone within the crystal lattice shown as 50% displacement ellipsoids; hydrogens and carbon chains atoms have been omitted for clarity.

Confirmation of this phenomenon comes from derivatisation studies when conversion of the C-5 hydroxyl to a C-5 sulfonate or C-5 acyl leads to loss of thecrystalline structure to afford liquids at room temperature.

The process of intermolecular hydrogen bonding between the C-3 carbonyl of the molecule and the C-5 hydroxyl group of another molecule may explain why derivatives of lupulone such as O-tosyl and O-acyl derivatives are liquid at room temperature as this derivatisation disrupts hydrogen bonding.

This phenomenon of intermolecular hydrogen bonding also explains the crystalline nature of humulone (mp 80 °C) as this molecule contains the same moieties as lupulone (22) (Figure 97).

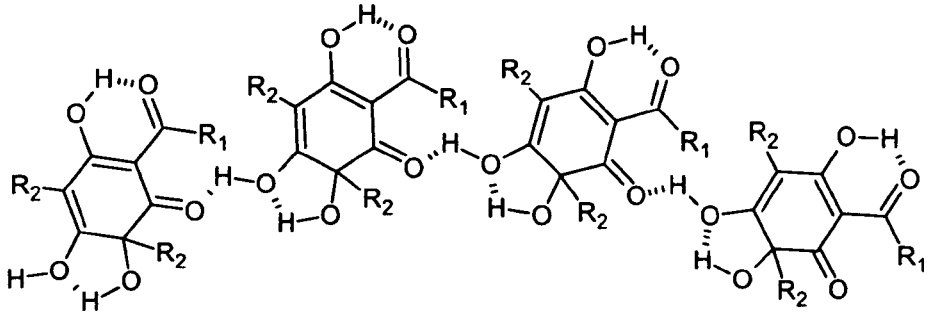


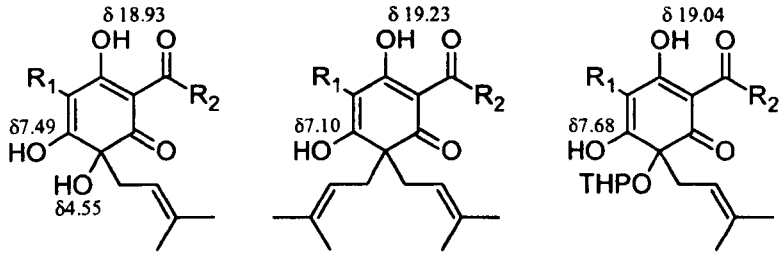
Figure 97 Proposed hydrogen bonding within the crystal structure of humulone (8)

Attempts to form a suitable crystal of humulone (8) for X-ray analysis have failed to produce a unit cell. This is likely to be due to this intramolecular hydrogen bonding randomly throughout the crystal preventing uniform repetition of the unit cell.

The hydrogen bonding character of lupulone and humulone, allowing for crystal formation, is of great interest as it has allowed for a greater understanding of why the synthetic derivatives of humulone and lupulone are not solids at room temperature.

Of all of the attempted derivatives of humulone only one formed a crystalline solid which was the THP ether of humulone (76). The observation that this was a solid led us to hypothesise that the protecting group had selectively reacted with the tertiary hydroxyl function. As was expected the reaction with dihydro-*2H*-pyran formed a new chiral centre, which, due to the chiral nature of the starting material the product was a diastereomeric mixture with a d.e. of 20 %. Separation of the diastereoisomers was possible by preparative chromatography, the isomers were (76_a) (76_b), based on the order by which they are eluted by normal phase chromatography using ether / hexane as the eluent. Diastereomer (76_a) is the isomer

formed in higher abundance and analysis by ^{1}H NMR spectroscopy confirmed that the tertiary hydroxyl function had been selectively protected (Figure 98).



(8) (22) (76_a)

Fig 98 Humulone (8), lupulone (22) and THP protected humulone (76a) respectively

The C-5 hydroxyl function of the mixture of the new derivative shows a resonance of δ 7.68ppm for diastereomer (76_a) and δ 8.9ppm for diastereomer (76_b). Another indication of the protection of the tertiary hydroxyl function is the presence of tautomeric forms seen in the earlier ^{1}H NMR spectra in $CDCl_3$. Indeed the aforementioned enol function shows two resonances; (76_a) shows a second at δ 6.72 ppm and (76_b) shows a second resonance at δ 7.1 ppm. The ratios of these diastereomers is as follows: (76_a) major : minor 97:3, (76_b) major : minor 92:8.

The observation that the THP ether of humulone (76) exists in a crystalline form allowed for a suitable sample for X-ray to be obtained. This confirmed that the C-4 hydroxyl group was selectively protected (Figure 99).

While it is known that the lack of any heavy atoms within the crystal lattice makes the determination of the absolute configuration inaccurate [124] it is not impossible for light atom containing structures to allow for absolute configuration determination [125, 131, 132]. The following structure was calculated using SHELXL (Figure 99).

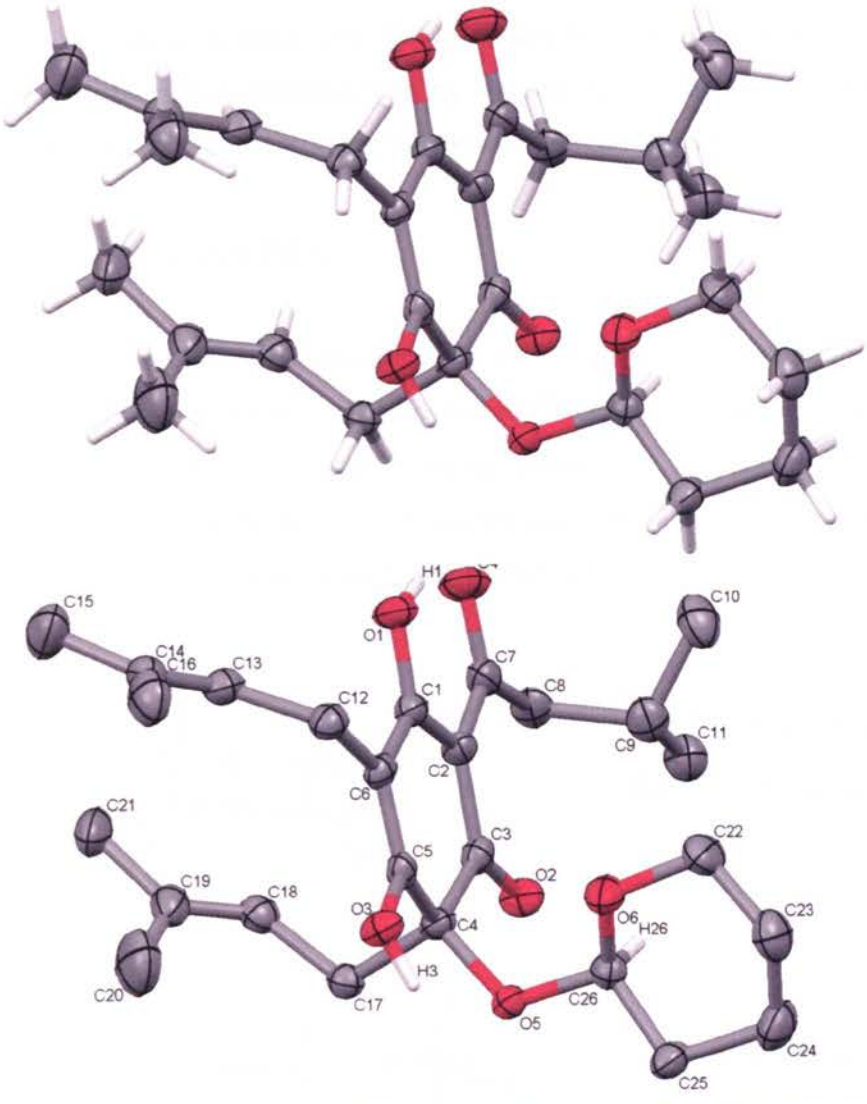


Fig 99 X-ray diffraction of the THP protected humulone (97a) represented as 50% displacement ellipsoids.

From these data we can observe the hydrogen bonding of the hydroxyl function at C-1. We can also observe the stereochemistry at C-4 and C-26. Interestingly the stereochemistry about C-4 appears to be in the (S)-configuration, this is in contrast to previously collected data. The stereochemistry at C-26 is in the (R)-configuration.

These cannot give irrefutable proof of the absolute configuration, however it does cast a shadow of doubt over the configuration that was previously suggested [45]. The conformation determination by Verzele and De Keukeleire was based on the techniques of Optical Rotatory Dispersion (ORD) and chemical degradation.

3.3.2.4 The Cotton Effect

The Cotton effect was discovered in 1957 for the determination of the absolute configuration of α-halo cyclohexanones [133] and later extended to saturated ketones [134]. This method relies heavily on the octant rule. The octant rule is a method of displaying a hexanone by observing the molecule along the carbonyl double bond so that the ring appears as a rectangle (Figure 100).

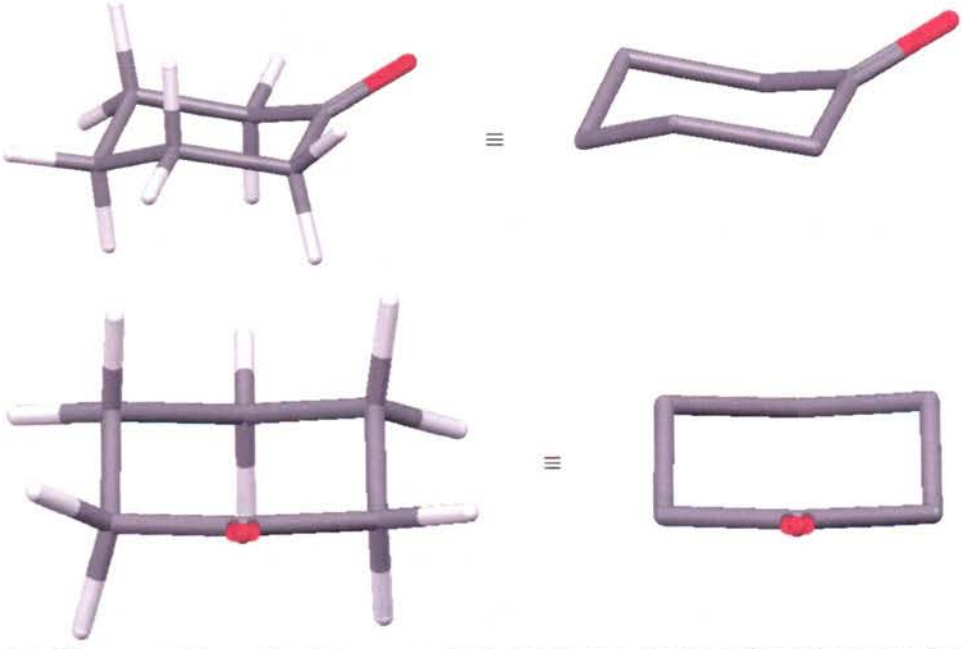


Fig 100 3D representations of cyclohexanone (top), octant representation of cyclohexanone (bottom)

The diagram is then subdivided into quadrants and each quadrant is allocated a +ve or –ve sign as detailed overleaf (Figure 101).

Fig 101 Octant diagram of cyclohexanone

If the ring is α-substituted, the substituent with the highest electronegativity is given priority and the quadrant this substituent resides in dictates the Cotton effect of the ORD curve. Humulone (8) gives a negative Cotton effect by ORD thus the following octant was proposed by Verzele for humulone (8) (Figure 102).

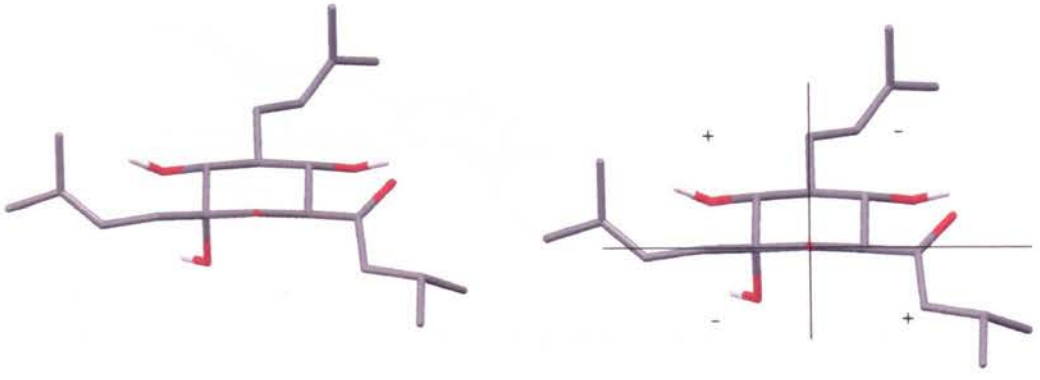
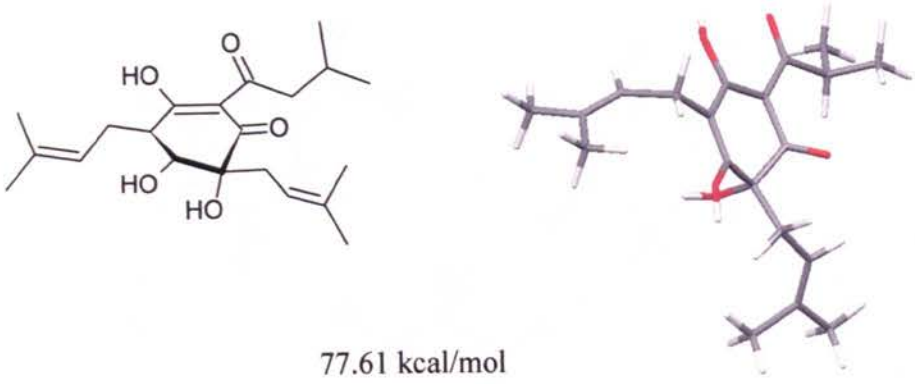


Fig 102 Octant representation of humulone (8)

If we superimpose the quadrants above then we can see that the tertiary hydroxyl function is in a negative quadrant thus the absolute configuration is correct. However, upon closer investigation we can see that the hydroxyl group has been placed in an axial position making a highly distorted ring. This conformation was confirmed using a Dreiding model. Using computer modelling to recreate this conformation the following conformation was produced (Figure 103).



77.61 kcal/mol

Fig 103 Energy of humulone in unfavoured conformation

If we rotate this structure to give the octant representation there is much greater distortion than observed with the Dreiding model (Figure 104).

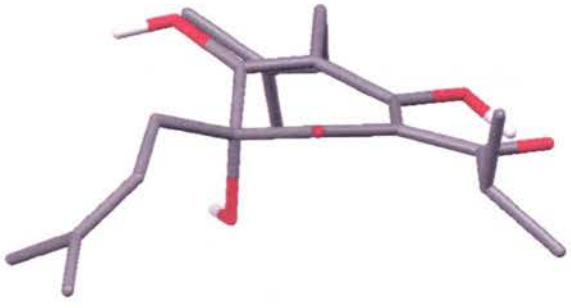


Fig 104 Octant representation of humulone in energetically unfavoured conformation

The tertiary hydroxyl group is still located in the negative quadrant so the Cotton effect still stands.

The above conformation does not take into account the intramolecular hydrogen bonding in the humulone molecule. Indeed even in the THP protected molecule (76) there is evidence of a great deal of intramolecular and intermolecular hydrogen bonding (Figure 105).

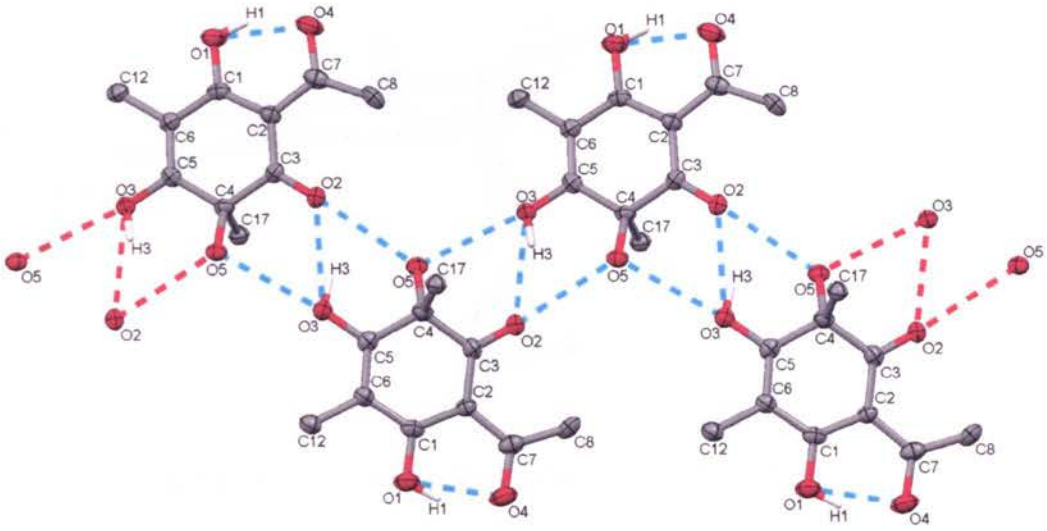
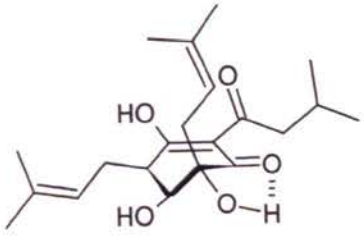
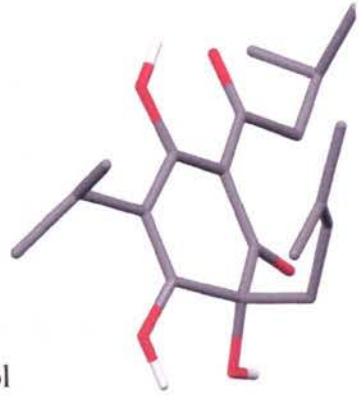


Fig 105 Single crystal X-ray of humulone THP (76) represented as 50% thermal ellipsoids, hydrogens and side chains have been omitted for clarity hydrogen bonds represented by dotted lines

Therefore if we re-model humulone and include the hydrogen bonding observed above (Figure 106).





53.84 kcal/mol

Fig 106 Energy of humulone in favoured conformation

The conformation is greatly different to the above structure, this gives the following octant representation (Figure 107).

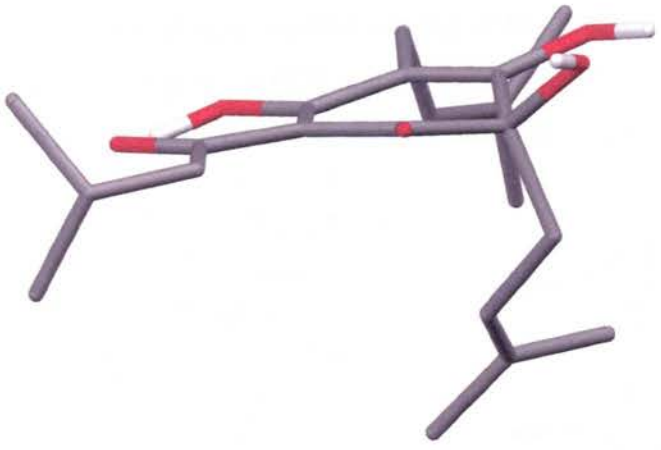
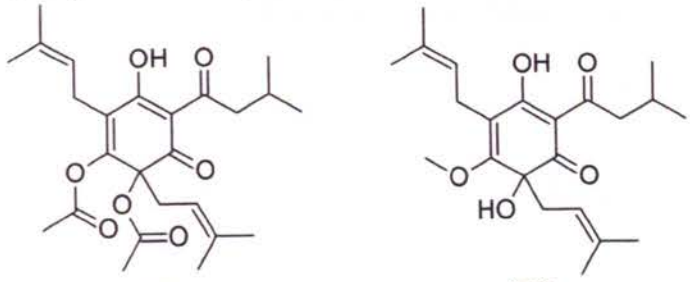


Fig 107 Octant representation of humulone (8) in the favoured conformation

While the tertiary hydroxyl is still within a negative quadrant this does not fulfil the criteria of an octant diagram as the ring is almost planar. This theory is supported further by the ^{1}H NMR spectra of humulone (8), humulone diacetate (77) and 5-O-methyl humulone (78) (Figure 108).

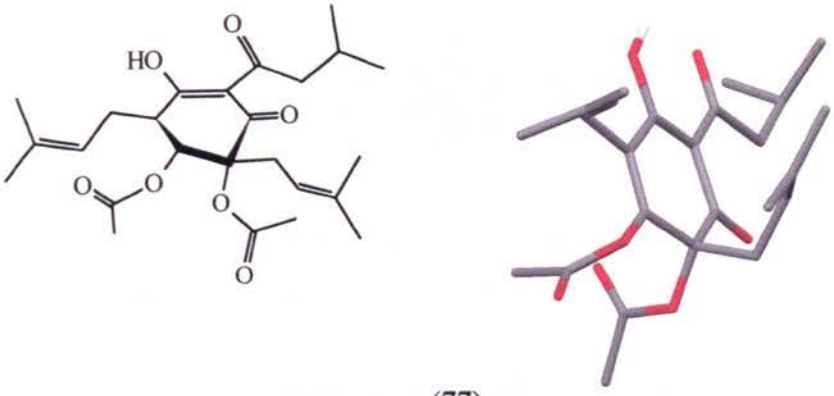


(77) (78)

Fig 108 diacetyl humulone (77) and methyl humulone (78) respectively

By ^{1}H NMR spectroscopy there are 6 protons in the region between 2.4 and 3.0 ppm. These correspond to protons found on (C1) of both of the isoprenyl side chains and those found on (C2) of the isovaleryl side chain. The NMR of O-methylhumulone (78) has the same pattern of peaks in approximately the same region. Presumably the molecule has not been greatly affected by the loss of an OH group. i.e. there has been little significant change to the overall configuration. The diacetylhumulone NMR shows no such pattern of peaks, indeed all six of the peaks are integrated together as a complex multiplet spanning between 2.5 and 3.1 ppm.

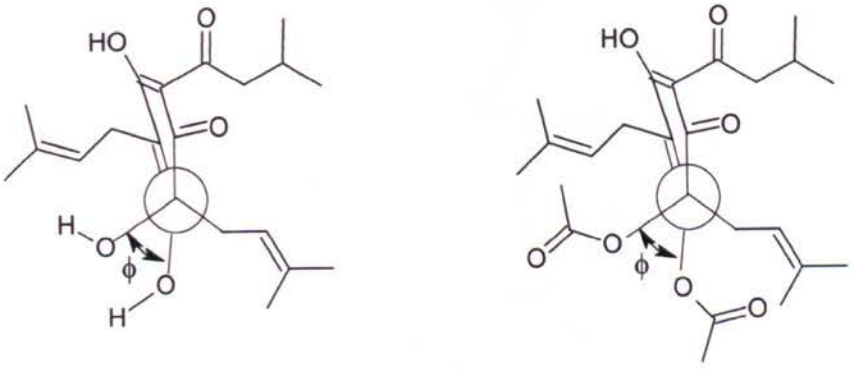
This indicates that some sort of change in configuration has taken place. This would be explained by the blocking of any possible hydrogen bonding between the tertiary OH group at (C4) and the carbonyl at (C3), forcing the isoprenyl group into the aforementioned equatorial position (75) (Fig 109).



(77)

Fig 109 Possible configuration of diacetylhumulone (77) CACHE model of diacyl humlone

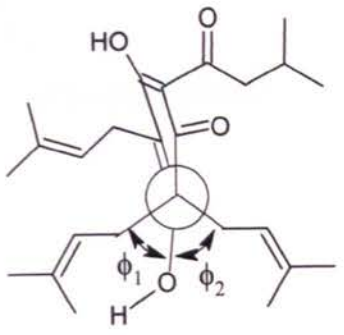
The calculated dihedral angle (ϕ) between the two oxygens of humulone and diacetyl humulone along the C4 – C5 bond are as follows (Figure 110):



$\phi = 35.40$ $\phi = 53.25$

Fig 110 Dihedral angles of humulone (8) and diacetyl humulone (77) respectively, energy minimisation was conducted using CONFLEX/MM3 (extensive search)

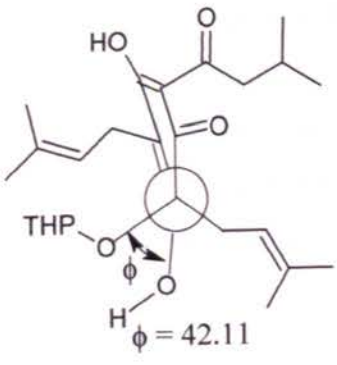
If we now compare these figures to those found from the X-ray diffraction of lupulone (Figure 111):

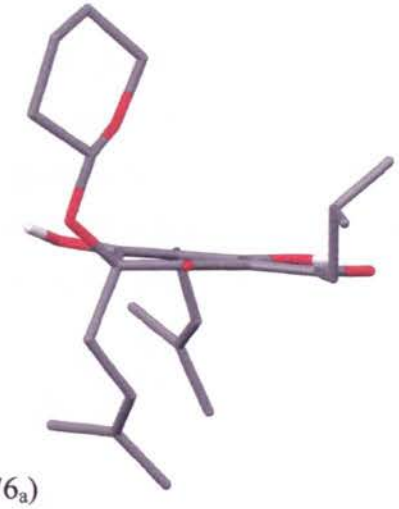


$\phi_1 = 57.11$ $\phi_2 = 62.69$

Fig 110 Torsion angles of lupulone from X-ray crystal structure

Below is the torsion angle for the THP ether of humulone (76a), and the octant representation as acquired by Single Crystal X-ray analysis (Figure 112).





(76_a)

Fig 112 X-ray diffraction of Humulone THP ether (76_a) represented as capped stick model

The second technique employed by Verzele was chemical degradation. Ozonolysis of tetrahydrohumulone (13) yielded a chiral α-hydroxy acid (15) in 30-50% ee. The reaction cascade for this reaction is detailed below (Figure 113):

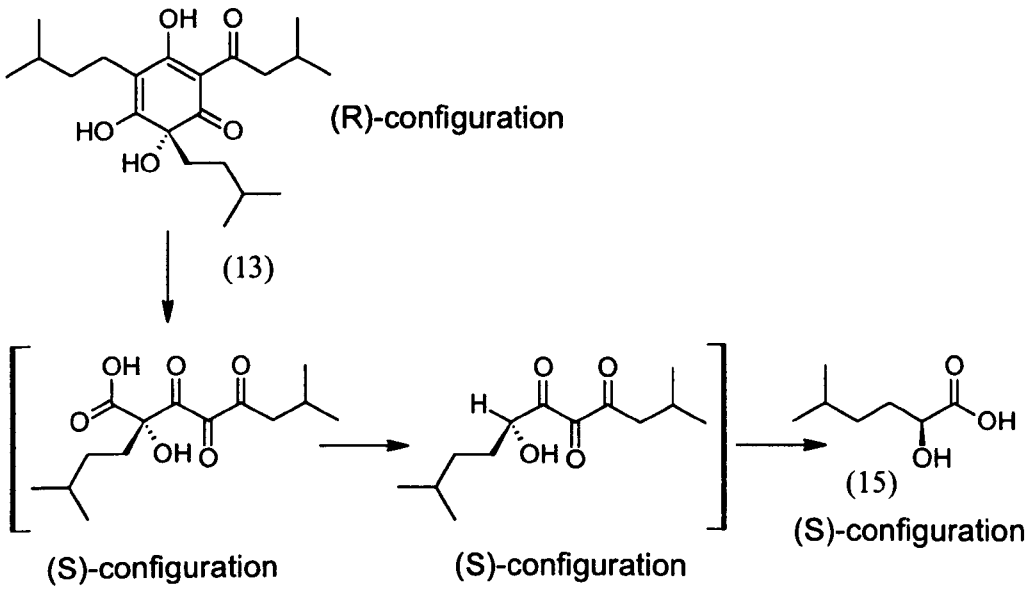


Fig 113 Ozonolytic degradation of tetrahydrohumulone (13)

In order for Verzele's theory to be correct there needs to be retention of configuration at the decarboxylation step. The authors claimed that there are examples where retention of configuration was observed, however, these examples involved fused ring systems or tertiary structures where enol formation could not take place. The following mechanism is likely to occur (Figure 114):

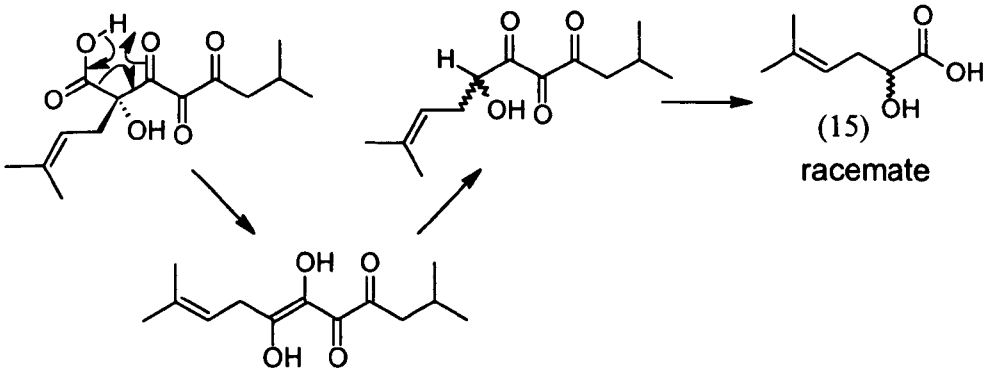


Fig 114 Proposed mechanism for the decarboxylation of β-keto acids

While there is no doubt that Verzele did recover a chiral product his suggested mechanism could not have taken place.

These data therefore suggest that a shadow of doubt hangs over the absolute configuration of humulone (8). The X-ray data that we have obtained coupled with computer modelling suggests the need for additional investigation.

The investigation into the NMR assignment and structure of these compounds is of great importance, it not only allows for greater identification of the derivatives of the α-and β- acids but it also has applications outside of hop chemistry. There is an ever increasing number of natural compounds being identified from plants and bacteria which rely on data which is, on closer inspection, incorrect.

3.4 Synthesis of Humulone (8)

The synthesis of humulone (8) from phloroglucinol (17) is not a novel process, though the yields are often modest at best. The standard Friedel Crafts reaction of phloroglucinol has been adapted over the years to provide enhanced yields. The main issues with this synthesis are the fact that phloroglucinol contains water of crystallisation, indeed it is available as a dihydrate commercially. However even normal phloroglucinol is not completely anhydrous and must be meticulously dried over P_2O_5 under vacuum. Alternatively water of crystallisation can be removed by re-crystallisation from toluene / ethyl acetate. If this water is not removed then the conversion of the phenone to deoxyhumulone is greatly affected.

As previously described the phlorisovalerophenone molecule is very reactive towards isoprenyl bromide, forming a complex mixture of products, two of those being lupulone and deoxyhumulone, the separation of which can be very difficult, usually achieved by their difference in pKa and / or solubility in hexane.

It was noted that a patent, describing the synthesis of deoxyhumulone (19), details the use of isoprenyl chloride and potassium iodide [135]. The yields are greatly reduced if the bromide is used. Presumably this is due to the chloride being a poor leaving group and hence not reacting with the isovalerophenone. Potassium iodide

substitutes the chloride forming the alkyl iodide, which is considerably more reactive. The bromide is intermediately reactive and probably produces a competing reaction.

During the course of this project it was discovered that the bromide can be used if it is allowed to completely convert to the iodide prior to the addition of the substrate. This is achieved by refluxing in acetone with an excess of sodium iodide followed by filtration.

It was decided that to deactivate the phloroglucinol (17) molecule protecting groups could be added, although some chemo selectivity was required to keep at least one hydroxyl group free for the alkylation step. The literature shows that acetylation is not selective; indeed, the acetylation of phlorpropiophenone (79) takes place at all three hydroxyl groups to give the triacetate (80) and can also force the remaining carbonyl into the enol acetate (81) (Figure 115) [136].

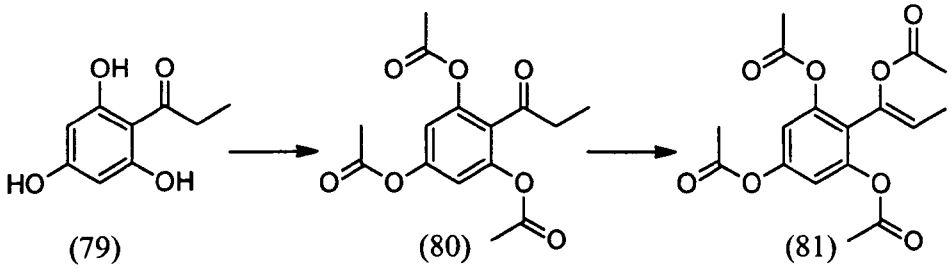


Fig 115 Acetylation products from phlorpropiophenone

It was decided to use substituted methyl ethers, namely methoxymethyl (MOM) and methoxyethoxymethyl (MEM) under mildly basic conditions (Hunigs base). Only the two non-hydrogen bonded hydroxyl protons can be removed to form the di-substituted compounds (82) and (83) (Figure 116).

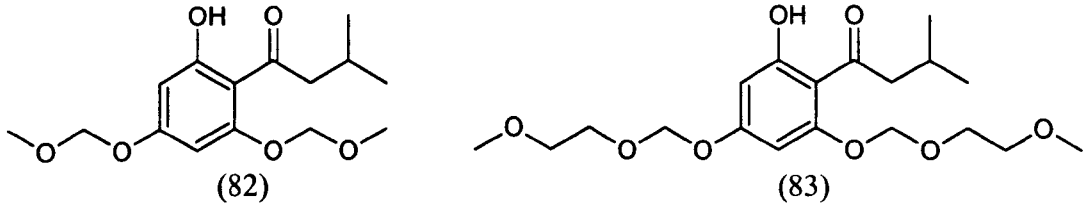
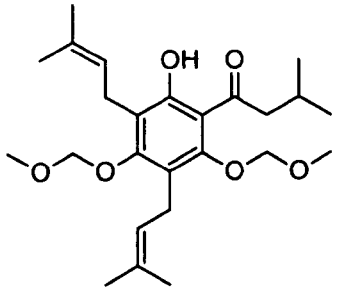


Fig 116 Bis-protected phlorisovalerophenones MOM and MEM respectively

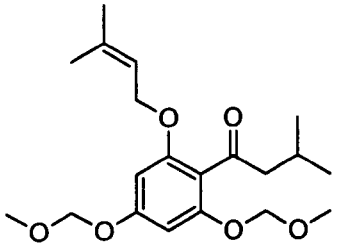
It was then hoped that either of these could then be C-alkylated to form the bis protected deoxyhumulone (84) (Fig 117).



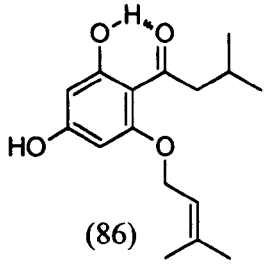
(84)

Fig 117 Bis-MOM-protected deoxyhumulone

On analysing the product it was discovered that only one isoprenyl group was present. On closer examination it was discovered that the ^{1}H signal for the CH_2 of the isoprenyl group was much further down field that predicted and there is also a significant shift in the ^{13}C DEPT of this group. This information doubled with the apparent loss of the remaining hydroxyl signal indicates that the compound had been O-alkylated (85) (Figure 118).



(85)



(86)

Fig 118 O-alkylated derivative of phlorisovalerophenone and the de-protected product

Deprotection of this compound should liberate the O-alkylated product (86) (Figure 118).

It has been demonstrated that substrates similar to the above compound can undergo a Claisen rearrangement (87) to give the required C-alkylated compound (89) [137, 138] (Figure 119).

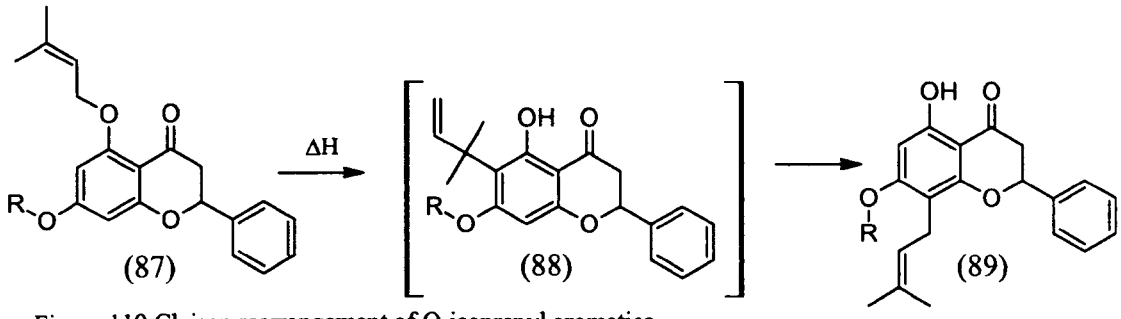
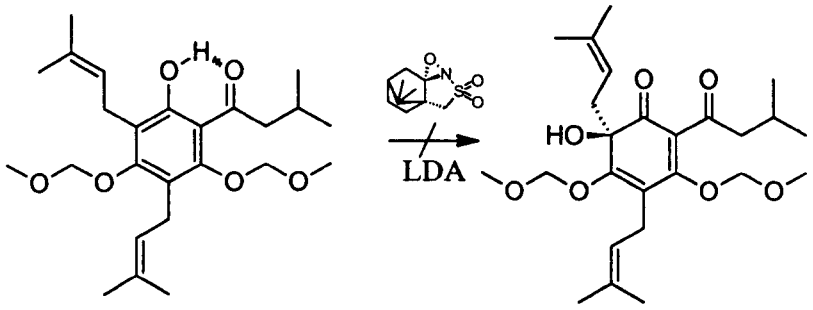


Figure 119 Claisen rearrangement of O-isoprenyl aromatics.

The intended product of the isoprenylation reaction (84) (Figure 117) was still of interest, as the bis-protected nature removes the symmetry of deoxyhumulone (19). It was hoped that using Davies [139] oxidising agent that we would be able to oxidise this compound to give some degree of stereoselectivity. Thus reacting deoxyhumulone (19) with MOMCl in the presence of DIPEA we were able to form the bis-O-protected precursor (84). Davies-oxidising reagent [139,140] can be used to asymmetrically oxidise prochiral centres α- to carbonyl compounds. As the conversion of deoxyhumulone to humulone is an oxidation, it was decided to attempt this conversion asymmetrically.

The first step of this type of oxidation is deprotonation of the carbon α- to the carbonyl with a strong base. At room temperature conditions using LDA as the base no reaction was observed (Figure 120). This apparent lack of reactivity could be attributed to the lack of nucleophilic activity on the carbon centre adjacent to the hydroxyl bearing carbon. If we consider the attempts to form a C-alkylated product from the di-O-alkylated phlorisovalerophenone, we can see that by protecting two of the hydroxyl functions that the remaining hydroxyl oxygen becomes more nucleophilic towards isoprenyl bromide.

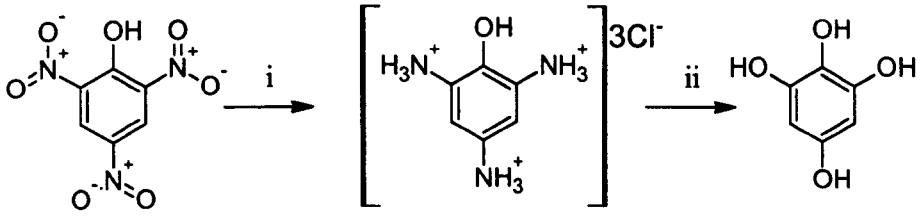


(84) (90)

Fig 120 Proposed oxidation product of (84) using an asymmetric Davies oxidising reagent

This change in the nucleophilic atom is no doubt responsible for compound (84) not reacting with Davies oxidising agent.

Possibly one of the better approaches to the synthesis of humulone is derived from the acylation and subsequent alkylation of 1,2,3,5-benzene tetrol (25). This method, however, is not without its shortcomings. First and foremost is the fact that 1,2,3,5-benzene tetrol (25) is not commercially available, thus it must be synthesised. The group that ultimately synthesised humulone from this material started with the reduction and subsequent hydrolysis of 2,4,6-trinitrophenol or picric acid (91) [141, 142] (Scheme 17).



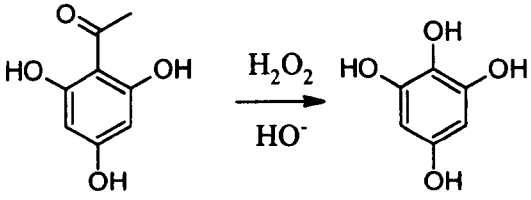
(91) (25)

Reagents : i) H_2, PtO, HCl ii) H_3O^+ ΔH

Scheme 17 Synthesis of 1,2,3,5-benzenetetrol (25) from picric acid (91)

Picric acid is known to be highly unstable when dry, however the conditions of the reaction are aqueous so at no time is there a danger of an explosion. Picric acid itself is hazardous and must be checked regularly for drying out. Consequently it is not useful as a reagent in large scale synthesis.

Another method for the synthesis of benzenetetrol (25) is the oxidation of 2,4,6-trihydroxyacetophenone (92) via the Dakin reaction, an oxidation of aromatic aldehydes and ketones to afford phenols similar to the Bayer-Villager synthesis of esters from ketones (Scheme 18) [143]. The main problems associated with this are the solubility of the product in water, hence a requirement for large volumes of solvent to extract the material and the apparent formation of many unwanted by products. These points considered the yields are no higher than 6 % and even then, chromatography has to be conducted over cellulose to yield pure material.



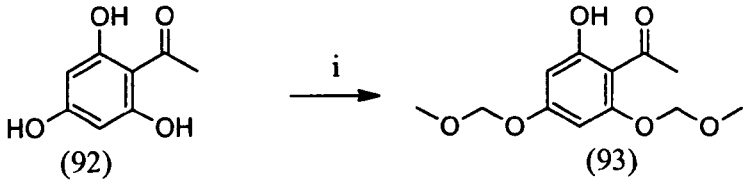
(92) (25)

Scheme 18 Dakin reaction of phloracetophenone (92) to 1,2,3,5-benzene tetrol (25)

The authors quoted that if a glucoside of an acylphloroglucinol is used then the yields were greatly improved, ca 80%. This is most likely due to one of the hydroxyl groups being effectively protected. This has also been demonstrated in a paper outlining the synthesis of catechols via salicaldehydes [144].

It was decided that it may be possible to improve the yields by the additional use of protecting groups; these would have to be stable to the conditions of the reaction, i.e. sodium hydroxide and hydrogen peroxide, and must be easily removed. Thus it was decided to attempt to use the MOM protecting group. This group was used to make compound (93) from phloracetophenone (92) using the literature procedure [165] successfully using DIPEA, THF and MOMCl. However, the reaction time is long and the protecting reagent MOMCl is highly toxic. More importantly the contaminant present in MOMCl from manufacture, chloromethylether $O(CH_2Cl)_2$ is even more toxic! The result of this toxicity is that supply of this reagent is strictly controlled.

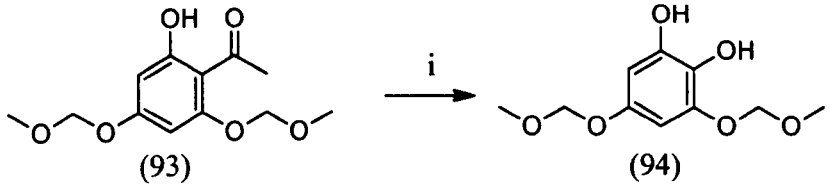
To circumvent this problem it was decided to use the, apparently, less toxic MOMBr. The result of using this reagent was that the reaction was complete in 1 hour and the work up yielded far fewer by products (Scheme 19).



Reagents i) DIPEA, MOMCl, THF

Scheme 19 Protection of phloracetophenone (92) with methoxymethyl protecting groups

This compound was then oxidised, using the Dakin reaction [144] to give 3,5-bis(methoxymethoxy)benzene-1,2-diol (94) in 60% yield (Scheme 20).



Reagents i) NaOH, H_2O_2

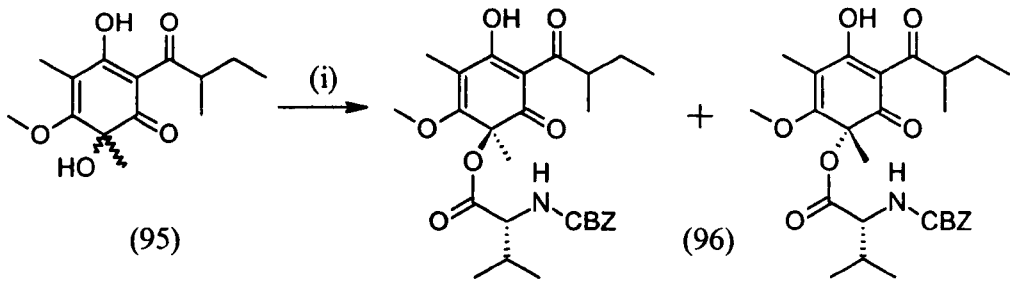
Scheme 20 Dakin reaction of bis-MOM-protected phloracetophenone to compound (94)

Deprotection of (94) produces an extremely polar compound which is very difficult to isolate, particularly as the best procedure for carrying out this reaction involves concentrated HCl in methanol at reflux. This deprotection has not been fully investigated.

It was hoped that following the literature methodology of alkylating the isovaleryl derivative using a chiral phase transfer catalyst (PTC), such as Lygo's Catalyst [145], that stereoselective alkylation could be achieved. This has yet to be investigated. Attempts to form the isovalerylphenone directly from this compound have so far not produced satisfactory results.

Even though there have been no successful stereoselective synthesis of humulone we have achieved the first recorded chiral resolution of humulone. A resolution of an analogous compound used in the synthesis of wasabidienone A (59) (Figure 42) [146] was employed. This involved the reaction of a methylated derivative (95) with

an N-protected amino acid. Chromatography of the product (96) over silica gel followed by deprotection yielded the appropriate isomer (Scheme 21).



Reagents: i) DCC, DMAP, Z-valine

Scheme 21 Chiral resolution of compound (95)

Attempts to apply this type of reaction on humulone were unsuccessful demonstrating that it is necessary for the methoxy group to be present.

Natural humulone (8) complexes with 1R,2R-diaminocyclohexane (71_R) to form a crystalline solid. If 1S,2S-diaminocyclohexane (71_S) is employed then an oily product is obtained. This oily material is soluble in acetonitrile, whereas the solid complex is insoluble in acetonitrile. This has allowed us to resolve synthetic (±) humulone into its separate optical isomers, in particular this has allowed us to isolate, for the first time, unnatural (+)-humulone. This has also been derivatised into a THP ether. An X-ray performed on this material shows the following configuration (Figure 121):

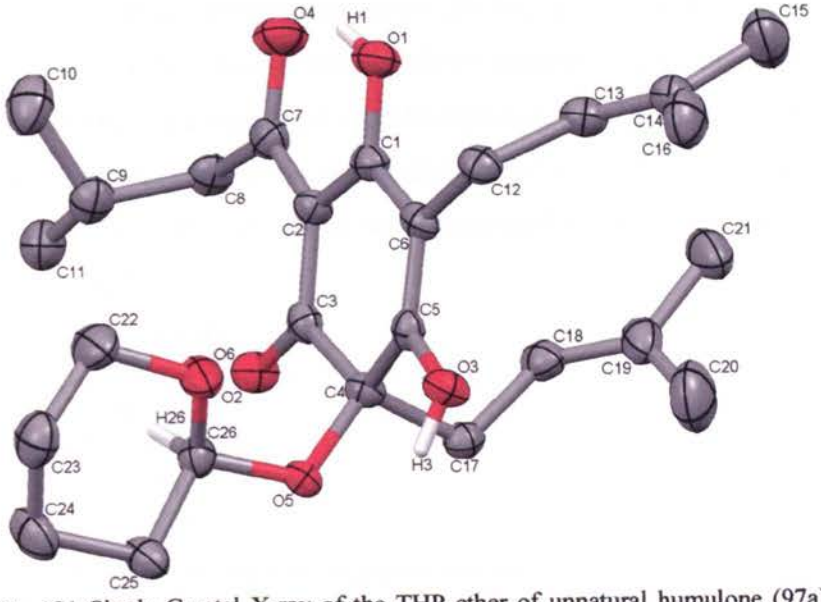


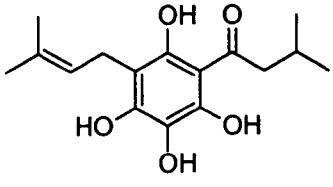
Fig 121 Single Crystal X-ray of the THP ether of unnatural humulone (97a) represented as 50% displacement ellipsoids.

This shows the opposite configuration to the natural humulone (8), further clarifying our theory that the currently accepted configuration of humulone (8) is incorrect. As this is not unequivocal evidence for the purposes of this report we will continue to refer to natural humulone having the R-configuration as is currently accepted.

3.4.1 Racemisation of (-)-humulone (1) to (±)-humulone (8)

A small-scale racemisation reaction was attempted by heating humulone (8) in toluene until the optical rotation had been reduced to zero. However, the product, upon removal of the solvent, was highly contaminated with numerous degradation products as shown by HPLC analysis. This was probably due to light or oxygenic degradation. A larger scale racemisation was attempted by refluxing a crude liquid CO_2 extract of hops in toluene for approximately 20 hours. The progress of the reaction was monitored by optical rotation, once the rotation of the toluene solution was determined to be zero the racemisation was assumed complete. The racemic humulone was then isolated, using the phenylene diamine complex method, upon

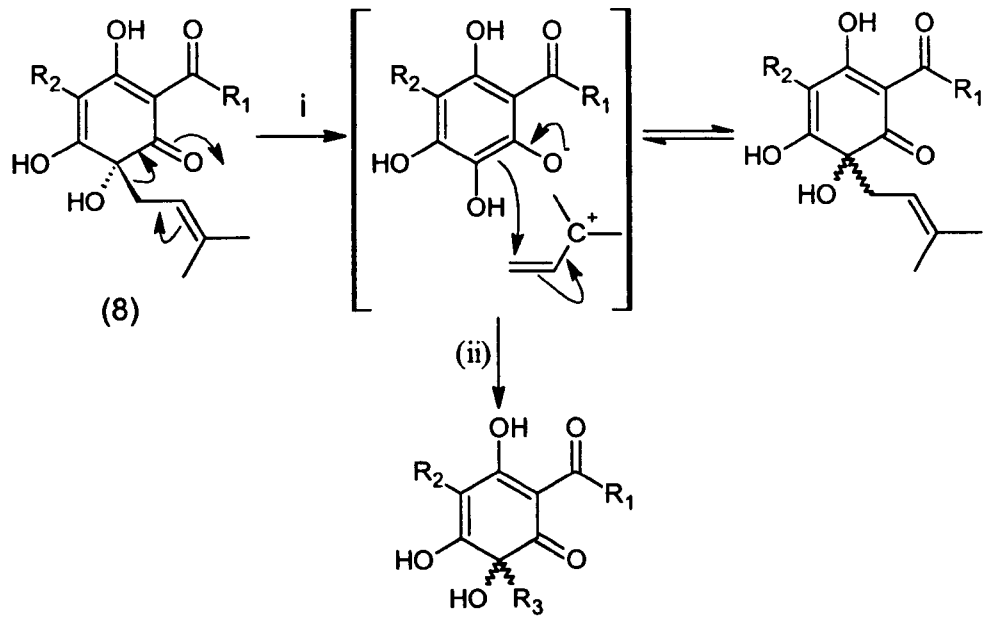
decomposition, gave (±)- humulone (8). We then wanted to investigate whether the stabilised ionic alkenyl group, which fragments and subsequently becomes re-attached during racemisation, could effectively be 'trapped' and thus clarify that the commonly accepted ionic intermediate was correct. This would provide a derivative substituted by proton giving an unsaturated humulohydroquinone derivative (97) (Figure 122).



(97)

Fig 122 Unsaturated humulohydroquinone derivative (97)

It was thought that it may be possible to achieve this by using pyridine as the solvent during the racemisation process. By monitoring the reaction, using optical rotation and HPLC, it was clear that after only a few hours reflux the humulone (8) was being rapidly degraded. It was decided that instead of removing the alkyl group it may undergo a reaction with a suitable haloalkane. As the α–β unsaturation is no longer present, it could not form the stable cation required for fragmentation and the reaction would go to completion (Scheme 22). Again, reverse phase HPLC analysis showed high levels of degradation. However, the presence of a new peak with a considerably lower polarity was slowly being formed. This compound had a similar retention time to that of O-methylhumulone (78). No purification work has been attempted.



Reagents i) Toluene, heat ii) R_3-X, R_3= α–β saturated

Scheme 22. Proposed mechanism for C4 substitution

Of course there is no real evidence that this reaction proceeds via the ionic intermediate. Other potential mechanisms could be either a concerted mechanism or a radical reaction. Evidence of this is that the reaction can be conducted in either isooctane or dioxane. Indeed it would be more likely that the above reaction would proceed in dioxane in which ionic compounds would be more stable. In isooctane (2,2,4-trimethylpentane) or toluene one would expect there to be a concerted reaction whereby the 6-membered transition state below would be achieved (Figure 123).

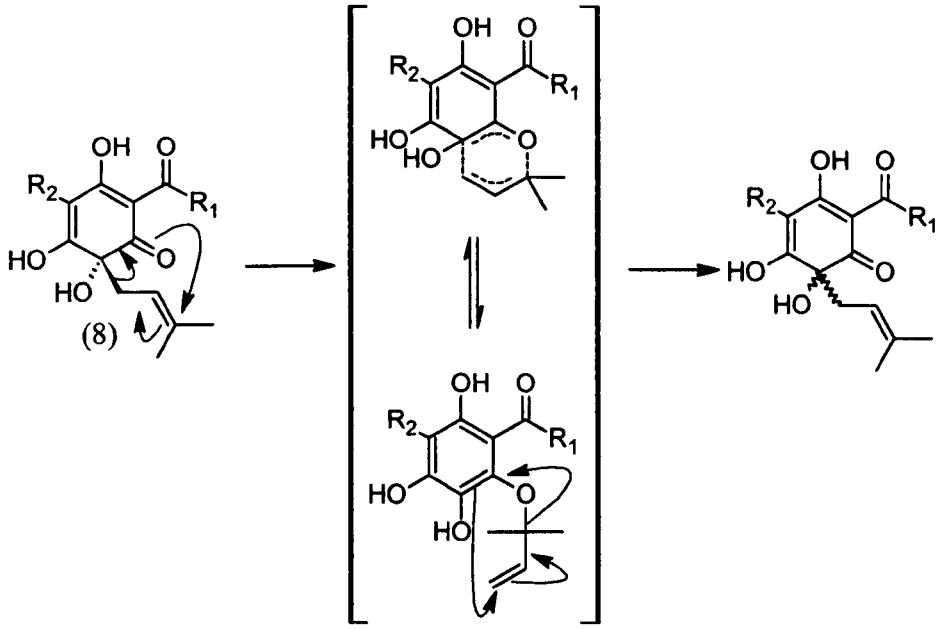


Fig 123 proposed 6-membered intermediate in the racemisation of natural humulone (8)

A racemisation reaction was conducted in toluene (d_8) and the ^1H, ^{13}C, and DEPT NMR spectra were collected before and after refluxing for 15 hours. After this time a small amount of a new compound was present, evident by a new hydrogen bonded hydroxyl peak at δ 19.1 ppm. When compared to the resonance that corresponds to humulone (8) at δ 19.55 ppm this new compound constitutes about 5% of the mixture.

If this new compound were the intermediate Claisen product (98) then we would expect a downfield methylene group corresponding to the terminal alkene of (98). This is not the case, when observing the DEPT spectra there are new resonances in the correct region, however these do not correspond to a CH_2.

3.5 Isomerisation of humulone (8)

Several novel and non-novel derivatives of humulone have been synthesised for biological screening.

Isohumulone (27) was synthesised as an orange oil, subsequent complexation with DCHA gave white crystals of *trans*-isohumulone complex (99) (Figure 124). However, the yields were modest at best as a consequence of the *cis*-isomer being formed in preference to the *trans*-isomer using Mg^{2+} catalysed isomerisation. It was decided that an alternative approach using an industrial preparation of Isohop ™, may provide access to *trans*-isohumulone. An aqueous solution of the potassium salts of the iso-α-acids, (isohumulone, isocohumulone (100) and isoadhumulone) (Figure 124) was acidified to give the free acids and then treated with DCHA. The subsequent complex, consisting of all of the above *trans*-iso-α-acids, was recrystallised from ethyl acetate a number of times with the aim of producing pure *trans*-isohumulone (27_{trans}) in a similar manner to that of our humulone isolation. Curiously enough after 3 recrystallisation steps it was obvious that it was in fact *trans*-isocohumulone (100) (Figure 124) that was the major constituent. As soon as this became apparent the experiment was abandoned.

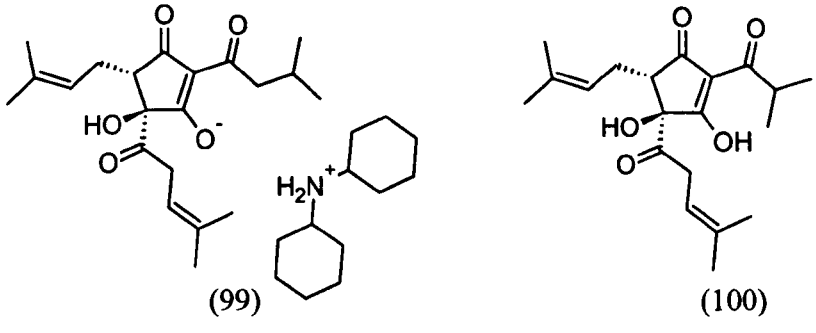


Fig 124 *trans*-isohumulone complex (99) and *trans*-isocohumulone (100)

3.6 Novel Derivatives of the bitter acids humulone (8) and lupulone (22)

It is well known that many of the problems associated with the derivatisation of humulone (8) i.e. the apparent sensitivity to oxygen, acid, base and ultraviolet radiation, make any derivatisation challenging. Isomerisation to isohumulone (27) and other stable 5 membered ring structures of the derivatives, seen earlier, are among the unwanted by products. It was decided that in order to avoid this problem it would seem appropriate to 'protect' the molecule. It seems an obvious step to

protect the enol function at C5 in order to prevent the molecule from isomerising and then to carry out the following transformations:

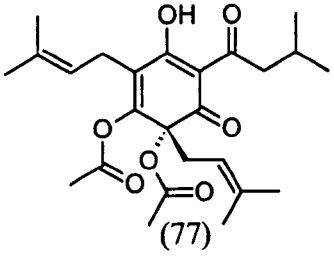
i) Esterification (3.6.1)

ii) Etherification (3.6.3)

iii) Acetal / ketal formation (3.6.4)

3.6.1 Enol Protection-Acylation of Humulone (8)

Our approach to the acetylation of humulone (8) to afford humulone diacetate (77) (Figure 125) was initially attempted using acetic anhydride in pyridine with a catalytic amount of DMAP. The results of this were a complex mixture of materials which proved impossible to separate by chromatography. A successful approach was to use acetic anhydride in excess and sodium acetate.



(77)

Fig 125 Proposed structure of diacetyl humulone

With gentle heating the presence of a new compound became evident by reverse phase HPLC, after a few minutes. This compound seems to have a greater polarity to that of humulone (8) as it is eluted earlier. After about 10 minutes a second new peak began to form. This compound has a lower polarity to that of humulone (8). After 30 minutes it became apparent that the initial product slowly converted to a new material. After 1 hour the reaction was complete and neither humulone (8) or the first product were detectable by HPLC. This finding is of great interest, as there are 3 possible sites for acetylation. It was deduced that only 2 of these were actually acetylated under these conditions. Indeed ^{1}H NMR spectroscopy of the sample suggests the presence of only 6 new protons all in the region of acetyl protons and this begs the question as to which of the three hydroxyl groups were acetylated. The

enol function involved with the β-triketo function seems unaffected as the new molecule can still bind to Fe (III) ions to give a cherry red colour. This binding would not take place if the free hydroxyl was effectively blocked. The resonance for the strongly hydrogen bonded proton remained intact at δ18.9 ppm. This only leaves the second enol function at C5 and the tertiary hydroxyl at C4 as candidates for acetylation. From the HPLC we deduced that one hydroxyl is acetylated before the other, otherwise we would see two mono-acetyl compounds and one diacetyl. Instead we only appear to have one mono-acetyl compound and one diacetyl compound. This method requires the mixture to be heated; this in turn produces degradation products which are difficult to remove by preparative chromatography. Experiments with the acetylation of the analogous β-acid, lupulone (22) using pyridine as the solvent shows a new compound with a lower polarity to that of the starting material. Upon extraction of this material, using hexane, a solid precipitate was observed (101). Upon filtration and drying, fine white needles were acquired. Closer examination of this product showed that there were three acetyl groups present by NMR. Previously we showed that acyl phloroglucinol derivatives undergo keto-enol tautomerisation to the corresponding alcohol which may then be acetylated under basic conditions. This apparently also occurs with lupulone using pyridine in the presence or absence of catalytic amounts of DMAP (in the absence of DMAP the reaction times are greatly increased). This reaction has been known to occur with natural compounds analogous to lupulone, isolated from *Myricia gale* (102). Acetylation of this product gave an analogous triacetate (103) (Figure 126) [147].

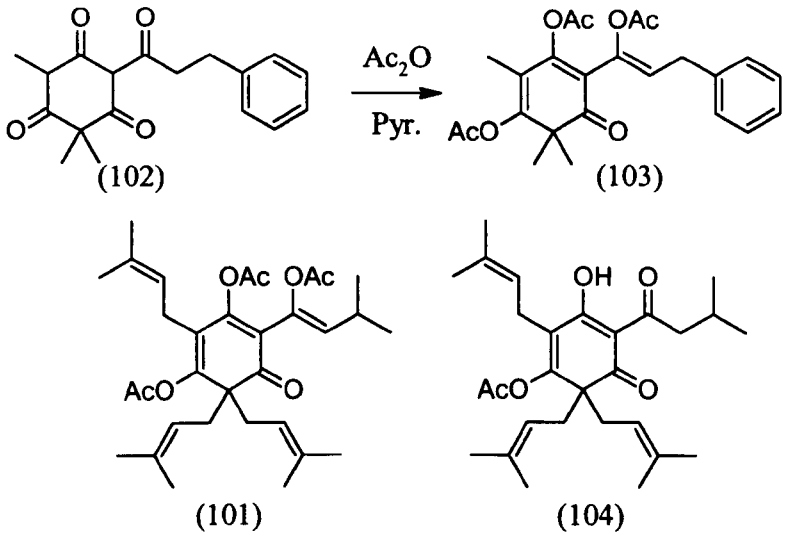


Fig 126 Conversion of a lupulone (102) analogue to a tri-acetate (103) and the proposed structures of lupulone triacetate (101) and lupulone monoacetate (104)

The purpose of these experiments was an attempt to see if we could selectively protect humulone (8) or lupulone (22) with acetyl groups to increase their activity in biological systems. The advantage of the tri-ester of lupulone (101) is that the chelating function is blocked. This compound may not be recognised by the proteins present in cell walls that expel chelating acids from the membrane before they have gained access to the intracellular fluid. These esters, if chosen carefully, would ideally hydrolyse in the relatively high pH conditions of the cell interior and exert their efficacy.

Of course from our studies into lupulone and colupulone the structures are likely to exist in a similar tautomeric form to lupulone (22). If, instead of using pyridine as the solvent, acetic anhydride was used as the solvent, and only a catalytic amount of pyridine was added then the reaction product from lupulone (22) was the monoacetate (104) (Figure 126). This compound has previously been reported [148], however the spectral data does not match that found by our studies. Their synthesis used pyridine as the solvent with acetyl chloride. It was carried out on a mixture of β-acids and the product purified by preparative HPLC. Their data did not suggest that they observed more than one tautomeric form, however other than NMR spectra there was no other structural characterisation carried out.

This monoacetyl derivative of lupulone (104) also exists in two tautomeric forms however the ratio has shifted from 70 : 30 for lupulone (22) to 45 : 55 as shown when comparing the ^{1}H NMR spectra (Figure 127)

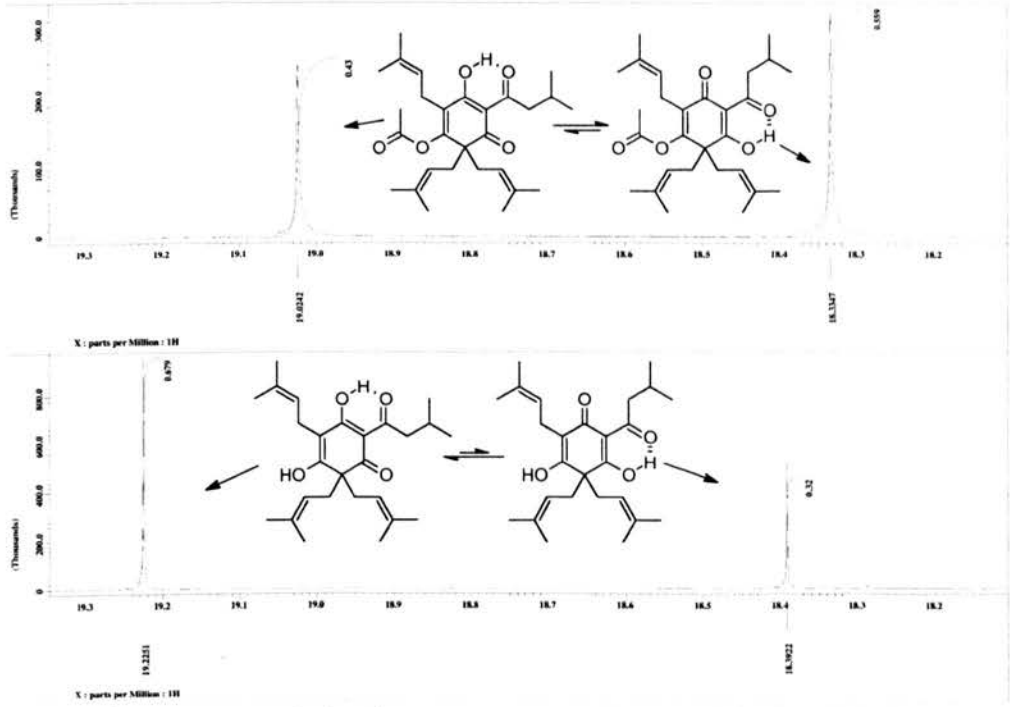
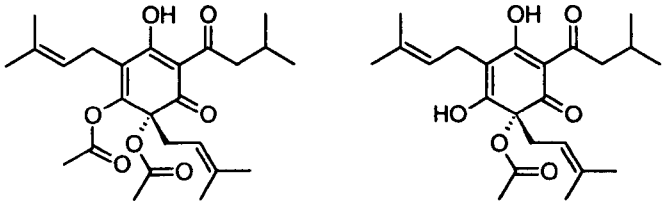


Fig. 127 ^{1}H NMR spectra for lupulone monoacetate (104) (top) and lupulone (22) (bottom) showing the hydrogen bonded enolic proton

Even in a polar solvent such as methanol the presence of two tautomeric forms is evident by a doubling of the acetyl resonance in the ^{1}H spectra. This is in contrast to lupulone (22) which is found in only one tautomeric form in polar solvents of this type.

As lupulone (22) does not contain the same tertiary OH group, only the enol function at C5 can be acetylated to give acetyl lupulone (104). Although these results are not entirely conclusive, it is reasonable to predict that it is the tertiary hydroxyl (C4), which is acetylated first (105) (Figure 128).



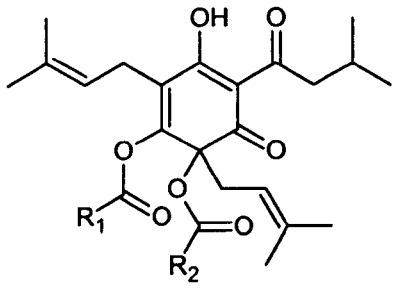
(77) (105)

Fig 128 humulone diacetate (77) monoacetylhumulone (105)

As we anticipated the novel di-acetylated compound (77) does not undergo the isomerisation reaction characteristic of humulone (8). It is stable to heating in the presence of divalent magnesium cations and can from water soluble species in the presence of aqueous sodium hydroxide solution. Upon refluxing diacetyl humulone in alkali solution (8) we would expect hydrolysis of the ester functions and subsequent isomerisation to isohumulone (27).

This successful protection of humulone (8) should allow for in-depth manipulation of the alkenyl side chains in order to derivatise further. The sodium acetate catalyst used was later replaced by a catalytic amount of pyridine. This gave the same compound with no degradation products at ambient temperature after 1 hour. After the reaction was deemed complete, by HPLC analysis, the mixture was brought to reflux temperature. After a further 1 hour no appreciable degradation could be detected by HPLC. Prior to completion of the reaction these reaction conditions would have caused significant degradation.

In order to produce a 'library' of structural analogues for screening a number of other acyl derivatives have also been synthesised (Figure 129). All of these derivatives were isolated as oils, as we predicted would occur with the loss of the hydrogen bonding capacity.



1)	$R_1 = R_2 = CH_2CH_3$	propanoyl	(106) 83%
2)	$R_1 = R_2 = CH_2CH_2CH_3$	butanoyl	(107) 81%
3)	$R_1 = R_2 = CH_2CH_2CH_2CH_3$	pentanoyl	(108) 79%
4)	$R_1 = R_2 = CH_2CH_2CH_2CH_2CH_3$	hexanoyl	(109) 77%
5)	$R_1 = R_2 = CH_2CH_2CH_2CH_2CH_2CH_3$	heptanoyl	(110) 75.5%

Fig 129 Acyl derivatives of humulone

Interestingly the bis-esters of humulone exist in two tautomeric forms in $CDCl_3$, whereas in methanol there is only one tautomer present. In chloroform the minor tautomer can be quantified by means of the integrals of the hydrogen bonded proton. All of the derivatives give the same ratio of 90 : 10 of major : minor tautomers.

3.6.2 Enol Protection - Sulphonates of Hop Acids

Using an adaptation of a literature method [149], treatment humulone with tosyl chloride in the presence of pyridine at 50 °C gave a new product (111) which was observed as a yellow oil. This viscous oil was identified as a monotosylate by ^1H NMR spectroscopy and found as a mixture of tautomers in the ratio 90 : 10. Lupulone can also be converted to the toluenesulfonate (112) using the same procedure to afford a ratio of tautomers of this compound of 57 : 43 (Figure 130).

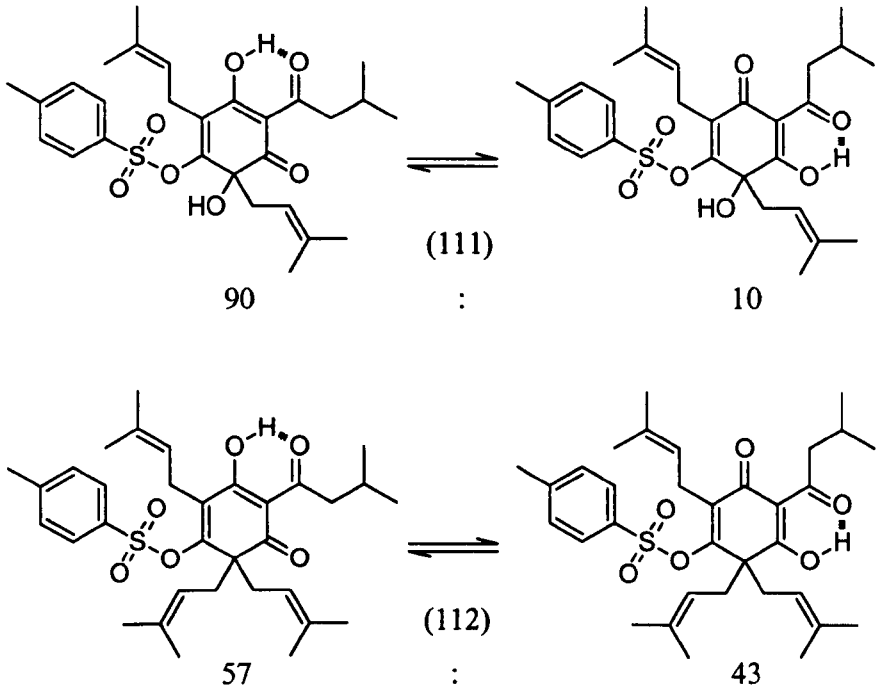
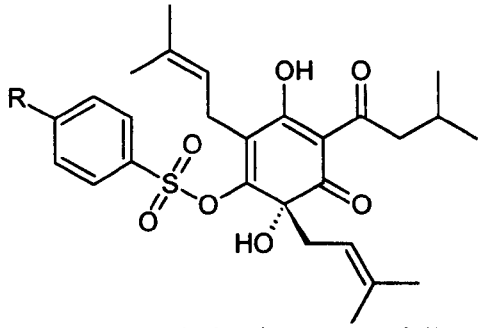


Fig 130 Tautomeric forms of humulone toluenesulfonate (111) and lupulone toluenesulfonate (112)

A range of seven sulphonate esters were synthesised from humulone (Figure 131). All in good yield selectively to the C5 enol function;



The isolated derivatives are as follows and all were formed as oils.

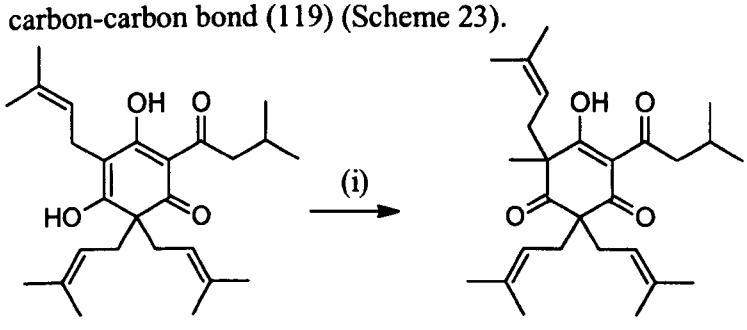
1) R = Me	(111) 71%	5) R = Br	(116) 78.6%
2) R = CF_3	(113) 57%	6) R = I	(117) 70.5%
3) R = NO_2	(114) 57%	7) R = Ph	(118) 31%
4) R = Cl	(115) 80.5%		

Fig 131 Sulfonate esters of humulone

3.6.3 Ether Synthesis from Humulone (8)

One of the oldest and traditional methods for producing unsymmetrical ethers from alcohols is the Williamson ether synthesis [150]. This reaction proceeds *via* an S_N2 type reaction between an alkyl halide and an oxygen anion. In order to deprotonate the hydroxyl it is often necessary to use a strong base, though the hydroxyl proton of the humulone molecule is relatively labile, thus it can be removed easily using metal hydroxides in aqueous solution.

Lupulone (22) has been reacted under these conditions [151], however, the product of the reaction was not the expected ether. As opposed to the oxygen centre acting as the nucleophile, the adjacent carbon reacted with the alkyl halide producing a new carbon-carbon bond (119) (Scheme 23).



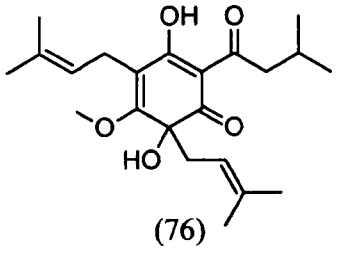
(22) (119)

Reagents: (i) $KOH_{(aq)}$, $(CH_3)_2SO_4$

Scheme 23 Methylation of lupulone (22)

If humulone (8) is treated in the same way there appears to be many competing reactions, however if dimethyl sulphate (DMS) is used in place of the halide a new compound (78) (Figure 131) with a lower polarity was obtained, albeit in a low yield (<10%). This low yield was due to the reaction conditions, many competing reactions resulted in the formation of isohumulone and humulinic acid among other things. It was difficult to predict the outcome of this reaction, although the enolic hydroxyl group is activated by the addition of NaOH it was still quite feasible for the new methyl group to attack the carbon at C5 as seen in lupulone (22). The ^1H

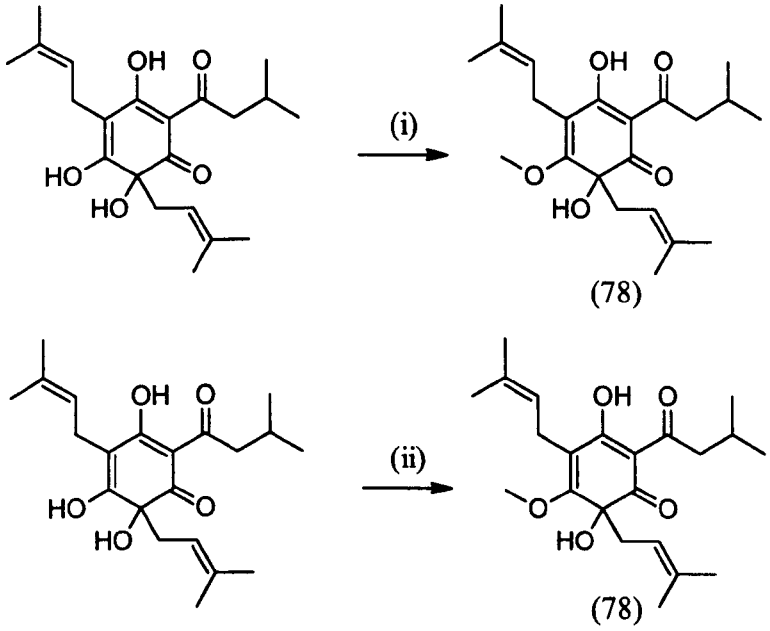
NMR spectrum indicated the presence of a downfield resonance at δ 4.2 ppm. This would suggest that the molecule has been O-methylated.



(76)

Fig 131 O-methylhumulone (78)

If DMS was replaced with Meerwein salt, trimethyloxonium tetrafluoroborate [152, 153] then some of the degradation products were avoided, though yields were still only modest at best. (Scheme 24).

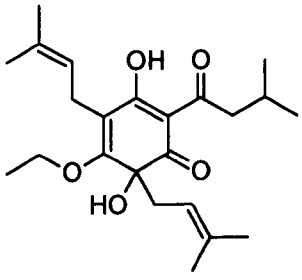


Reagents i) NaOH, CH_3I, ii) $O(CH_3)_3^+ BF_4^-$, Proton Sponge

Scheme 24 Methylation of humulone (8)

During attempts to synthesise the ethyl ether analogue of humulone using the corresponding Meerwein salt, triethyloxonium tetrafluoroborate, the product of this reaction was a complex mixture. Attempts to purify the material by preparative chromatography resulted in a single spot by TLC, however ^1H NMR spectroscopy indicated that a paramagnetic compound had been produced making structural

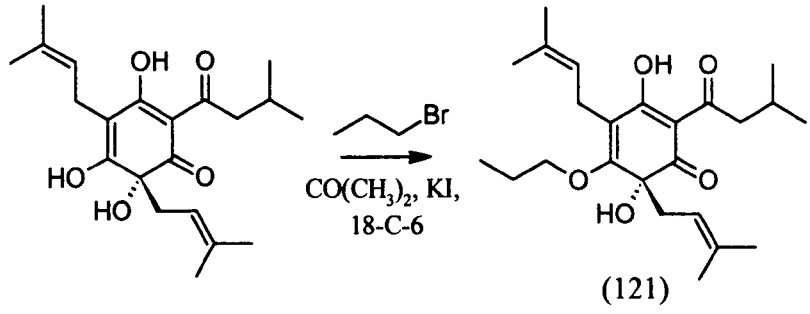
elucidation by NMR impossible. It was then decided to investigate other methods for the formation of ethers. Using alkyl halides in acetone in the presence of potassium carbonate has been shown to be an effective method for the production of methyl ethers from phenols [154]. This procedure however was not suitable as prolonged refluxing (several days) produced competing degradation reactions. If, however, dialkyl sulfates were used then the reaction time was reduced. An addition of the crown ether, 18-crown-6 in catalytic amounts, further reduced the reaction time. This new approach was successful for the synthesis of both the methyl (78) and ethyl ethers (120) (Figure 132) in good yields.



(120)

Fig 132 O-ethylhumulone (120)

However, as no other alkyl sulfates are available commercially an alternative approach was needed. In order to synthesise further alkyl ether derivatives of humulone (8) the use of propyl bromide in the presence of potassium iodide, to facilitate the conversion to the alkyl iodide, was investigated. Using the same conditions, employing 18-crown-6 as a PTC the conversion was slow (ca 10 % conversion after 1 week reflux) though this could no doubt be optimised. This type of reaction has allowed access to further derivatives of humulone (121) (Scheme 25).



(121)

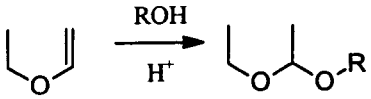
Scheme 25 O-propylation of humulone (8)

All of these ethers exist in one tautomeric form in $CDCl_3$.

3.6.4 Hydroxyl Protection *via* Substituted Ethyl Ethers

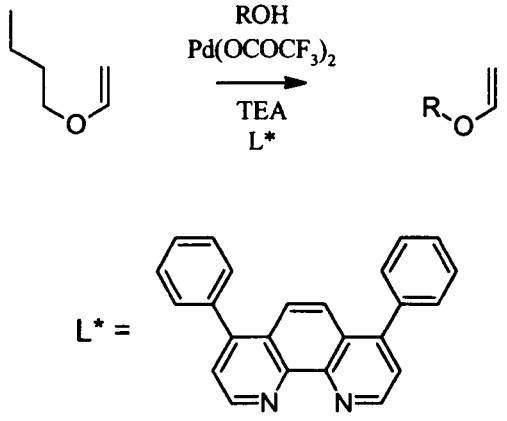
After the discovery that THP ethers show selectivity towards the tertiary hydroxyl function and provide a crystal morphology that is suitable for Single Crystal X-ray crystallography, a number of substituted ethyl ethers were synthesised. The substituted ethyl ethers were chosen as they bear a close relationship to the THP ether (Figure 133) and due to their ease of synthesis from vinyl ethers which again is analogous to the synthesis of THP ethers (Scheme 26).

Fig 133 Structures of a THP ether and a substituted ethyl ether, similarities are shown by in bold



Scheme 26 Synthesis of substituted ethyl ethers from ethyl vinyl ether

1-Chloroethyl vinyl ether is available commercially which would fulfil the criteria for the Flack Parameter, used in X-ray crystallography to determine absolute configuration, [124] to be applicable. It is possible to synthesis a whole range of vinyl ethers using palladium catalysed substitution reaction (scheme 27).



Scheme 27 Synthesis of vinyl ethers by palladium catalysis

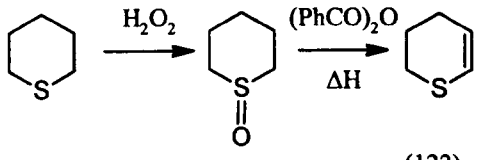
Using this method [155] it was possible to produce a menthyl vinyl ether and react this with humulone. The products from all of these reactions have proven to be highly unstable and quickly break down into acetaldehyde, menthol and humulone upon exposure to even trace amounts of water. This made NMR analysis impossible and no products were available for X-ray analysis. The instability of these compounds is probably due to the acidic nature of the adjacent enol function of the protected humulone molecule. It was hoped that the use of the more stable 1-(2-chloroethoxy)ethyl ether would produce a stable product as these compounds have been shown to be more stable to acid hydrolysis than the THP ether [156]. Unfortunately this material also seemed very susceptible to hydrolysis.

3.6.4.1 Formation of THP ethers

The THP ether was synthesised using the literature methods [157, 158] however, neither method allows for complete conversion, even when the reagent or catalyst was used in excess or if extended reaction times were used.

As previously mentioned the THP ether has the advantage over other protecting groups, used during the course of this research, in that it shows selectivity towards the tertiary hydroxyl group at C4.

This selectivity has already been utilised in the crystal morphology being close to that of the α- acid lupulone allowing for a single crystal X-ray to be produced for one of the diastereoisomers. It was decided to attempt to form the thio derivative for dihydro-*2H*-pyran, dihydro-*2H*-thiopyran (122) (Scheme 28). This was synthesised from pentamethylene sulphide *via* the oxide [159] although it may be synthesised via pyrolysis of acyloxy derivatives [160].



(122)

Scheme 28 Synthesis of tetrahydro-*2H*-thiopyran

The literature method for producing tetrahydrothiopyran (THTP) ethers uses TFA as the acid. It is well known that humulone will not tolerate TFA at elevated temperatures, although it seems that DHTP dimerises [161] under the same conditions that it reacts with alcohols. The product of this reaction was in very low yield and contained mostly the dimerised product. However there was evidence of a small amount of a product derived from humulone, but there was an insufficient quantity to perform any further purification.

Purification of humulone can also be achieved using the THP ether of humulone (74). If crude alpha acids are converted to their corresponding THP ethers then they can be recrystallised many times from heptane to afford pure humulone THP ether in a similar manner to the purification of lupulone and colupulone.

De-protection of the THP can be easily achieved using the literature method [162] by stirring at room temperature in a 4:2:1 v/v mixture of acetic acid, THF and water respectively to yield humulone (8) with no alteration to the stereochemistry.

Aside from the apparent selectivity of the THP ether, another important feature of blocking the tertiary hydroxyl group of humulone is that the molecule has a similar reactivity to that of lupulone. This has allowed manipulation of the humulone molecule in a way that has, until now, remained impossible. We have shown that if humulone is reacted with acetic anhydride with a catalytic amount of pyridine then a di-acetate is formed. The ^{1}H NMR of this compound can be seen in Figure 134. If humulone is treated with a 5 fold excess of acetic anhydride in pyridine, with or without a catalytic amount of dimethylaminopyridine (DMAP), a complex mixture

of products (Figure 134) was observed that was difficult to separate by common preparative techniques.

Figure 134 shows a comparison of the ^{1}H NMR spectrum of diacetyl humulone (77) with that of the spectrum of the complex mixture formed during the synthesis using pyridine as the solvent. Figure 135 shows an expansion of the resonances that correspond to acetyl protons. A small amount of the diacetyl compound is detectable in the complex mixture by NMR as shown by the comparison with the NMR spectra of the diacetyl compound.

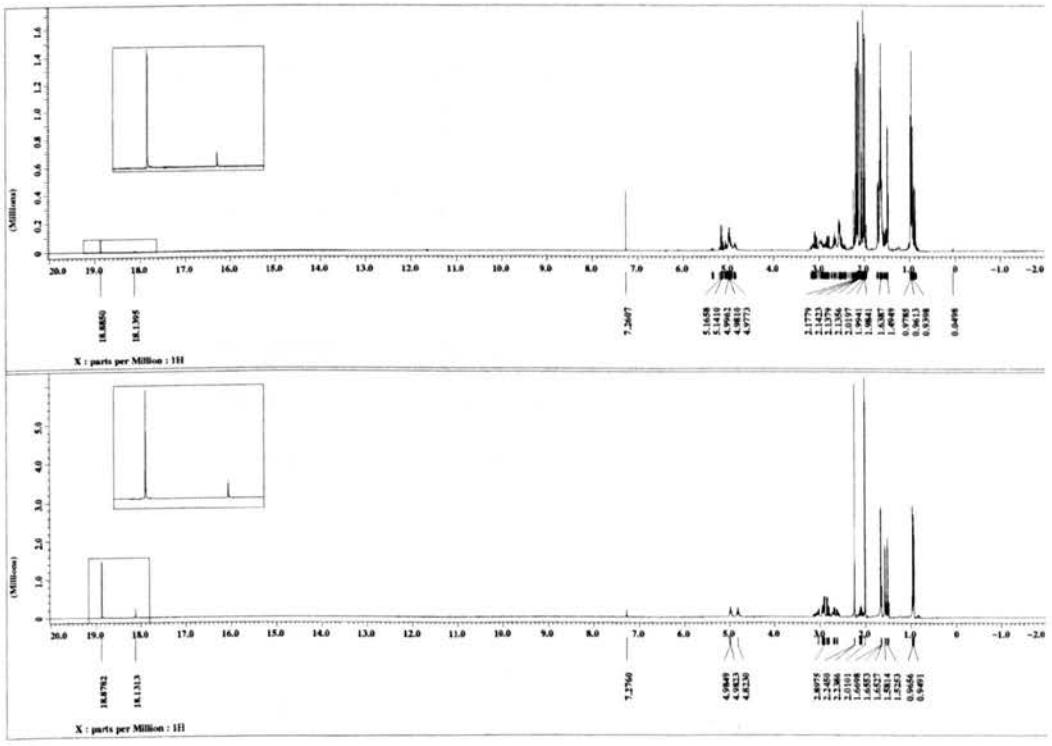


Fig 134 Comparison of the ^{1}H spectra of the complex mixture of acetylcompounds (top) and humulone diacetate (77) (bottom)

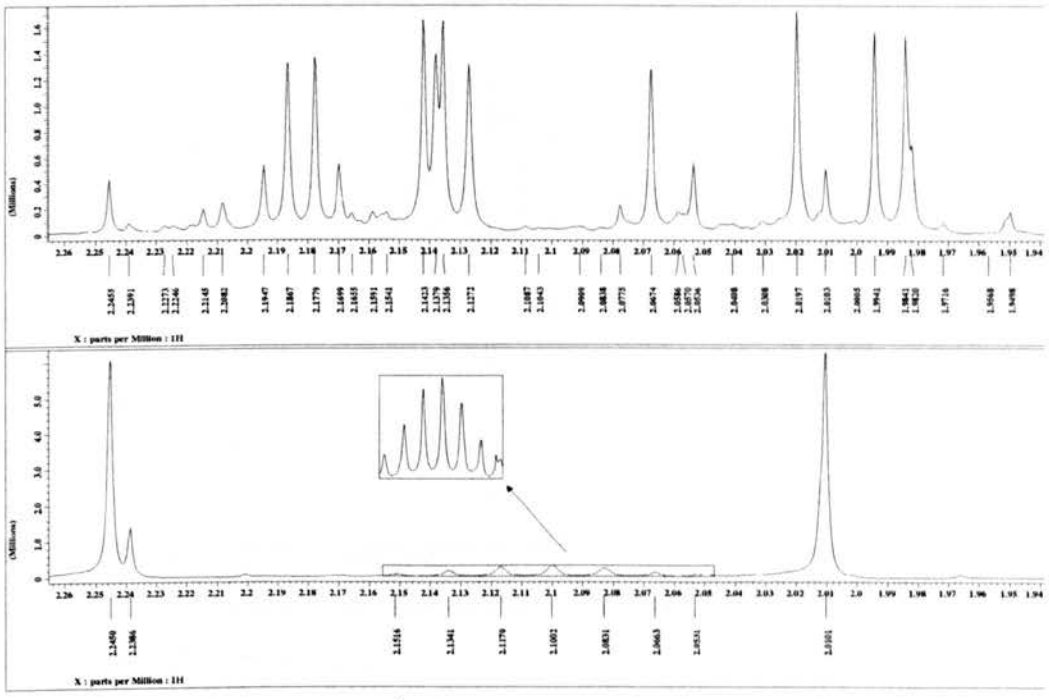
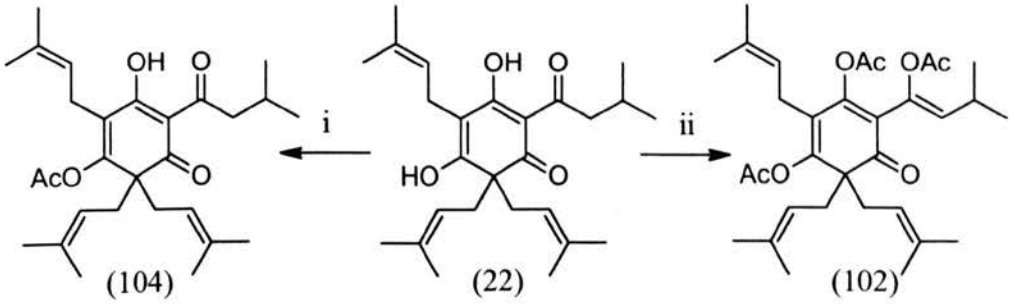


Fig. 135 Comparison of the expanded ^{1}H spectra of the complex mixture of acetylcompounds (top) and humulone diacetate (77) (bottom)

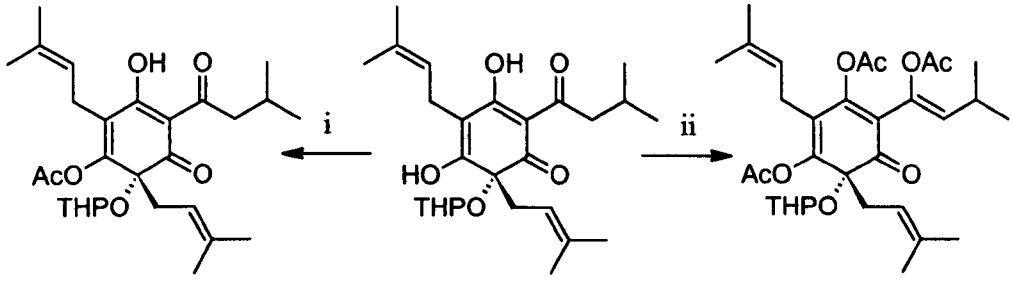
We have already demonstrated that using the above conditions, with pyridine as the solvent, it is possible to form a tri-acetate of lupulone (102). By altering the reaction conditions to catalytic pyridine it is possible to form the monoacetate of lupulone: (104) (Scheme 29).



i) Ac_2O, Pyridine (cat) ii) Ac_2O, pyridine (excess)

Scheme 29 Acetate esters from lupulone.

This selectivity has been observed with the THP ether of humulone (123), (126) as well (Scheme 30):



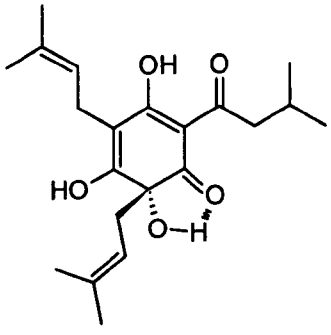
(123) (76) (126)

i) Ac_2O, Pyridine (cat) ii) Ac_2O, pyridine (excess)

Scheme 30 Acetate esters from humulone THP ether

The tautomeric forms of lupulone and its derivatives have been looked into using ^1H NMR spectroscopy and the monoacetate of lupulone (104) shows a ratio of 45:55 major to minor form.

As we have already established with humulone, the presence of the tertiary hydroxyl group restricts the tautomerisation possibly by hydrogen bonding with the neighbouring (C3) carbonyl group (Figure 136):



(8)

Fig 136 Proposed intramolecular hydrogen bonding of humulone (8)

We have also established from the THP ether of humulone (76), in which this hydrogen bonding capacity is blocked, there is evidence of two tautomeric forms albeit in different ratio to that of lupulone.

Due to the relatively small concentration of the minor tautomer (ca 3-8 % in $CDCl_3$ depending on which diastereoisomer is present) this makes the interpretation of the

NMR of the diastereomeric mixture easier to assign. This coupled with the fact that the isomers have been separated by preparative chromatography and identified by X –ray crystallography. If we are to assume that Verzele's proposed configuration, 4-(R), is correct then we can say with absolute certainty that the major diastereomer formed in a 20% d.e. has the (2S)-configuration on the THP ring (Figure 137). By process of elimination the minor diastereoisomer will have the (2R)-configuration.

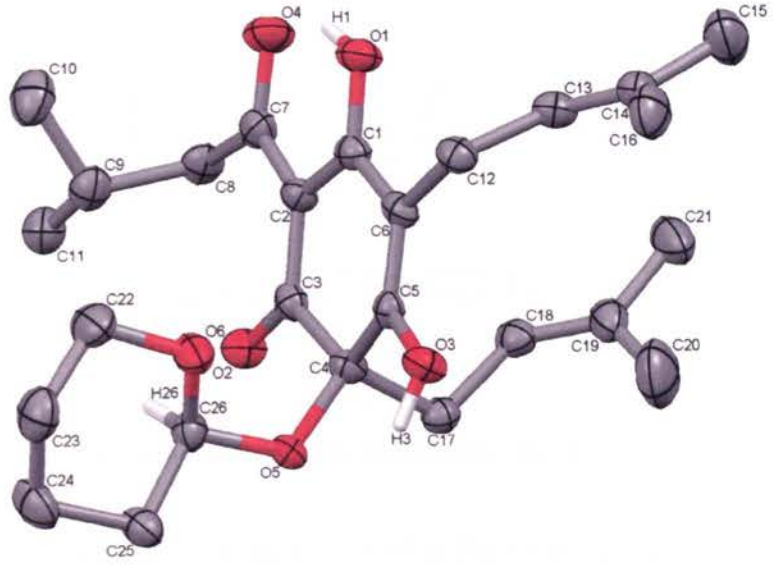


Fig 137 X-ray diffraction of (6R)-3,5-dihydroxy-4,6-bis-(3-methyl-but-2-enyl)-2-(3-methyl-butyryl)-6-[(2S)-(tetrahydro-*2H-pyran*-2-yl)oxy]-cyclohexa-2,4-dienone (76) shown as 50% displacement ellipsoids. The hydrogens have been omitted for clarity with the exception of the hydroxyl and the chiral centre on the THP ring

The monoacetyl product (123) (Figures 140 and 141) was formed directly from the mixture of diastereoisomers of the THP ether of humulone (76). It was thought that the mixture of diastereoisomers of the monoacetate (123) would be relatively simple however this was not the case. Indeed there were 4 hydroxyl signals observed in the down field region as expected for the strongly hydrogen bonded proton for the two tautomeric forms of the two diastereoisomers (123_a, 123_b). However what was not expected were the observed ratios of these resonances (Figure 138).

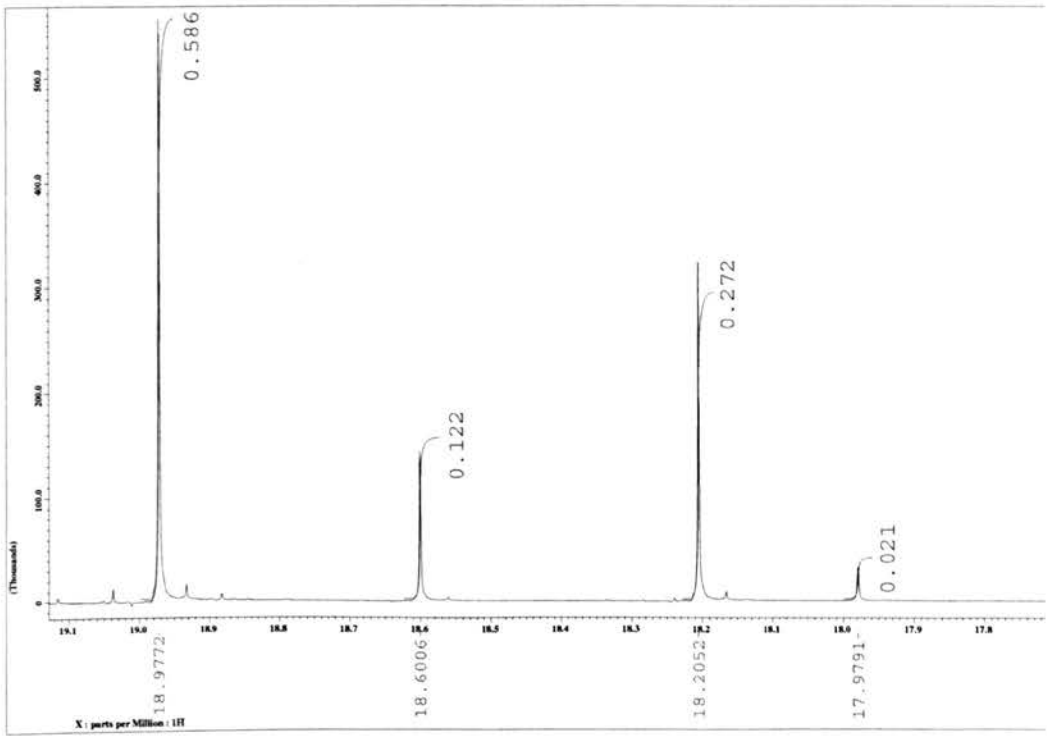
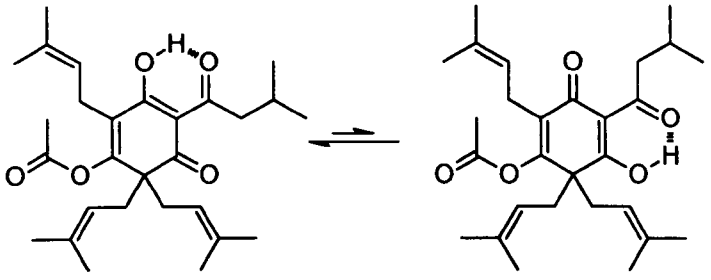


Fig 138 Expansion of the ^{1}H NMR spectra of the mixture of humulone THP acetates 123_a and 123_b

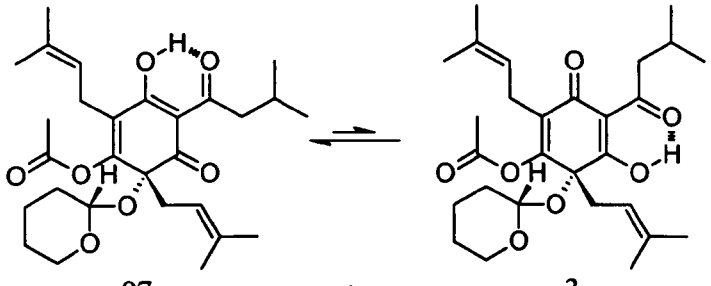
From first glance it would seem that the resonances at δ 18.98 and δ 18.60 ppm correspond to one diastereoisomer and the signals at δ 18.21 and δ 17.98 ppm correspond to the second diastereomer. However if we add the respective integrals then we see that the d.e is not the same as the starting material. i.e. a d.e= 41%. However the same de as the starting material is observed if resonances δ 18.98 and δ 17.98 ppm correspond to the major diastereomer and resonances δ18.60 and δ 18.21 ppm correspond to the minor diastereoisomer. Due to their similarity to other compounds investigated during the course of this research we have to assume that the minimum energy calculations are correct for lupulone acetate.



52.8829 kcal/mol. 73.4545 kcal/mol.

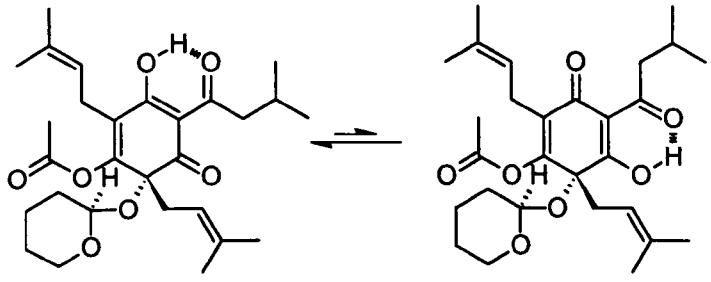
55 : 45

Fig 139 tautomeric forms of lupulone monoacetate (104)



97 : 3

Fig 140 123_a (6*R*)-3-hydroxy-4-(3-methyl-butyryl)-2,6-bis-(3-methyl-but-2-enyl)-5-oxo-6-[(2S)-tetrahydro-2*H*-pyran-2-yloxy]cyclohexa-1,3-dien-1-yl acetate



69 : 31

Fig 141 123_b (6*R*)-3-hydroxy-4-(3-methyl-butyryl)-2,6-bis-(3-methyl-but-2-enyl)-5-oxo-6-[(2*R*)-tetrahydro-2*H*-pyran-2-yloxy]cyclohexa-1,3-dien-1-yl acetate

While the presence of the minor tautomer of 123_a has a negligible impact on the ^{13}C NMR the presence of three compounds in the ratio of 60:38:13 makes the ^{13}C NMR very complicated and difficult to interpret. This is evident in a relatively clear region of the DEPT 135 spectrum, corresponding to the olefinic protons of the isoprenyl side chains of the molecule. Figure 142 shows a comparison of the DEPT 135 of compound (123) and humulone (8).

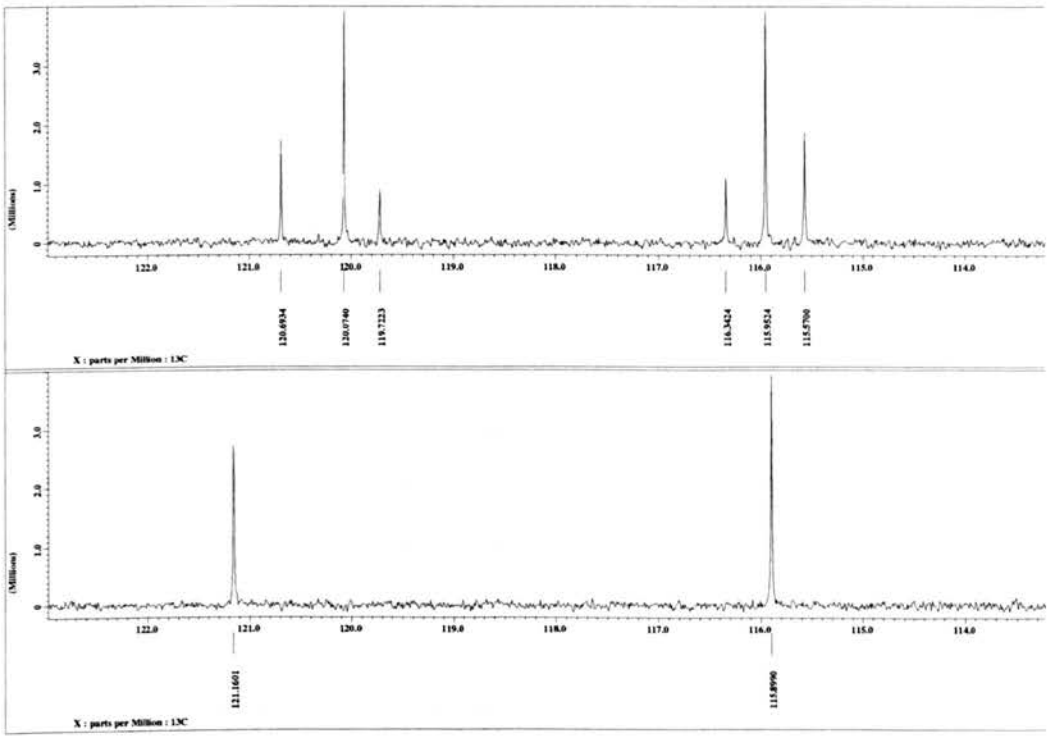
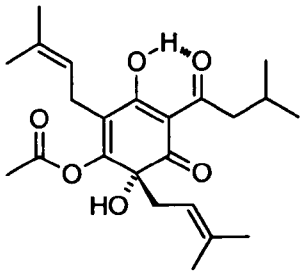


Fig 142 comparison of the DEPT 135 NMR spectra of compounds 123_a and 123_b (top) and humulone (8) (bottom)

It is possible to form both diastereomeric forms of this compound from the corresponding THP ether in order to make structural elucidation by ^{1}H and ^{13}C NMR more simple. This however is unnecessary as it is clear from the proton NMR that the resonances which correspond to the methyl group of an acetate group integrates for three protons only, suggesting that only one acetate group has been added.

Deprotecting this compound using acetic acid : THF : water in a 4:2:1 ratio at room temperature effectively removes the THP group to yield the monoacetate of humulone (125) (Figure 143).



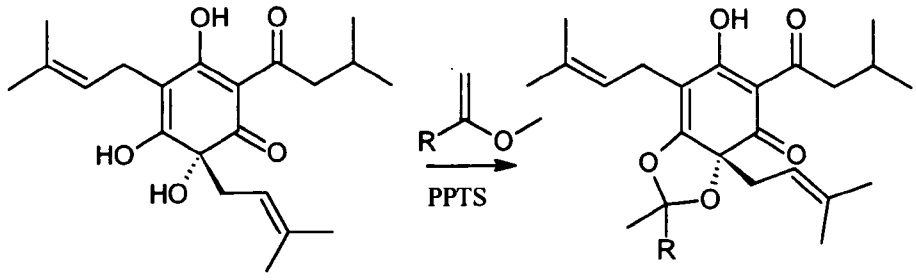
(125)

Fig 143 Humulone monoacetate (125)

This compound will allow for further derivatisation of the humulone molecule at C4. It is hoped that by silylating the free hydroxyl and removal of the acetate group would give a silylated analogue of the β-acid lupulone (22), hence give a crystal structure containing an heavy atom.

3.6.4.2 Enol protection –Cyclic Acetals and Ketals

It was predicted that the diol function of humulone could be used to form acetals and ketals by reaction with aldehydes and ketones respectively. Our primary attempts resulted in no products being afforded. After complete consumption of the starting material, determined by TLC no distinct products could be isolated. This was due to the strong acids and high temperatures needed to complete the reaction. Some dimethoxy acetals and ketals were also tested with similar results. However, upon using methyl or ethyl enol ethers in the place of the carbonyl the reaction proceeds at room temperature using weak acids such as pyridinium *para*-toluenesulphonate[163] (Scheme 31).



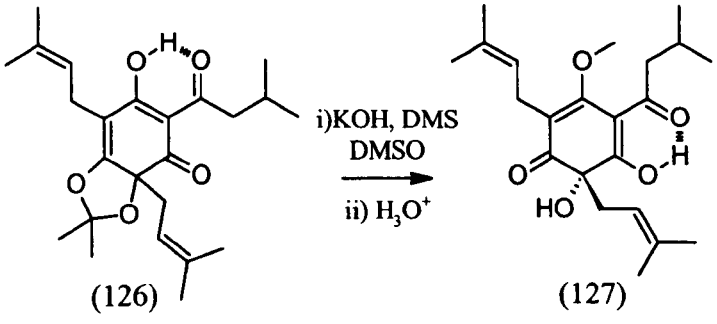
Scheme 31 Synthesis of humulone acetonides

The only isolated derivative of this nature has been the acetonide derivative (R = CH_3) (126). This acetonide derivative has allowed for selectivity to be accomplished with methylation reactions. Two of the three hydroxyl functions have now been effectively protected to basic conditions, conditions by which humulone normally undergoes conversion to five membered ring products.

Attempts to synthesise a methyl ether using sodium hydride in THF in the presence of DMS, a common method for the methylation of alcohols, did not meet with success, even when a large excess of the base was used.

It was then decided to use a method that is commonly used for the synthesis of methyl ethers from alcohols with DMS in DMSO in the presence of KOH. This did not show any sign of reactivity at room temperature (often a large exotherm is evidence of the reaction taking place) thus the material was heated to 80 °C.

After 1 hour the mixture was worked up with HCl and the product contained around 75% humulone, 23% humulone acetonide and about 2% of a new compound by NMR, this new compound was identified by the strongly hydrogen bonded proton being much less deshielded due to the loss of one of the carbonyl groups in the triketo system (127) (Scheme 32).



(126) (127)

Scheme 32 methylation of humulone acetonide (126)

3.7 Outcomes from The Derivatisation Studies

From our studies into the novel derivatives of humulone we can see that using humulone as a precursor for the synthesis of new compounds is not as simple as it first seems.

Throughout the analysis of these compounds it has become apparent that in order for just one tautomer to be present in non-polar solvents there needs to be a free hydroxyl function at C4 in order for the tautomer to be "locked". We have also seen that, in the case of lupulone, if the enol function at C5 is protected then the tautomers exist in approximately 50 : 50 ratio.

Interestingly, many of these compounds appear to exist in only one form if placed in polar media. This is most useful if we are to consider biological activity as these compounds will mostly be in an aqueous environment. However the esters, acyl and sulfonyl, of lupulone do not preferentially form one isomer in polar media, indeed they are observed in an almost 50 : 50 ratio in very polar and very non-polar media.

Chapter 4

Physical Properties and Biological Activity of the Hop Acids

4.0 Physical Properties and Biological Activity of the Hop Acids

4.1 Structure Activity Relationship (SAR)

When a drug compound is screened against a biological target a number of factors can affect the activity. Many molecules contain an active moiety, such as the β-triketo function seen in hop acids, the rest of the molecule may or may not play a role in the activity. However, it is known that by changing the carbon skeleton of a compound one can, sometimes dramatically, change the efficacy of a drug molecule. This change in activity with respect to the structure of a compound is known as the Structure Activity Relationship or SAR.

4.2 Metal Chelation

Hop acids (HAs) are capable of binding to both divalent and trivalent metal ions in a process known as chelation. The hop acids act as bidentate ligands this is to say that there are two moieties which participate in chelation.

When a trivalent metal cation complexes with three anions, i.e. chloride, and coordinates with three neutral species, i.e. ammonia, the result is an octahedral arrangement about the central metal ion (Figure 144).

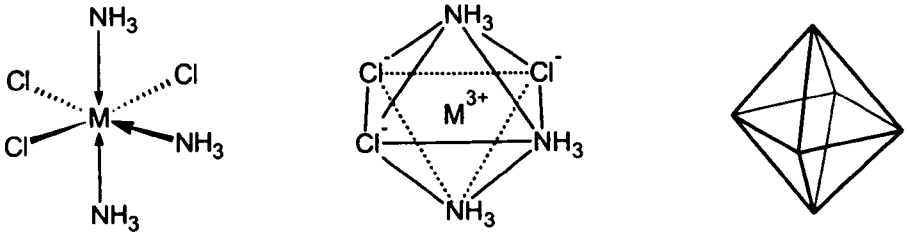
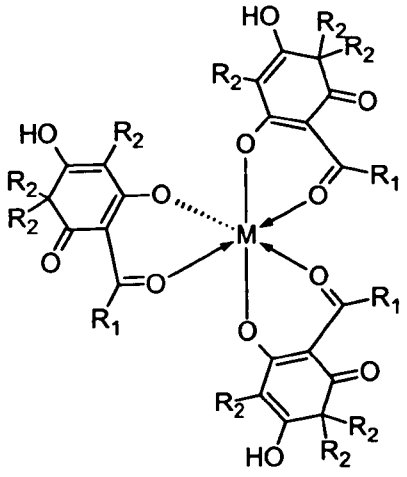


Fig 144 Octahedral arrangement of trivalent metal cations

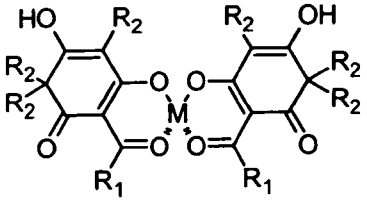
As the hop acids are bidentate they act as both the anionic and neutral species in their chelate complexes (Figure 145).



$= ML_3$

Fig 145 ML_3 complex of hop acids with trivalent metal cations

Bidentate ligands complex with divalent molecules and form square planar complexes. For instance hop acids complex with divalent metal cations in the following complex (Figure 146):



$= ML_2$

Fig 146 ML_2 complex of hop acids with divalent metal cations

It is the ability of these compounds to chelate with divalent species within bacterial cells, such as Mn^{2+} or Fe^{2+}, that has been attributed to their efficacy in causing cell death [98]. The relationship with manganese chelation and biological activity has led us to develop an assay to determine the efficacy of hop acids as chelators of manganese ions.

4.2.1 Cell Walls

Bacterial cell walls are comprised primarily of phospholipids, these compounds are amphipathic, possessing non-polar long chain fatty acids bonded to a negatively charged phosphate group and a positively charged choline (Figure 147).

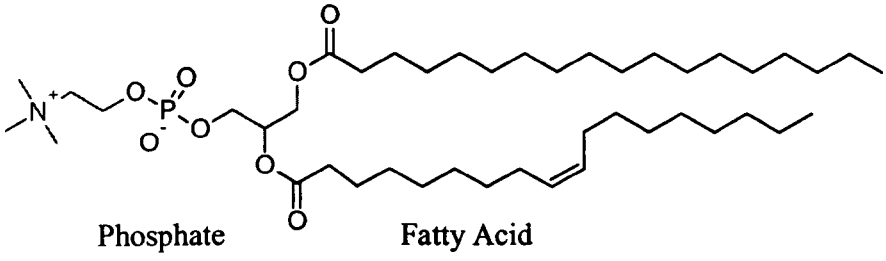


Fig 147 Structure of a phosopholipid

The amphipathic nature of lipids produce the membrane bi-layer of cell walls. The polar 'head' is hydrophilic, therefore making up the external and internal walls that are in contact with the water. In contrast the non-polar 'tail' made up of the fatty acids carbon chain is very hydrophobic and therefore makes up the internal part of the cell wall (Figure 148).

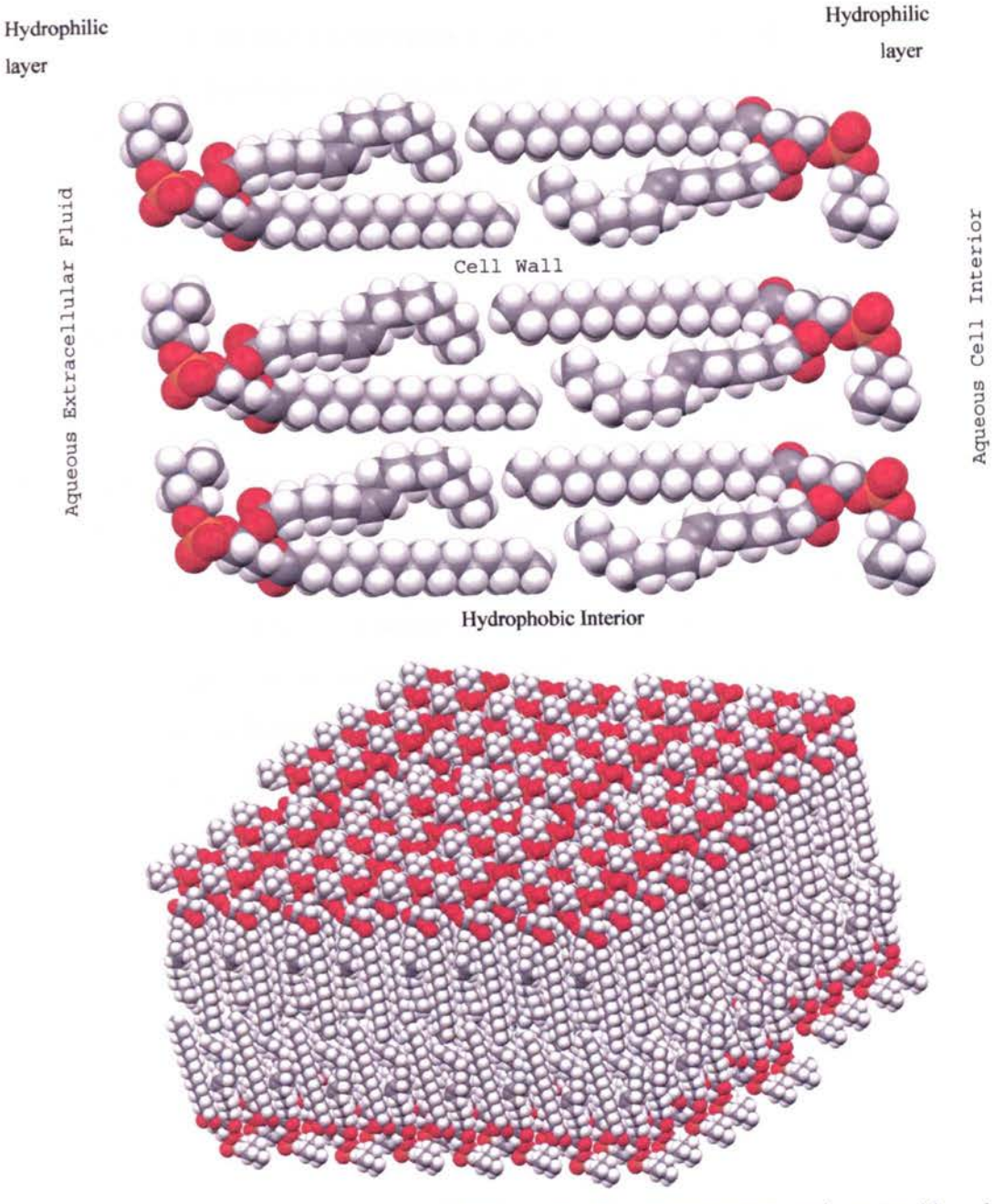


Fig 148 A simplified representation of the lipid bi-layer; cell wall components such as steroids and proteins have been omitted for clarity [166]

In order for a drug substance to be effective against living cells it must be able to pass through the hydrophobic membrane interior. If the drug molecule is to pass through into the aqueous cell interior it must be soluble in both the hydrophobic and the hydrophilic phases. Strongly hydrophilic compounds will not pass into the

cell membrane as they have a very poor solubility in non polar media. In contrast to this strongly hydrophobic compounds will be freely soluble in the non-polar membrane but have a poor solubility in the aqueous phase, hence once they have passed into the membrane they will be unlikely to diffuse out into the interior of the cell and ultimately they will be unlikely to reach their target.

4.2.2 Partition Coefficient (LogP)

A drug like molecule must have an affinity for both hydrophobic and hydrophilic conditions. This affinity for both conditions is measured by way of a compound's partition coefficient. This coefficient, or LogP, an equilibrium constant, is a ratio of the distribution of the drug in either phase. It is determined by measuring the partition of a drug across a biphasic mixture of immiscible solvents usually an aqueous layer and octanol. Octanol is commonly used because it mimics the lipid bi-layer of living cells at the partition (Figure 149).

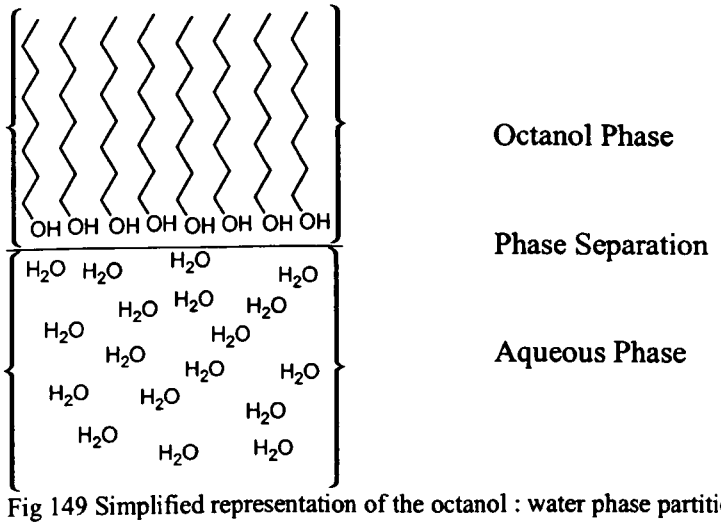


Fig 149 Simplified representation of the octanol : water phase partition

Thus a measurement of the concentration of the drug in the organic phase against the concentration in the aqueous phase will give the LogP:

LogP or the Partition coefficient is calculated by the following formula (Equation 1):

$$\text{LogP} = \frac{\text{Concentration in octanol}}{\text{Concentration in water}}$$

Equation 1

One would assume that the LogP of ionic manganese chloride to be practically zero, however, the calculated LogP for the complex of lupulone with manganese is 5.9, it is assumed that the complex is in the ML_2 form.

In the ML_2 form, the lupulone complex can exist as either *cis* or *trans* geometric isomers around the manganese (Figure 150).

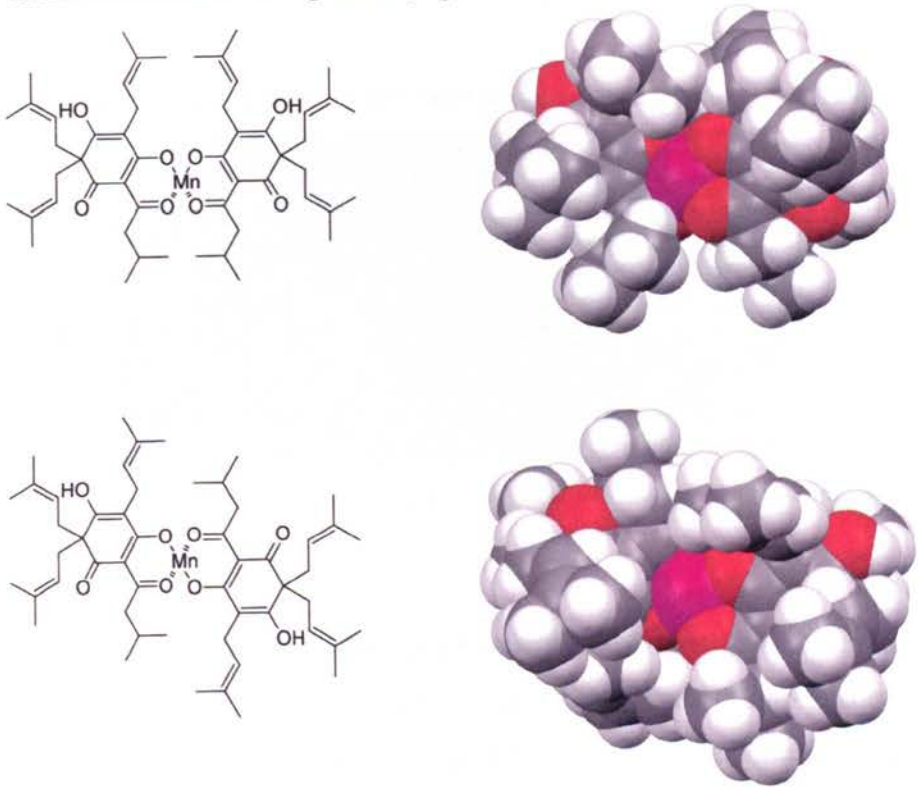


Fig 150 Geometric isomers of lupulone manganese complexes.

As lupulone or humulone manganese complexes are not strongly coloured, the complex with humulone is a light green colour and is observed at relatively strong

concentrations (> 100 ppm). This is in contrast to the complexes formed with iron (III) which are all strongly coloured, even at 5 ppm these are visible to the naked eye, hence an iron (III) chloride solution is often used to visualise these compounds during TLC analysis [34]. In order to evaluate the assay for determining LogP, a preliminary trial was conducted.

4.2.2.1 Assay Evaluation

A solution of iron (III) chloride was made in deionised water to give a concentration of 10 ppm w/v with respect to iron i.e. 0.01mg/ml. This was mixed for 15 hours with an octanol solution of lupulone. Four concentrations of lupulone provided different molar equivalents of the ligand with respect to the iron. Equivalents were as follows: 0.5, 1.0, 3.0 and 5.0.

Visual inspection of the bilayers was readily accomplished (Figure 8).

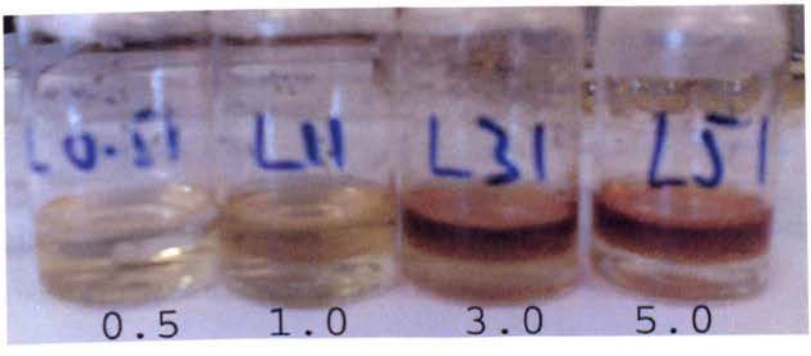


Fig 151 The bottom row of numbers in the figure above indicate the molar equivalents of lupulone in relation to iron.

The results confirm that there is a relationship between the concentration of ligand present in the octanol and the amount of metal that passes into the organic phase. Unfortunately for our assay iron proved to be an unsatisfactory candidate as iron (III) acts as an electron donating oxidising agent and unless the solution is kept acidic iron oxide is formed. This was evident in the experiment by the formation of an insoluble precipitate at the phase partition.

Manganese is the most biologically important metal with respect to the hop acids activity towards bacterial cells, thus this was chosen for these studies.
The LogP of a compound, however, is not a convenient method for this assay, because the concentration is often measured by UV as this is a very sensitive method.

From the visual inspection of the iron chelates in Figure 151 the visible range has changed. We can, therefore, predict with some certainty that there would be a measurable change in absorption in the UV region of the electro magnetic spectrum that would allow us to quantify the concentration of complex in the organic phase using the Beer Lambert equation (Equation 2).

$A = \varepsilon CL$

Where A = absorbance

ε= extinction coefficient ($l\ g^{-1}$ cm-1

C = concentration ($g\ l^{-1}$)

L = Path Length. (cm)

Equation 2 Beer Lambert Equation

The extinction coefficient ε is individual for any given compound and, unless known, must be calculated using a standard.

The main problem encountered with using UV spectroscopy to determine the concentration of complexes is associated with speciation. With bidentate ligands and trivalent metal cations there are three different species that can be formed: ML, ML_2 and ML_3. Each of these would have a different λ max and as they are different compounds they would have different extinction coefficients. This makes quantification very difficult without prior knowledge of a number of physical parameters such as pKa and binding affinities. We could use UV spectroscopy to determine the concentration of iron remaining in the aqueous phase, however, there is a distinct possibility that there would be a significant concentration of ligand in

the aqueous phase which would interfere with the UV absorbance and this could not be quantified for the same reason as above.

4.2.2.2 AAS

It was decided to use atomic absorption spectroscopy (AAS) as this would be able to quantify the amount of metal in the aqueous phase without any interference from any dissolved ligand. AAS involves passing a solution of metal ions through a flame ioniser, this in turn produces a change in the electromagnetic absorbance of the flame. The absorbance is measured and is directly proportional to the concentration of metal ions.

The first trial was conducted, in triplicate, on humulone (8) and lupulone (22). An aqueous solution of manganese chloride tetrahydrate was dissolved in water to give a manganese concentration of 50 ppm. This was then shaken with an octanol solution of ligand with the following molar equivalents 0.0 (blank), 0.5, 1.0, 3.0, 5.0 After the solutions had been allowed to mix for 15 hours an aliquot of the aqueous phase was removed and diluted 1 :10 with 5% hydrochloric acid. This solution was then analysed by AAS and the absorption measured with respect to the blank and against a standard calibration curve to give the concentration of manganese in the octanol by difference (Figure 152).

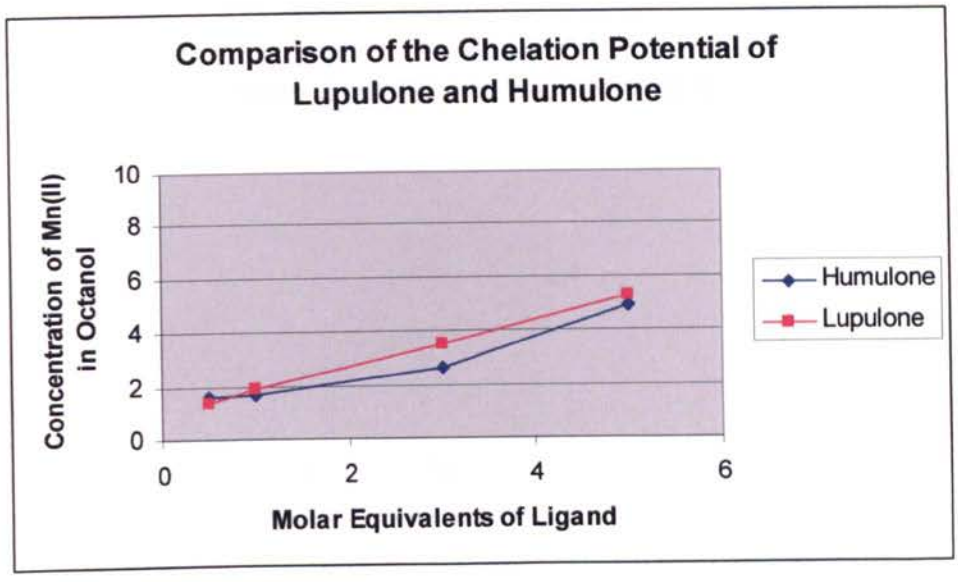


Fig 152

From these data we can see a relationship between the molar equivalents of the ligand and the concentration of manganese in the octanol layer. We can see quite clearly that the relationship is linear for lupulone and humulone shows a nearly linear response. There is, however, no particularly significant difference between the two compounds.

It was decided to examine the same compounds but change the molar ratios to 1.0, 10.0 and 15.0 equivalents. These data were as follows (Figure 153):

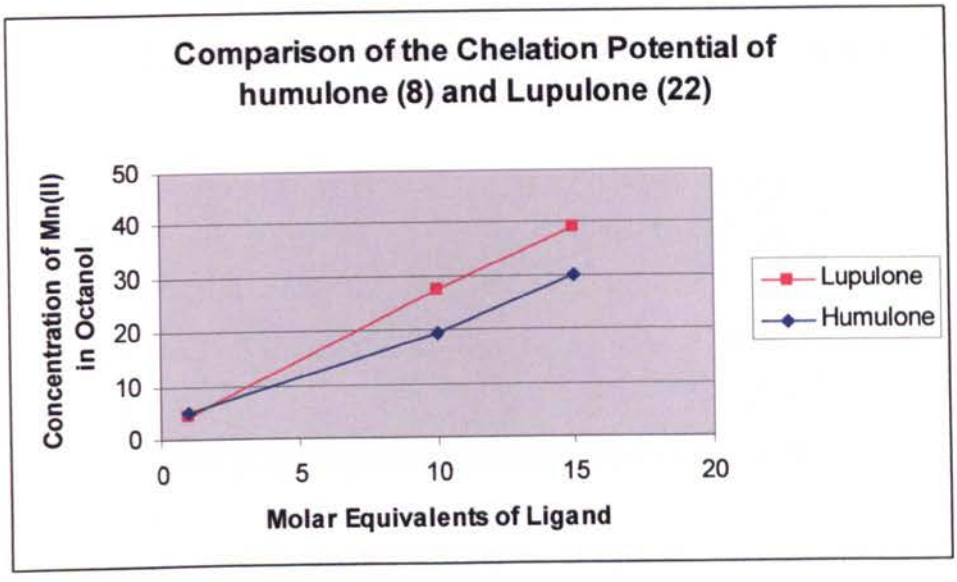


Fig 153

We can now observe from these data that there is a significant difference between humulone and lupulone at 15 times the molar equivalence of ligand.

As The starting concentration of manganese (II) was 50 ppm in the aqueous phase we can consider that the maximum concentration of manganese that we could find in the octanol layer would be 50 ppm. Therefore if we know the concentration in the aqueous phase and we can then assume the concentration in the octanol we can express the experimental values as LogP of manganese (II) (Figure 154).

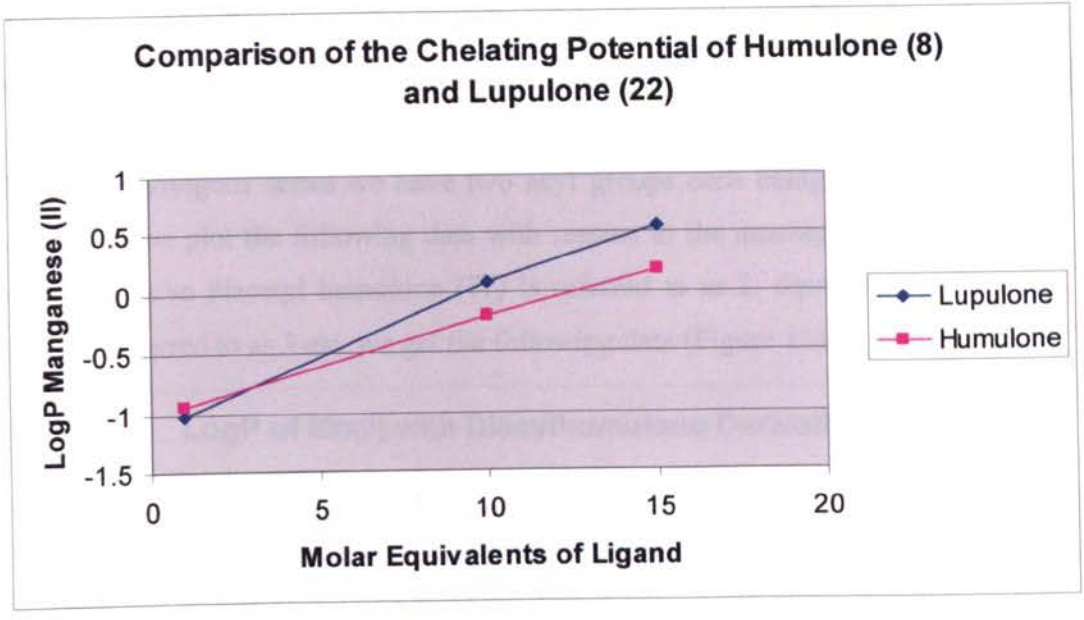


Fig 154

Having optimised a convenient method for the synthesis of the diacetate ester of humulone (77) a small library of analogues were synthesised. Five of these were tested under the same conditions to those with humulone and lupulone, and the Log P of the manganese complexes were plotted below (Figure 155):

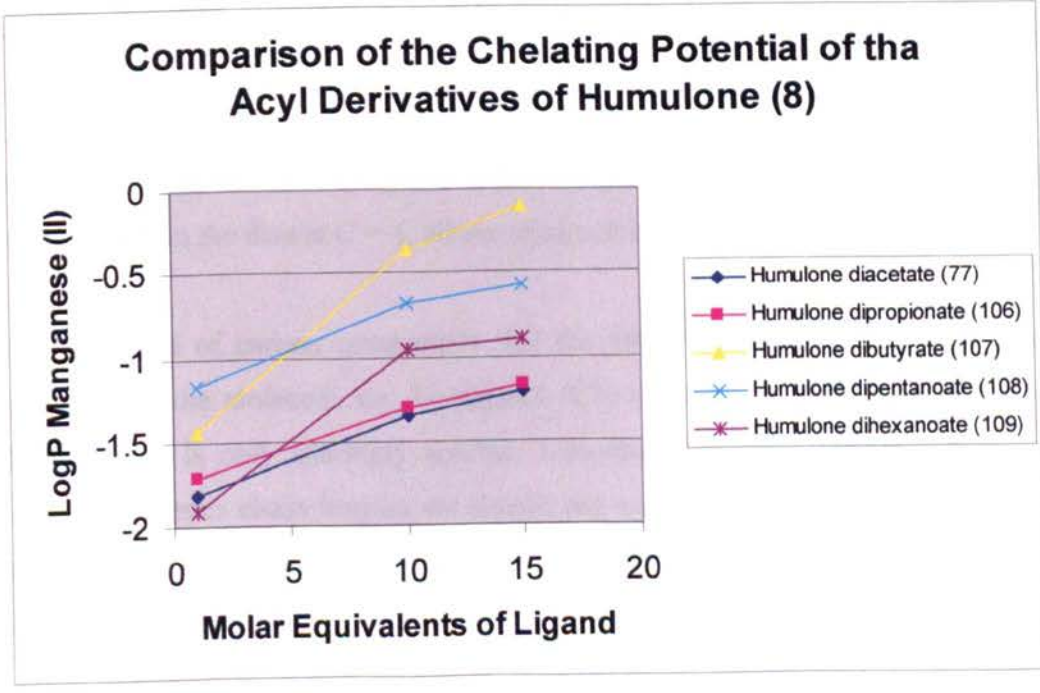


Fig 155

Plotting the Log P removes the linearity of the data, however from these data we can see a change in the LogP with changes in the molecule.

In this homologous series we have two acyl groups each being increased by one carbon. If we plot the following data with respect to the number of carbons in the ester chains so diacetyl humulone (77) is referred to as 2, dipropionyl humulone (106) is referred to as 3 etc. we get the following data (Figure 156):

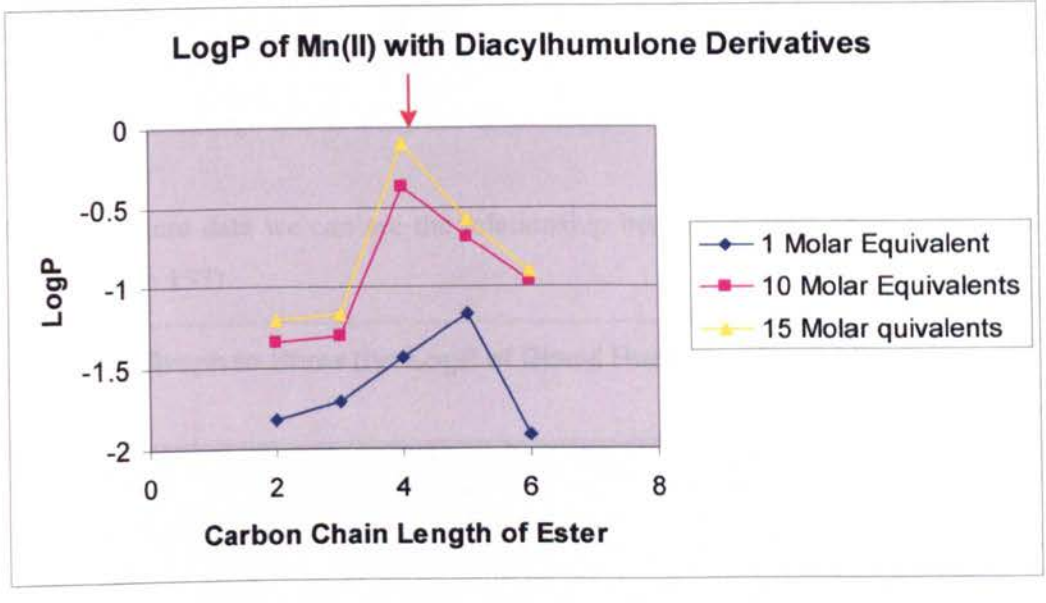


Fig 156

From these data we can clearly see that at 10 and 15 molar equivalents there is a distinct peak in the data at C = 4, dibutyrylhumulone (107).

It is expected of carbon compounds that the longer the carbon chain the more hydrophobic the molecule. i.e. 1-propanol (C3) is freely soluble in water and 1-octanol (C8) is only sparingly soluble. Likewise we would expect that as we increase the ester chain lengths we should see a decrease in the water solubility, hence a shift in the partition between octanol and water and therefore a change in the compound's LogP.

The Log P data below (Table 10) was collected using QUANTUM CaCHE project leader.

Compound	LogP (calculated)
humulone acetate (77)	0.91
humulone propionate (106)	1.934
humulone butyrate (107)	2.958
humulone pentanoate (108)	3.751
humulone hexanoate (109)	4.544

Table 10 Calculated LogP data for diesters of humulone (8)

If we plot these data we can see the relationship between carbon chain length and LogP (Figure 157).

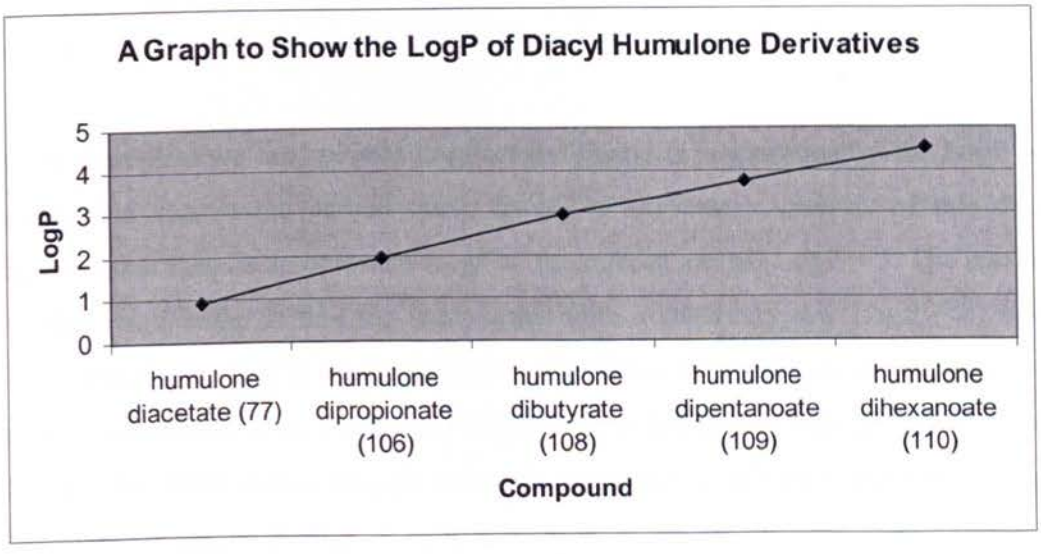


Fig 157

From these data we can see that as the acyl chain length increases, the LogP increases. We may therefore anticipate the LogP of the resultant manganese complex (ML_2) would increase in the same linear manner.

This may be the case but in this water / octanol system we are seeing a different relationship. If we plot LogP of the ligand against the LogP of the manganese in the

presence of the ligand at 15 molar equivalents we get the following relationship (Figure 158).

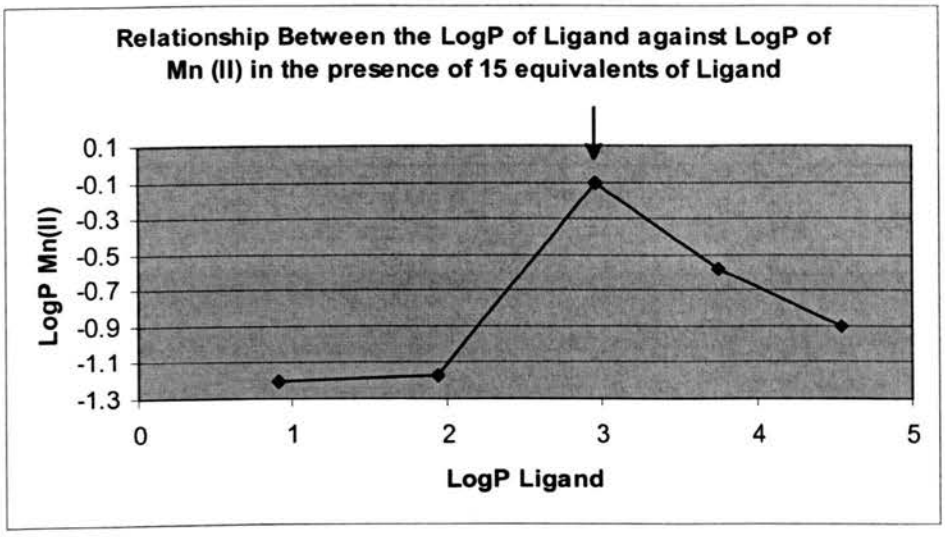


Fig 158

From these data we can see that LogP of the ligand is proportional to the LogP of manganese against the carbon chain length of the ester. Analysis of this data suggests that there is an optimum LogP of the ester at around LogP = 3. The reason for this relationship is that the compounds with a relatively low LogP (<3) will form complexes with a relatively low LogP hence they remain in the aqueous phase. Compounds with a relatively high LogP (>3) are too hydrophobic to enter the aqueous phase, hence they do not come into contact with the manganese cation and so do not form a chelate.

A range of other derivatives were also investigated and the LogP plotted against the LogP of manganese in the presence of the ligand (Table 11) (Figure 159).

Compound	LogP Calculated	LogP of Mn (II) Experimental
Methyl Humulone (78)	1.029	-1.272520424
Humulone tosylate (111)	2.475	-0.977480313
Lupulone (22)	3.461	0.554835264
Lupulone tosylate (112)	4.939	-0.986231679

Table 11 Calculated LogP data and experimental Mn(II) LogP data of selected ligands

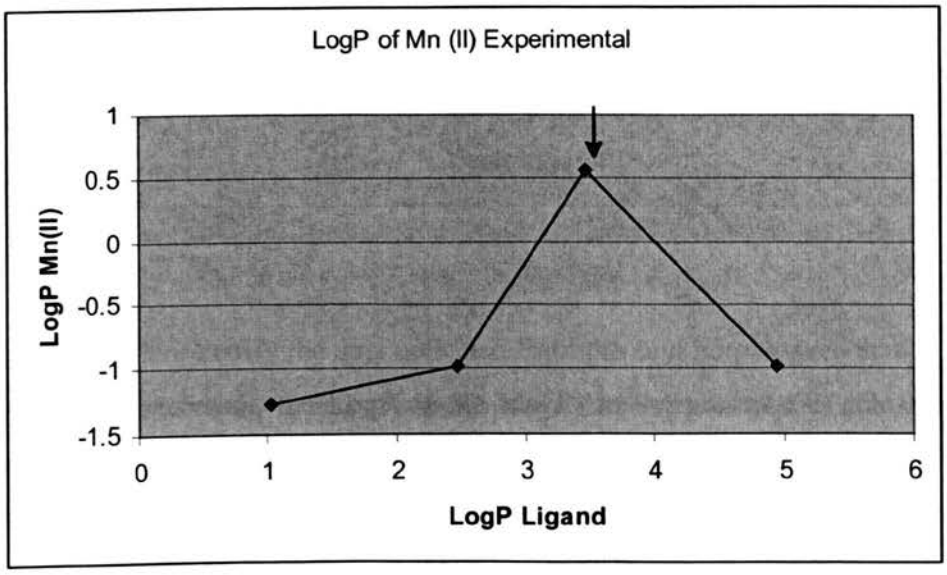


Fig 159

This again shows a similar trend with a ligand Log P of 3.5 being optimum. If we now add these data with the previously collected data from the Acyl derivatives then we achieved the following plot (Figure 160).

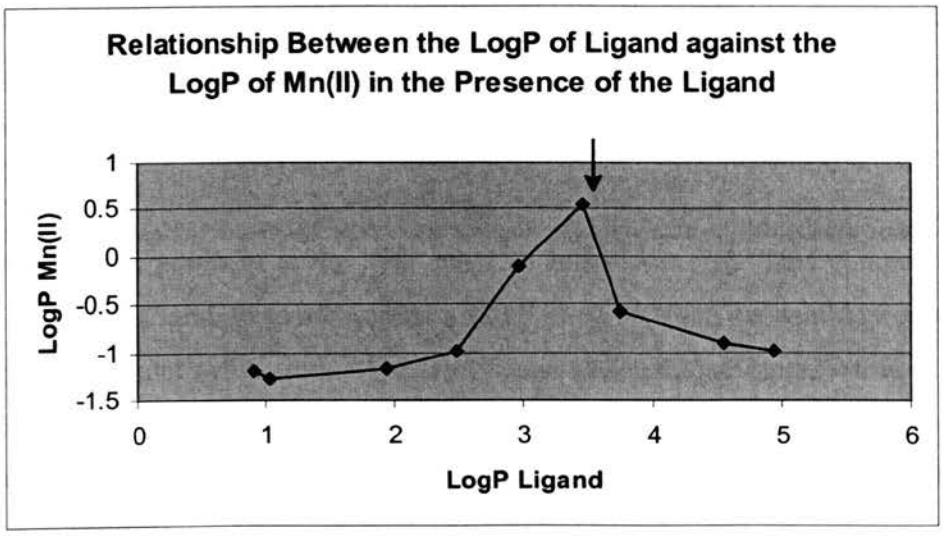


Fig 160

We can further clarify the data collected from the acyl homologues that as LogP of the ligand increases, the LogP of the Mn(II) in the presence of that ligand also increases to a maxima at around 3.5 at which point the Mn (II) LogP begins to fall. With all of this data we can see that the chelating potential of these compounds is closely related to their Log P which in turn is related to the structure of the compound.

Not all of the hop acids and their derivatives fit this model. For instance humulone (8) has a very low LogP, however manganese has an anomalously high Log P in the presence of humulone (8). Humulone (8) has a calculated LogP of 0.997 which would be expected to give manganese a LogP of around -1.25, however it gives manganese a LogP of +0.17. This result is considerably higher than the dibutyryl derivative (107). This suggests that there is an important moiety present in humulone which is not present in the other derivatives.

Humulone (8) is known to form 1:1 complexes with 1,2-phenylendiamine and lead (II) both of these have been attributed, not to the β-tricarbonyl but to the diol function at (C4-C5). This implies that humulone (8) can chelate the manganese in more than one position. Perhaps this forms multi unit complexes hence surrounding

the manganese cation with a large hydrophobic group. Whatever the reason behind this anomalous reading it does suggest that the diol of humulone (8) must remain intact to allow for optimum chelating activity.

The second anomaly is the THP ether of humulone (76). This compound has a LogP of 1.97 and gives manganese a LogP of -0.34 when we would have predicted this compound to be around the same value as humulone dipropionate -1.17, this increase in activity is most likely due to the easily hydrolysed THP group being removed during the experiment to yield a mixture of humulone (8) and the THP ether of humulone (76).

The final compound to not fit the data was lupulone acetate (104) this has a calculated LogP of 3.25 and gives manganese a LogP of -1.21 at 15 equivalents.

Lupulone (22) has a LogP of 3.46, so we would have expected that the acetate (104) would be very similar in its chelation potential.

So why is this compound not allowing manganese to pass into the octanol phase? This cannot be explained in the same way as the difference between humulone (8) and acetyl humulone (77) as we have shown that blocking the enol function still allows the chelation of manganese and it is only the LogP which is the factor which effects activity.

The main characteristic feature of lupulone acetate, (104) which distinguishes it from humulone dibutyrate (107), the humulone analogue with the closest LogP, is the presence of two tautomeric forms even in very polar media. We have previously demonstrated that diesters of humulone exist in tautomer ratio of 9 : 1 in $CDCl_3$ but in CD_3OD there is clearly only one isomer present. Lupulone acetate (104), however, exists in close to a 1 : 1 ratio of tautomers in both $CDCl_3$ and CD_3OD. So by blocking the enolic hydroxyl of lupulone the molecule is not held in one

tautomeric form in polar media. This possibly lowers the molecules ability to form stable chelates with metal ions.

From the above trial we can deduce that by derivatising humulone (8) we drastically reduced the molecule's ability to abstract metal ions from aqueous media into non-polar solvents. We have also deduced that the diol moiety of humulone is essential to its activity.

4.3 Biological Evaluation

Once we had deduced the chelating potential of the derivatives the next logical step was to test hop acids activity against bacterial samples. This work was carried out as a preliminary trial due to time constraints. The initial test was using the Kirby-Bauer disk diffusion test [167]. This is a test which uses antibiotic-impregnated discs of filter paper, at different concentrations to test whether particular bacteria are susceptible to specific antibiotics. The bacteria are grown in petri dishes on agar with the impregnated filter paper placed on the agar (Figure 161):

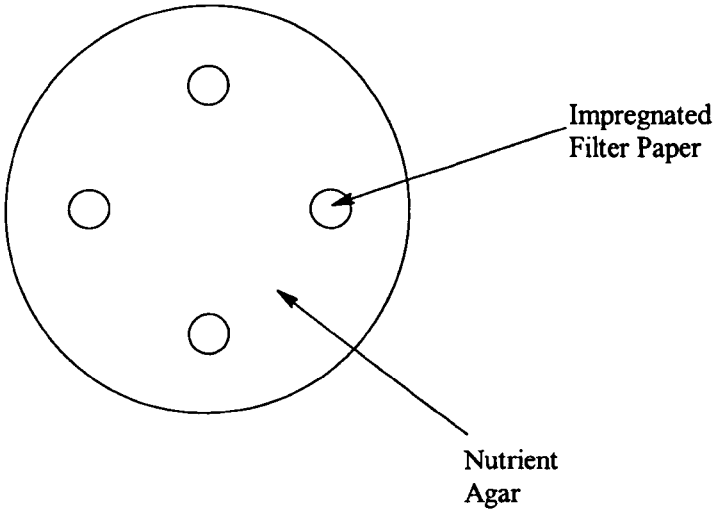


Fig 161 A simple Bauer disc diffusion test

The plates are allowed to incubate for 24 hours as the bacteria grow on the agar culture. The areas around the antibiotic impregnated discs do not allow growth if the bacteria is susceptible to the antibiotic.

Two micro organisms were tested;

i) *Staphylococcus aureus* (4.3.1)

ii) *Streptococcus faecelis* (4.3.2)

4.3.1 *Staphylococcus aureus (Staph aureus)*

Staphylococcus aureus is a relatively safe bacteria thus it can be handled outside of a biological safety cabinet. It is commonly used as a preliminary test for antibiotics [168] as it is sensitive to a vast array of anti bacterial agents.

Staph. aureus is a Gram-positive bacteria one of the most common causes of food poisoning. The bacterium produces a toxin which, when indigested, causes nausea, vomiting and diarrhoea. The symptoms often have a rapid onset, a property which distinguishes them from salmonellosis (poisoning from salmonella) and unlike salmonellosis, poisoning from *Staph. aureus* is seldom fatal [169].

There are many reports, both in the scientific literature and in the media with respect to Methicillin Resistant *Staph aureus*, (MRSA) [170], a strain of the bacteria which has developed a resistance to most licensed antibiotics. However, due to the dangerous nature of these pathogens they have not formed a part of this study.

4.3.2 Streptococcus faecelis (Strep. faecelis)

The *Streptococcus* are Gram-positive bacteria, along with the *Staphylococcus* they are not regarded as dangerous pathogens so can be handled outside of a biological safety cabinet [171].

During this study we investigated *Strep. facealis*, these bacteria are commonly found in human and other animal faeces. These organisms can survive for long periods of time in faeces-polluted water thus are commonly used as indicators of sewage or faecal pollution.

The *Streptococcus* are also closely related to the *Lactobacillus* type bacteria making them an ideal substrate to examine SAR of hop acids.

The results of the disk diffusion test were encouraging, two compounds were tested against *Staphylococcus aureus* and *Streptococcus faecelis*; humulone (8) and lupulone (27) showed a definite kill zone around the disks (bactericidal effect), there was also evidence of an area where the bacterial growth had been significantly slowed (bacteriostatic effect).

The disc diffusion test is a useful and fast method for determining susceptibility of bacteria to the antibiotic effects of drug molecules qualitatively. After we had determined that there was activity of the two natural compounds it was decided to use a more quantitative method.

4.3.3 Basic MIC Test

The method used was the broth dilution MIC test [164]. This experiment uses a liquid broth of molten agar which is inoculated with bacteria. The broths are also treated with known concentrations of antibiotic. The broth is then allowed to incubate for 24 hours after which time bacterial growth is observed by turbidity in the sample. A blank is prepared which contains no antibiotic which has the highest turbidity.

The turbidity of the solutions is determined by measuring the absorbance of visible light. This absorbance is directly proportional to the number of bacterial cells present. Therefore the higher the turbidity, the more cells have grown, thus the less

effective the antibiotic. The number of cells is measured directly by comparison with standard solutions.

The lowest concentration which has no turbidity, hence no bacterial growth, is known as the minimum inhibitory concentration (MIC). This quantitative measurement allows us to compare the biological activity of antibiotic by a comparison of the MIC, the lower the MIC the higher the activity.

During our preliminary trial we tested a number of hop acids and hop acids derivatives against *Staph. aureus* and *Strep. faecelis*, against a known antibiotic, chloramphenicol (Figure 162). The MIC of each compound was measured in mM concentrations.

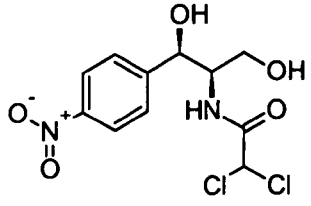


Fig 162 Chloramphenicol

This experiment produced the following data (Figure 163):

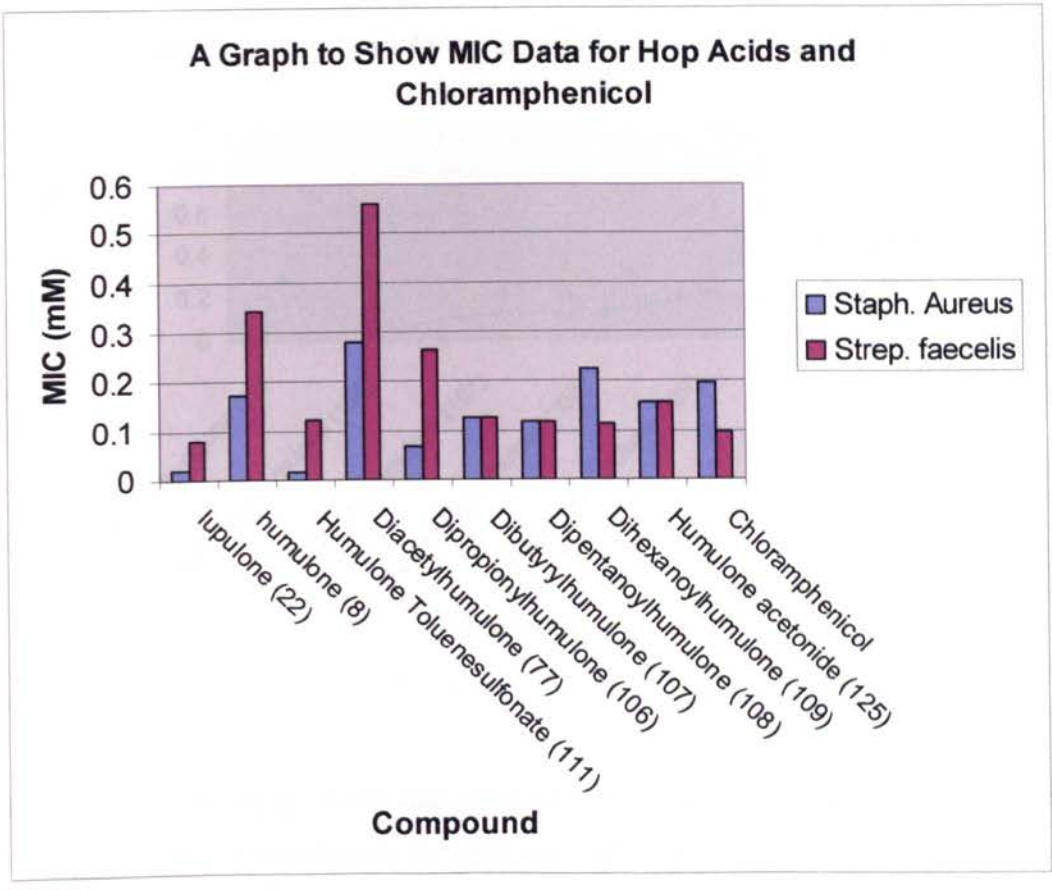


Fig 163

From these data we can see that many of the compounds have a comparable activity to chloramphenicol against *Strep. facelis.* However, candidates (111) and (108) showed enhanced activity against *Staph aureus* compared to chloramphenicol. As these two micro organisms are different we would expect there to be different activities of the compounds tested. If we look at the pattern of these compounds we can see a similar pattern to the chelation studies, in particular, the effect of the acyl derivatives against *Staph. aureus* (Figure 164):

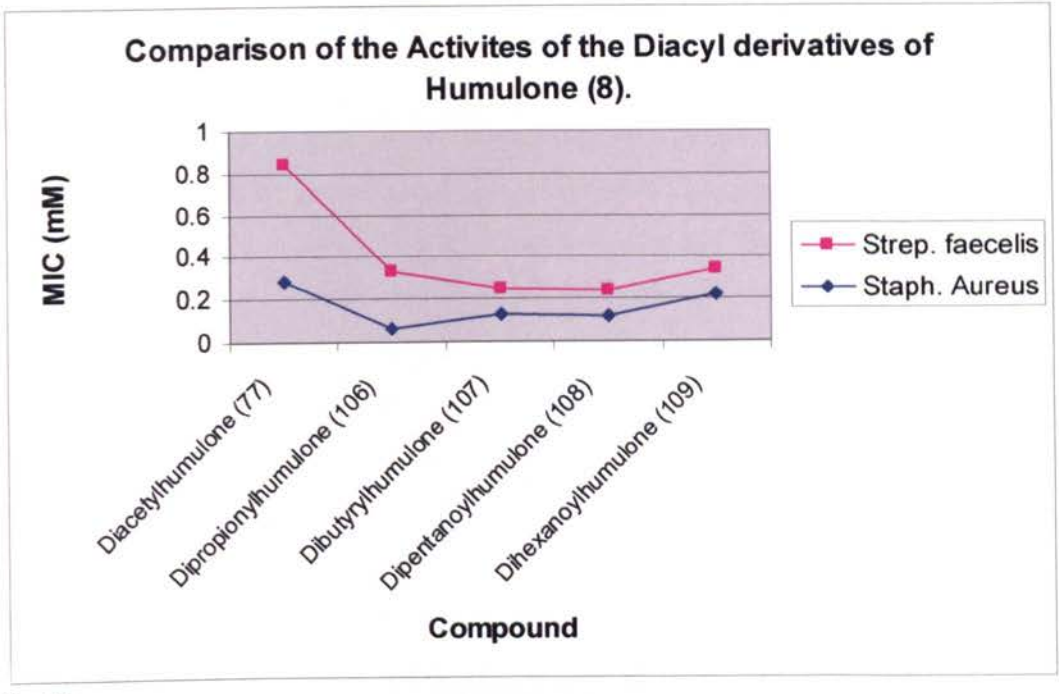


Fig 164

While we do not see the same sharp increase in activity with dibutyryls humulone we do see a trend where there is an optimum LogP range.

One of the most interesting features of the data collected is that the toluenesulfonate ester of humulone shows a significant activity against *Staph aureus* therefore confirming the SAR and the different mode of action compared to *Strep facelis*. This information was not observed with the chelation data. From this observation we can speculate that there may be more to the activity of the hop acids than just the chelating of manganese ions.

In conclusion we can see that there is some evidence that the hop acids have more than one mode of action against certain bacteria. In particular we are seeing heightened effects with the sulfonate ester of humulone; this work should be investigated further.

Chapter 5

Achievements

5.0 Achievements.

During the course of this project we have pushed the boundaries of hop chemistry away from the brewing industry and into mainstream chemistry. We have managed to remove all ambiguity from the spectroscopic data for humulone, lupulone and possibly any compound, natural or synthetic, which shares the same nucleus as these compounds. We demonstrated that synthetic humulone (8) can be resolved into its respective enantiomers, and we have shown that the potential derivatives of humulone are not restricted to the complex mixtures of oxidation and reduction products found during the brewing of beer.

We have shown that synthetic derivatives of humulone have activity against bacteira and we have shown that humulone contains moieties which make it very effective at chelating divalent metal cations.

5.1 Future Directions

i) This work has only brushed the surface of the new applications of these derivatives We have seen many reports in the literature, and from personal communications that the hop acids have potential uses in the fight against cancer [172]. This work should act as a firm grounding to begin screening of these hop acid derivatives against the biological targets.

ii) From our investigations we have demonstrated that derivatisation of the hop acids has a detrimental effect on their ability to chelate metal ions. Any future synthetic work should involve the synthesis of hop acid derivatives from simple materials whereby the acyl (R_1) and alkyl (R_2) side chains are manipulated. This should allow for fine tuning the LogP to aquire a range of more efficacious compounds.

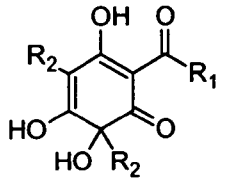


Fig 165 Novel side chain analogues of the α-acids

iii) The use of hop acids, in particular humulone (8), against cancer cells is going to be hampered by the questions raised by the absolute configuration of humulone (8). This is still an area which needs unambiguous proof to contradict or confirm the previously collected data. This has been started in the use of 4-hydroxy protection of humulone (8) works which were not carried out during this research due to time constraints were to form a silyl ether from compound (125) and selectively cleave the acetyl group to form a silylated analogue of lupulone. This would hopefully fulfil the criteria for a crystalline product and the heavy atom requirements for the Flack Parameter in X-ray determination of absolute configuration.

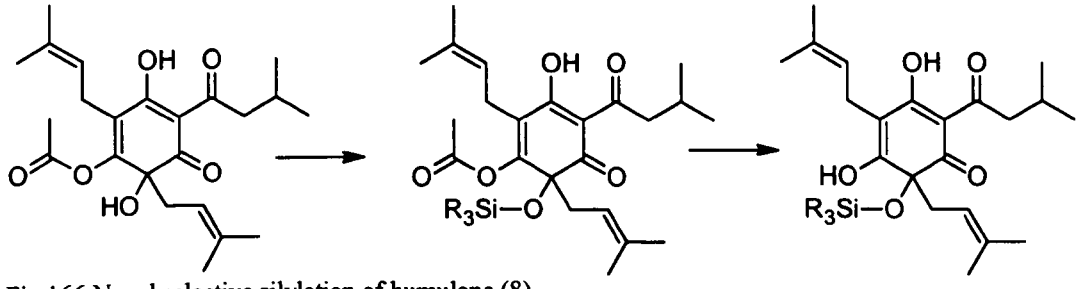


Fig 166 Novel selective silylation of humulone (8)

Chapter 6

Experimental

6 Experimental

6.1 General Procedures.

Starting materials were used as obtained from Sigma-Aldrich unless otherwise stated. Organic extractions were performed using unpurified solvents and the organic extracts were dried over anhydrous sodium sulphate and concentrated using a Bucchi rotary evaporator. TLC was conducted on silica gel with an aluminium backing with a fluorescent indicator UV_{254}. TLC plates were visualised using shortwave UV radiation, heating after immersion in potassium permanganate solution or heating after immersion in a solution of iron (III) chloride. Flash chromatography was conducted using the methods outlined by Leonard [173], using unpurified solvents.

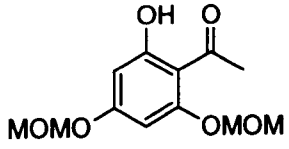
6.2 Instrumentation

HRMS were carried out at EPSRC unit in Swansea using a Finnigan MAT900XL-QTrap VG Autospec. CHN Microanalysis were performed and reported by the London School of Pharmacy using a Carlo-Erba EA 1108. HPLC analyses were carried out using a Shimadzu LC-20A equipped with a Phenomenex C18 Lunar 150 x 4.6 mm column, using a 90% methanol 10% buffer solvent system (buffer 0.05% phosphoric acid, 0.001% EDTA) at 1.5 ml/min and detection at 320 nm. Infrared spectra were collected using a Perkin Elmer Spectrum one Fourier Transform spectrometer fitted with an ATR-3 top plate. All materials were run without dilution. 300MHz NMR spectra was obtained using a Bruker AC-300 Fourier Transform Nuclear Magnetic Resonance spectrometer. 400 MHz NMR spectra were collected using a ^1H NMR data, using a JEOL Eclipse$^+$ 400 spectrometer and the 2D INADEQUATE data collected for lupulone (22) and humulone (8) were collected using a Bruker DRX 500 MHz Spectrometer. Single Crystal X-ray data was collected on a Bruker APEX 2000 diffractometer. Melting points are uncorrected and obtained using a Buchi 512 capillary tube apparatus. Bulb to bulb

distillations were carried out using a Buchi GKR-51 Kugelrohr apparatus. Optical activities were measured using an AA-10 polarimeter. AAS data were collected using a Varian AA-1275 Atomic Absorption Spectrometer, using an air-acetylene flame at 403.1 nm.

6.3 Synthesis

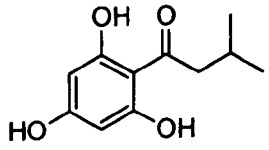
Synthesis of 1-[2-hydroxy-4,6-bis(methoxymethoxy)phenyl]-3-methylbutan-1-one1-{2,4-bis(methoxymethoxy)-6-[(3-methylbut-2-en-1-yl)oxy]phenyl}ethan-1-one (93)



(93)

Compound (93) was synthesized according to the literature procedure [165] all spectral data found were in agreement with literature values.

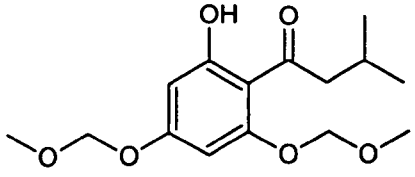
3-methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one (18)



(18)

Compound (18) was synthesised according to the literature procedure [17] , all spectral data were in agreement with the literature values.

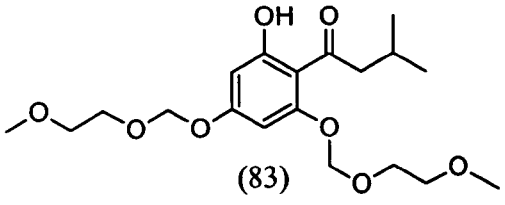
Synthesis of 1-[2-hydroxy-4,6-bis(methoxymethoxy)phenyl]-3-methylbutan-1-one1-{2,4-bis(methoxymethoxy)-6-[(3-methylbut-2-en-1-yl)oxy]phenyl}-3-methylbutan-1-one (82)



(82)

To a flask containing anhydrous THF (20 ml) was added via syringe DIPEA (13.2 ml, 95 mmol) at 0°C to this was added MOMCl (2.3 ml, 26.0mmol) slowly via syringe under nitrogen. This solution as stirred at 0°C under nitrogen for 15 minutes. After this time anhydrous phlorisovalerophenone **(18)** (1.75 g, 8.3 mmol) in THF (10 ml) was added and the solution was stirred at 0°C for 30 minutes. After this time the ice bath was removed and stirring was continued at room temperature over night. The reaction was then quenched by the addition of water (20 ml) and ethyl acetate (50 ml) was added. The solution was acidified using 5% HCl (50 ml) and the organic phase removed. The aqueous phase was extracted with ethyl acetate (50 ml) and the combined organic fractions were washed with 5% HCl (2 x 25 ml) and water (2 x 25 ml), dried (Na_2SO_4) and concentrated to give a yellow oil. Chromatography on silica with 15% ethyl acetate in hexane afforded **(82)** as white crystals (2.23g, 7.5 mmol) 90%. Mp 44 – 47 °C υ_{max} **(ATR)/cm^{-1}** 2960.52, 618.64, 1582.38, 1434.54, 1423.86, 1337.41, 1265.21, 1151.09, 1053.10 δ_H **(400MHz $CDCl_3$)** 0.97 (6H d, J 6.72 Hz) 2.22 (1H, m J 6.72) 2.89 (2H d, J 6.72 Hz) 3.47 (3 H, s) 3.52 (3H s) 5.16 (2 H s) 5.24 (2 H s) 6.25 (1H d, J 2.42 Hz) 6.27(1H, d J 2.42) 13.81 (1H s) δ_C **(100 MHz, $CDCl_3$)** 22.9 (q) 25.18 (q) 25.44 (d) 53.32 (t) 56.54 (q) 56.83 (q) 94.08 (t) 94.17 (d) 94.73 (t) 97.39 (d) 106.99 (s) 160.21 (s) 163.22 (s) 167.02 (s) 205.80 (s) MS calcd for $C_{15}H_{22}O_6$ 298.1411 $(M)^+$ found 298.1414

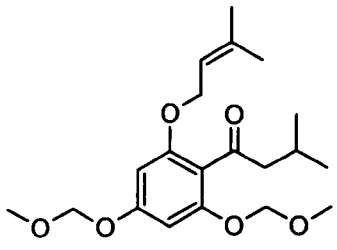
Synthesis of 1-{2-hydroxy-4,6- bis[(2-methoxyethoxy)methoxy] phenyl}-3-methylbutan-1-one (83)



(83)

To a dry flask containing THF (20ml) wass added DIPEA (13.2 ml, 95 mmol) at 0°C under N_2. The addition was followed by MEMCl (3.6 ml, 26 mmol) after stirring for 15 minutes at 0°C phlorisovalerophenone **(18)** (1.75 g, 8.3 mmol) in dry THF (10ml) was added *via* syringe. This solution was stirred for a further 15 minutes in ice. After this time the solution was warmed to room temperature and stirring continued for 18 hours. The reaction was quenched with water (10ml) and ethyl acetate (50ml) was added. The solution was acidified with 5% HCl (50 ml) and the organic phase removed. The aqueous phase was further extracted with ethyl acetate (50 ml) and the combined organic fractions were washed with 5% HCl (50 ml) and brine (2 x 25 ml), dried and concentrated to give a yellow oil. Chromatography over silica gel using hexane ethyl acetate (1 : 1) gave **(83)** as a pale yellow oil (2.64 g 82 %) υ_{max} **(ATR)/cm^{-1}** 2938.37, 1617.97, 1579.59, 1419.81, 1277.98, 1204.53, 1157.46, 1078.82, 1054.86, 1011.19 δ_H **(400MHz $CDCl_3$)** 0.93 (6H, d, J 6.72 Hz) 2.18 (1H, sept, J 6.72) 2.85 (2H, d, J 6.85) 3.34 (3H s) 3.36 (3H s) 3.52 (2H m) 3.56 (2H m) 3.77 (2H m) 3.81 (2H m) 5.23 (2H s) 5.31 (2H s) 6.25 (2H s) 13.77 (1H s) δ_C **(100 MHz, $CDCl_3$)** 22.85 (q) 25.31 (d) 53.26 (t) 59.10 (q) 59.12 (q) 68.22 (t) 68.53 (t) 71.54 (t) 93.04 (t) 93.75 (t) 94.29 (q) 97.40 (q) 106.95 (s) 160.14 (s) 163.18 (s) 166.96 (s) 205.69 (s) MS calcd. For $C_{19}H_{30}O_8$ 387.2013 $(M+H)^+$ found 387.2015

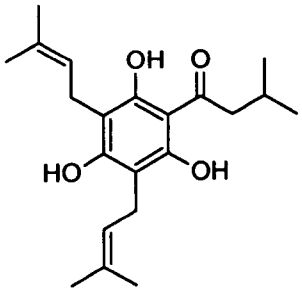
Synthesis of 1-{2,4-bis(methoxymethoxy)-6-[(3-methylbut-2-en-1-yl)oxy]phenyl}-3-methylbutan-1-one (85)



(85)

To a flask under nitrogen containing 1-[2-hydroxy-4,6-bis(methoxymethoxy)phenyl]-3-methylbutan-1-one **(82)** (100 mg, 0.33 mmol) and acetone (5ml) was added finely ground potassium carbonate (400 mg, 0.4 mol) and 3,3-dimethylallyl bromide (100 µl, 0.7 mmol) the mixture was brought to reflux for 15 minutes after this time further dimethylallyl bromide (50 µl) was added and refluxing continued for 5 minutes. The absence of starting material by TLC showed the reaction was complete. Filtration of the product followed by concentration gave (85) as a yellow oil (100 mg, 0.3 mmol 90%). υ_{max} **(ATR)/cm^{-1}** 2956.67, 1702.84, 1585.96, 1428.82, 1384.20, 1335.95, 1276.60, 1209.12, 1064.43 δ_H **(400MHz $CDCl_3$)** 0.94 (6H d, J 6.72 Hz) 1.72 (3H,s) 1.75 (3H, s) 2.22 (1H, m J 6.72 Hz) 2.63 (2H d, J 6.85 Hz) 3.44 (3H, s) 3.47 (3H, s) 4.47 (2H, d, J 6.58 Hz) 5.11 (2H, s) 5.14 (2H, s) 5.38 (1H, m, J 6.72 Hz) 6.29 (1H, d, J 2.42 Hz) 6.42 (1H, d J 2.42) δ_C **(100 MHz, $CDCl_3$)** 18.30 (q) 22.75 (q) 24.57 (q) 25.78 (d) 54.11 (t) 56.24 (q) 56.37 (q) 65.58 (d) 94.58 (t) 94.71 (t) 95.04 (d) 95.88 (d) 116.39 (s) 119.44 (t) 137.99 (s) 155.23 (s) 157.22 (s) 159.43 (s) 204.34 (s) MS calcd for $C_{20}H_{30}O_6$ 298.1411 $(M)^+$ found 298.1414

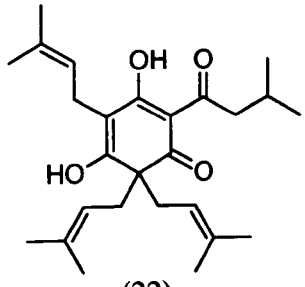
Synthesis of 3-Methyl-1-[2,4,6-trihydroxy-3,5-bis-(3-methyl-but-2-enyl)-phenyl]-butan-1-one (19)



(19)

Compound **19** was synthesized according to the literature procedure [135] all spectral data were in agreement with the literature values.

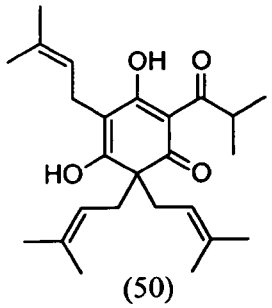
Synthesis of 3,5-Dihydroxy-4,6,6-tris-(3-methyl-but-2-enyl)-2-(3-methyl-butyryl)-cyclohexa-2,4-dienone (22)



(22)

Lupulone (22) were synthesized according the literature procedure [78] all spectral data was in agreement with the literature values.

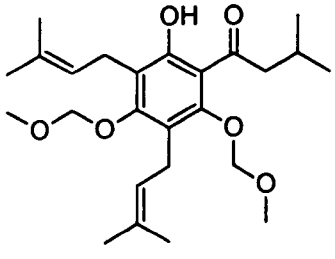
Preapration of 3,5-Dihydroxy-2-isobutyryl-4,6,6-tris-(3-methyl-but-2-enyl)-cyclohexa-2,4-dienone (50)



(50)

Colupulone (50) was isolated from a liquid CO_2 extract of East Kent Golding Hops by repeated crystallisation from heptane, the method was adapted from the literature method [1] all spectral data were in agreement with literature values.

1-[2-hydroxy-4,6-bis(methoxymethoxy)-3,5-bis(3-methylbut-2-en-1-yl)phenyl]-3-methylbutan-1-one (84)

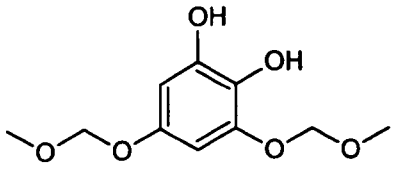


(84)

To a dry flask under N_2 was added dry THF (4 ml) via a syringe and DIPEA (0.5 ml, 3.6 mmol) this solution was cooled in an ice bath with vigorous stirring. To this was added chloromethyl methylether (100 µl, 0.7 mmol) slowly via syringe. After 15 minutes deoxyhumulone **(19)** (100 mg, 0.3 mmol) after 15 minutes stirring in an ice bath the solution was allowed to come to room temperature and stirred for 18 hours. After this time water (2 ml) and hexane (10 ml) were added to this mixture and the phases separated, the organic phase was washed with 5% HCl (2 x 10 ml) and brine (2 x 10 ml), dried (Na_2SO_4) and concentrated to give **(84)** as a yellow oil. (98 mg 78%) υ_{max} **(ATR)/cm^{-1}** 2961.65, 1585.96, 1428.82, 1209.12, 1106.87, 1064.43 δ_H **(400MHz $CDCl_3$)** 0.92 (6H, d, J 6.72 Hz) 1.69 (3H, d, J 1.07 Hz) 1.70 (3H, d, J 1.21 Hz) 1.74 (3H, d, J 0.81 Hz) 1.76 (3H, d, J 0.94 Hz) 2.22 (1H, m J

6.72) 3.00 (2H d, J 6.85 Hz) 3.34 (4H, tt, J 1.21 Hz 6.58 Hz,) 3.47 (3H s) 3.57 (3H, s) 4.87 (2H, s) 5.22 (1H tq J 1.48 Hz 6.18, Hz) 12.37 (1H, s) **δ_C (100 MHz, $CDCl_3$)** 18.11 (q) 18.19 (q) 22.84 (q) 23.66 (t) 24.04 (t) 25.6 (q) 25.76 (d) 25.80 (q) 25.84 (q) 52.23 (t) 57.74 (q) 58.37 (q) 101.18 (d) 101.55 (d) 114.13 (s) 120.19 (s) 120.69 (s) 122.71 (d) 123.87 (s) 131.61 (s) 132.10 (s) 155.08 (s) 160.00 (s) 160.48 (s) 207.85 (s) MS calcd for $C_{25}H_{38}O_6$ 434.2663 $(M)^+$ found 434.2663

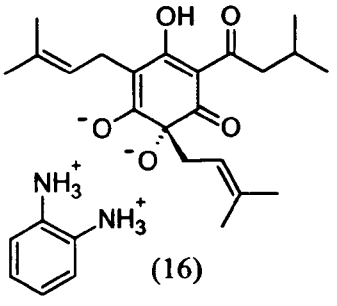
Synthesis of 3,5-bis(methoxymethoxy)benzene-1,2-diol (93)



(93)

To a flask containing 1-[2-hydroxy-4,6-bis(methoxymethoxy)phenyl]ethanone **(18)** (1 g, 3.9 mmol) and 0.5 M NaOH (25 ml) once all of the material had dissolved 30% H_2O_2 (0.5 ml, 4.4 mmol) this solution was stirred for 10 minutes, after this time 30% H_2O_2 (0.5 ml, 4.4 mmol) was added and the solution stirred for a further 20 minutes. After this time the reaction was quenched with 5% HCl (60 ml). The mixture was extracted with ether (4 x 50 ml). The combined organic fractions were washed with brine (2 x 20ml) dried and concentrated to give **(93)** as a dark viscous oil which solidified upon standing. (613 mg, 2.7 mmol) 68% **υ_{max} (ATR)/cm^{-1}** 3320, 2909.38, 1612.09, 1510.66, 1141.63, 1098.99, 1017.52 **δ_H (400MHz $CDCl_3$)** 3.46 (3 H s) 3.54 (3 H s) 5.07 (2 H s) 5.14 (2 H s) 5.60 (1 H s) 5.98 (1H, s) 6.40 (1H, d, J 2.69 Hz) 6.43 (1H, d, J 2.69 Hz) **δ_C (100 MHz, $CDCl_3$)** 56.08 (q) 56.69 (q) 95.33 (t) 96.61 (t) 98.06 (d) 99.26 (d) 129.05 (s) 145.17 (s) 145.24 (s) 150.96 (s) MS calcd for $C_{10}H_{14}O_6$ 230.0785 $(M)^+$ found 230.0788

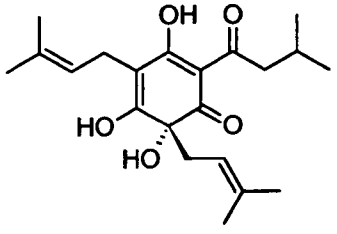
Isolation of Benzene-1,2-diaminium (1*R*)-4-hydroxy-1,3-bis(3-methylbut-2-enyl)-6-oxo-5-(3-methylbutanoyl)cyclohexa-2,4-diene-1,2-diolate (16)



(16)

To a 5 l flask was added a carbon dioxide extract of hops (1kg) and hexane (1.5lL) this was warmed to 50 °C. Once most of the solids had dissolved the water was removed and the extract was dried over sodium sulphate and filtered. To this solution was rapidly added a solution of 1,2-phenylenediamine (115 g) in ethyl acetate (200ml) at 45°C and the mixture was allowed to cool to room temperature. After about 2 minutes of standing the solution began to precipitate and on cooling the material was a thick sludge. Filtration of this material and washing with hexane gave crude yellow brown crystals (340g) 87.9 % yield. Recrystallisation of this complex 10 times from toluene (5ml per g) gave pure humulone complex (16) (67 g) as yellow needles. Required C(68.91%) H(8.14%) N(5.95%) found C(68.96%) H(8.11%) N(5.97%) υ_{max} **(nujol mull)/cm^{-1}** 3374.7, 2610.6, 1628.5, 1536.1, 1503.9, 1465.3, 1364.7 δ_H **(300MHz $CDCl_3$)** 0.89 (3H d, J 6 Hz) 0.91(3H d, J 6 Hz) 1.52 (3H, s) 1.69 (6H, s) 1.73 (3H, s) 2.14 (1H, m) 2.47 (2H, m) 2.77 (2H dd, J 2 Hz, J 7 Hz) 3.1 (2H, m) 3.9 (5H, m) 4.99 (1H t, J8 Hz) 5.12 (1H t, J 8 Hz) 6.72 (4H, s) δ_C **(75.5 MHz, $CDCl_3$)** 17.7 (q) 17.9 (q) 21.1 (t) 22.6 (q) 22.8 (q) 25.8 (q) 26.0 (q) 26.4 (d) 42.7 (t) 46.3 (t) 78.8 (s) 96.7 (d) 106.5 (s) 109.4 (s) 115.8 (d) 116.8 (d) 120.3 (s) 121.0 (d) 132.6 (s) 138.0 (s) 167.9 (s) 190.9 (s) 195.1 (s) 200.2 (s)

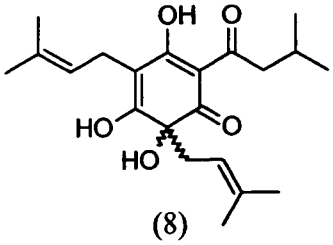
Synthesis of (6*R*)-3,5,6-trihydroxy-4,6-bis(3-methybut-2-enyl)-2-(3-methylbutanoyl)cyclohexa2,4-dien-1-one (8)



(8)

Humulone complex **(16)** (15 g, 31.9 mmol) was added to glacial acetic acid (45ml) and stirred until all of the solids had dissolved. After this time water (200ml) was added and the stirring continued for 2 minutes. The following solid / liquid mixture was extracted with pentane (3 x 20 ml) the combined organic fractions were dried over sodium sulphate and concentrated to give a semisolid resin which solidified on drying in air. Re-crystallisation from acetonitrile gave humulone (1) (10 g, 27.6 mmol 86%) as pale yellow amorphous crystals MP 79.1 – 81.3° C $[\alpha]_D^{20°C}$ -245.16 ° (c 0.5 in $CHCl_3$). υ_{max} **(nujol mull)/cm^{-1}** 3366.1, 1667.2, 1625.3, 1523.1, 1464.4, 1375.8, 1296.6, 1238.2 δ_H **(300MHz $CDCl_3$)** 0.89 (3H, d, J 7 Hz) 0.93 (3H, d, J 7 Hz) 1.46 (3H, s) 1.62 (6H, s) 1.66 (3H, s) 2.1(1H, m) 2.43 (2H, m) 2.7 (2H, dd, J 2 Hz, J 7 Hz) 3.0 (2H, m) 4.31 (1H, s) 4.91 (1H, t, J 8 Hz) 5.06 (1H, t, J 8 Hz) 7.81 (1H, s) 13.13 (1H, s) δ_C **(75.5 MHz, $CDCl_3$)** 17.7 (q) 17.9 (q) 21.1 (t) 22.5 (q) 22.7 (q) 25.6 (q) 25.9 (q) 26.4 (d) 42.7 (t) 46.3 (t) 78.8 (s) 106.5 (s) 109.4 (s) 115.8 (d) 121.0 (d) 132.6 (s) 138.0 (s) 167.6 (s) 190.9 (s) 195.1 (s) 200.2 (s)

Synthesis of (+/-)-3,5,6-trihydroxy-4,6-bis(3-methybut-2-enyl)-2-(3-methylbutanoyl)cyclohexa-2,4-dien-1-one (8)

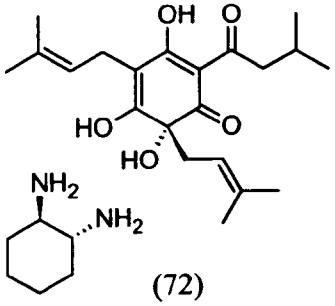


(8)

Hop Resin (500g) was dissolved in toluene (2.5 L) and brought to reflux for 18 hours. After this time the acidic fraction was extracted by continuous flow liquid liquid separation against 1M NaOH (2L). Once extraction was complete the aqueous phase was made acidic by the addition of 50% sulphuric acid (200 ml) slowly under external cooling (10 °C). once addition was complete heptane (1L was added and the mixture stirred vigorously with an overhead stirrer. The temperature was slowly increased to 50 °C the pH checked. The pH was adjusted to <1 with 50% sulphuric acid and the aqueous phase was removed. The organic phase was washed with water (2 x 250 ml) and saturated sodium sulphate solution (200 ml), dried and concentrated to give an orange viscous oil (200 – 300 g depending on the variety).

The above oil (200g) was dissolved in toluene (200 ml) and added to a solution of 1,2-phenylenediamine (50g) in toluene (100ml) at 50 °C) the solution darkened immediately and cooled to 10 °C. The resultant slurry was filtered and the solids recrystallised from toluene 10 times, the purity was monitored by ^{1}H NMR. After there was no appreciable cohumulone present the complex (20 g) was dissolved in acetic acid (80 ml) and water (200ml) was added. The aqueous phase was extracted with pentane (2 x 30 ml). the combined organic extracts were washed with water (4 x 10ml), dried and concentrated to give a yellow oil (15.1 g) all spectral data were in agreement with previously collected data for humulone. $[\alpha]_D^{20°C}$ 0.00° (c 0.5 in $CHCl_3$). All other spectral data were in agreement with previous data.

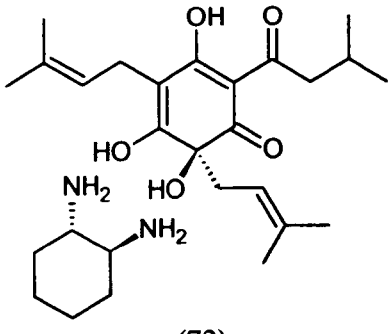
Synthesis of (6*R*)-3,5,6-trihydroxy-4,6-bis(3-methybut-2-enyl)-2-(3-methylbutanoyl)cyclohexa2,4-dien-1-one complex with (1R, 2R)-1,2-diamino cycohexane. (72)



(72)

To a solution of (-)-humulone (0.5 g, 1.4 mmol) in hexane (3 ml) was added (1R,2R) diaminocyclohexane (0.16 g, 1.4 mmol) in hexane (3 ml) the precipitate was collected by filtration and recrystallised from acetonitrile to give fine yellow needles. Mp 161.7 – 162.9 °C. (0.63g, 1.3 mmol 94%) $C_{27}H_{44}N_2O_5$ required C (68.04%) H (9.30%) N (5.88%) found C (68.10%) H (9.23%) N (5.93%) υ_{max} **(ATR)/cm^{-1}** 2956.08, 1636.73, 1581.76 1570.21, 1383.78, 1292.73, 1249.16, 1216.24, 1143.98, 1058.77 δ_H **(400MHz CD_3OD)** 0.92 (3H, d, J 6.72Hz) 0.94 (3H d, J 6.72 Hz) 1.31 (4H broad m) 1.49 (3H, s) 1.62 (6H, s) 1.70 (3H, s) 1.76 (2H, broad m)1.99 (2H, broad m) 2.07 (1H, sept J 6.72 Hz) 2.40 (2H, d J 7.66 Hz) 2.60 (1H, dd, J 6.58 Hz 12.89 Hz) 2.62 (2H, broad m) 2.75 (1H, dd, J 7.52 Hz, 12.89 Hz) 2.90 (1H, dd, J 6.98 Hz 14.37 Hz) 3.00 (1H, dd, J 6.98 Hz, 14.37 Hz) 4.92 (7H, s) 5.10 (2H, m) δ_C **(100MHz, CD_3OD)** 16.69 (q) 16.77 (q) 20.88 (t) 21.19 (q) 22.06 (q) 24.35 (t) 24.74 (q) 24.90 (q) 26.27 (d) 32.45 (t) 43.02 (t) 48.45 (t) 54.85 (d) 83.46 (q) 102.88 (q) 103.04 (q) 118.12 (d) 124.46 (d) 128.86 (q) 133.94 (q) 181.24 (q) 191.73 (q) 197.52 (q) 198.19 (q)

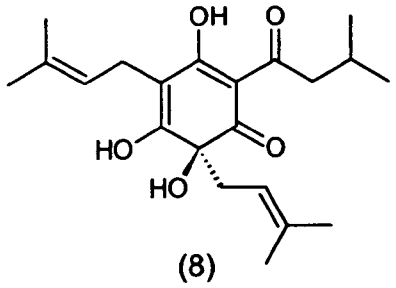
Synthesis of (6S)-3,5,6-trihydroxy-4,6-bis(3-methybut-2-enyl)-2-(3-methylbutanoyl)cyclohexa2,4-dien-1-one complex with (1S, 2S)-1,2-diamino cyclohexane. (72)



(72)

To a flask containing racemic (±) - humulone **(8)** (3.175g, 8.8 mmol) in acetonitrile was added a solution of (1S, 2S)-diaminocyclohexane (1.0 g, 8.8 mmol) in acetonitrile in two portions, after the first addition, the solution turned dark and lightened slowly over 10 minutes, the second portion was added slowly, after complete addition crystals were formed immediately. The crystals were collected by filtration, the mother liquor was concentrated to ½ volume and a second crop of crystals was collected. Recrystallisation from acetonitrile yielded the desired complex **(72)** (1.44g, 3 mmol 69%) M.P. 161.5 – 163.0 °C. $C_{27}H_{44}N_2O_5$ required C (68.04%) H (9.30%) N (5.88%) found C (68.34%) H (9.27%) N (5.88%) υ_{max} **(ATR)/cm^{-1}** 2931.15, 1637.33, 1581.22, 1570.00, 1466.53, 1382.91, 1292.69, 1249.05, 1216.23, 1143.56 δ_H **(400MHz CD_3OD)** 0.92 (3H, d, J 6.72Hz) 0.94(3H, d, J 6.72Hz) 1.31 (4H, broad m) 1.49 (3H, s) 1.62 (6H, s) 1.70 (3H, s) 1.76 (2H, broad m)1.99 (2H, broad m) 2.07 (1H, sept J 6.72 Hz) 2.40 (2H, d J 7.66 Hz) 2.60 (1H, dd, J 6.58 Hz, 12.89 Hz) 2.62 (2H, broad m) 2.75 (1H, dd, J = 7.52 Hz, 12.89 Hz) 2.90 (1H, dd, J 6.98 Hz, 14.37 Hz) 3.00 (1H, dd, J 6.98 Hz, 14.37 Hz) 4.92 (7H, s) 5.10 (2H, m) δ_C **(100MHz, CD_3OD)** 16.69 (q) 16.77 (q) 20.88 (t) 21.19 (q) 22.06 (q) 24.35 (t) 24.74 (q) 24.90 (q) 26.27 (d) 32.45 (t) 43.02 (t) 48.45 (t) 54.85 (d) 83.46 (q) 102.88 (q) 103.04 (q) 118.12 (d) 124.46 (d) 128.86 (q) 133.94 (q) 181.24 (q) 191.73 (q) 197.52 (q) 198.19 (q)

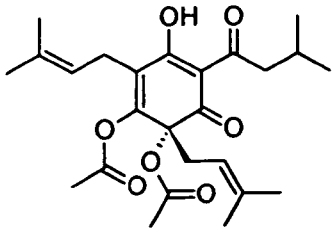
Synthesis of (S)-3,5,6-Trihydroxy-4,6-bis-(3-methyl-but-2-enyl)-2-(3-methyl-butyryl)-cyclohexa-2,4-dienone (8)



(8)

To a flask containing 1,2-diaminocyclohexane complex **(72)** (1.4 g, 2.9 mmol) was added glacial acetic acid (5 ml) under nitrogen. The mixture was stirred until all of the solids had dissolved. Stirring was continued for a further 10 minutes. After this time pentane (10 ml) was added and water (20 ml) was added slowly with stirring until phase separation was observed. The organic layer was removed and the aqueous phase re-extracted with pentane (2 x 10ml) the combined organic fractions were washed with water (3 x 20 ml), dried over sodium sulphate and concentrated to give (+) – humulone (8) as a pale yellow solid MP 79.1 – 81.3° C (0.94 g, 2.6 mmol 89%) $[\alpha]_D^{20°C}$ +244.98 ° (c=0.5 in $CHCl_3$) υ_{max} **(ATR)/cm^{-1}** 3356.10, 2958.79, 1666.26, 1622.74, 1517.80, 1464.38, 1342.95, 1295.38, 1250.1, 1100.97, δ_H **(400MHz $CDCl_3$)** 0.97 (3H, d, J 6.72 Hz) 1.00 (3H, d, J 6.72 Hz) 1.52 (3H, d J 0.94 Hz) 1.69 (6H, s) 1.73 (3H, d J 0.81 Hz) 2.14 (1H, sept J 6.72 Hz) 2.42 (1H, dd J 7.80 Hz, 13.96 Hz) 2.54 (1H, dd, J 7.92 Hz, 13.96 Hz) 2.77 (2H, m) 3.03 (1H, dd, 7.12 Hz, 14.51 Hz) 3.11 (1H, dd, 7.12 Hz, 14.51 Hz) 4.17 (1H, broad s) 5.00 (1H, t m 7.92 Hz 1.48 Hz) 5.13 (1H, t m 7.25 Hz, 1.48 Hz) 6.94 (1H, broad s) 18.89 (1H, sharp s). δ_C **(100MHz, $CDCl_3$)** 17.90 (q) 18.03 (q) 21.22 (t) 22.68 (q) 22.94 (q) 25.89 (q) 26.15 (q) 26.58 (d) 45.16 (t) 46.32 (t) 78.83 (s) 106.15 (s) 109.43 (s) 115.86 (d) 121.11 (d) 132.98 (s) 138.37 (s) 167.68 (s) 191.04 (s) 195.17 (s) 200.01 (s)

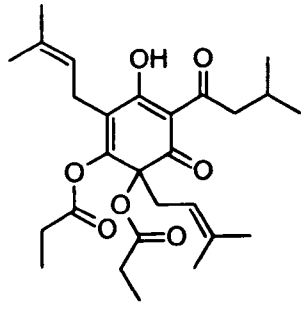
Synthesis of (1*R*)-2-(acetyloxy)-4-hydroxy-1,3-bis(3-methyl-but-2-enyl)-6-oxo-5-(3-methylbutyroyl)cyclohexa-2,4-dien-1-yl acetate (77)



(77)

To a flask containing acetic anhydride (2.5 g, 24.5 mmol) was added humulone **(8)** (1g, 2.8 mmol) once all of the solids had dissolved, pyridine (4 drops) was added. This mixture was stirred at room temperature for 1 hour. After this time water (10 ml) was added and sodium carbonate (1g) was added cautiously. This was stirred for 1 hour. After this time hexane (2 x 10ml) was added and the aqueous layer discarded. The combined organic layers were washed with water (3 x 10 ml) and dried over sodium sulphate. Concentration gave a brown oil which gave a red colour on treatment with methanolic iron (III) chloride solution. Chromatography on silica gel with pentane / ether)80 : 20 gave **(77)** as a pale yellow oil (950 mg, 2.1 mmol 77%) $[\alpha]_D^{20°C}$ -37.77 ° (c=0.5 in $CHCl_3$) υ_{max} **(ATR)/cm^{-1}** 3356.10, 2958.79, 1666.26, 1622.74, 1517.80, 1464.38, 1342.95, 1295.38, 1250.1, 1100.97, δ_H **(400MHz $CDCl_3$)** 0.93 (3H d, J 6.72 Hz) 0.94 (3H d J 6.72 Hz) 1.48 (0.5 H s) 1.51 (2.5 H s) 1.54 (0.5 H s) 1.57 (2.5 H s) 1.64 (1H s) 1.65 (5 H s) 1.99 (3 H s) 2.08 (1 H sept, J 6.72 Hz) 2.23 (3H s) 2.60 (1 H, dd J 7.52 Hz, J 13.30 Hz) 2.69 (1 H dd J 8.19 Hz, 13.30 Hz) 2.81 (1 H dd J 6.58 Hz, 13.83 Hz) 2.91 (1 H dd J 7.25 Hz, 13.83 Hz) 2.92 (1 H dd J 7.12 Hz, 15.04 Hz) 3.05 (1 H dd J 6.58 Hz, 14.9 1Hz) 4.81 (1 H tm J 8.06 Hz, 1.48 Hz) 4.97 (1 H tm J 6.72 Hz, 1.48 Hz) 18.82 (0.17 H s) 18.86 (0.83 H s) . δ_C **(100MHz, $CDCl_3$)** 17.88 (q) 17.90 (q) 20.48 (q) 20.52 (q) 22.74 (q) 22.78 (q) 23.34 (t) 25.77 (q) 25.97 (q) 26.01 (d) 36.56 (t) 47.49 (t) 79.94 (s) 110.01 (s) 114.36 (d) 119.65 (d) 127.96 (s) 133.43 (s) 137.81 (s) 155.46 (s) 166.88 (s) 69.02 (s) 189.05 (s) 190.20 (s) 202.71 (s) MS calcd for $C_{25}H_{34}O_7$ 447.2377 $[M+H]^+$ found 447.2375

Propionic acid 4-hydroxy-1,3-bis-(3-methyl-but-2-enyl)-5-(3-methyl-butyryl)-6-oxo-2-propionyloxy-cyclohexa-2,4-dienyl ester (106)



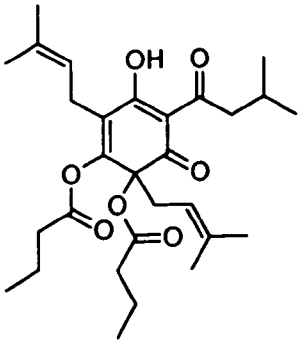
(106)

To a flask containing propionic anhydride (2.5 g, 19.2 mmol) was added humulone (1) (1g, 2.8 mmol) once all of the solids had dissolved pyridine (4 drops) was added. This mixture was stirred at room temperature for 1 hour. After this time water (10 ml) was added and sodium carbonate (1g) was added cautiously. This was stirred for 1 hour. After this time hexane (2 x 10ml) was added and the aqueous layer discarded. The combined organic layers were washed with 10% sodium hydrogen carbonate (3 x 10 ml) and water (3 x 10 ml) dried over sodium sulphate. Chromatography on silica gel with pentane ether (80 ; 20) gave **(106)** as a pale yellow oil (1.10 g, 2.3 mmol 83 %) $[\alpha]_D^{20°C}$ -40.38 ° (c=0.5 in $CHCl_3$) υ_{max} **(ATR)/cm^{-1}** 2963.12, 1774.27, 1753.40, 1680.60, 1531.37, 1451.15, 1165.52, 1077.54 δ_H **(400MHz $CDCl_3$)** 0.94 (3H d, J 6.58 Hz) 0.9 (3H d J 6.72 Hz) 1.08 (3 H t J 7.66 Hz) 1.22 (3H, t J 7.52 Hz) 1.49 (0.5H, s) 1.52 (2.5H, s) 1.55 (0.5H, s) 1.57 (2.5H, s) 1.64 (3H, s) 1.65 (3H, s) 2.09 (1H, sept, J 6.72 Hz) 2.23 (1H, dq J 16.79 Hz, 7.52 Hz) 2.33 (1H, dd J 16.92 Hz, 7.52 Hz,) 2.52 (2H, qd, 7.66 Hz, 1.21) 2.65 (2H, m) 2.76 – 3.08 (4H, m) 4.82 (1H, tm J 7.92 Hz, 1.21 Hz) 4.98 (1H, tm 6.85 Hz, 1.34 Hz) 18.14 (0.17H, s) 18.89 (0.83H, s) . δ_C **(100MHz, $CDCl_3$)** 8.73 (q) 9.18 (q) 17.91 (q) 17.94 (q) 22.77 (q) 22.81 (q) 23.34 (t) 25.78 (q) 25.97 (q) 26.0 (d) 27.06 (t) 27.49 (t) 36.63 (t) 47.54 (t) 79.87 (s) 110.04 (s) 114.46 (d) 119.75 (d) 127.88 (s) 133.40 (s) 137.75 (s) 155.72 (s) 170.48 (s) 172.44 (s) 189.07 (s) 190.37 (s) 202.78 (s) MS calcd for $C_{27}H_{38}O_7$ 475.2690 $[M+H]^+$ found 475.2691

General procedure for the synthesis of the remaining Acyl analogues:

To a flask containing the appropriate anhydride (5.0 g) was added humulone **(8)** (1 g, 2.8 mmol) and pyridine (2-3 drops) this mixture was stirred overnight. After this time the reaction was quenched by the addition of methanol (2 ml) and the solution was stirred for a further 24 hours to ensure complete reaction of the anhydride. This mixture was diluted with water (10 ml) and extracted with hexane (3 x 10 ml). The combined organic fractions were washed with 10% sodium hydrogen carbonate solution (2 x 10 ml) and 10% HCl (2 x 10 ml), dried over sodium sulphate and concentrated under vacuum. The crude product was purified by distillation of the contaminating methyl ester under high vacuum at 50 °C. Once the distillation had ceased the material was chromatographed over silica gel using ether : hexane as the eluent. Drying over night on a high vacuum line gave the desired product.

Butyric acid (R)-2-butyryloxy-4-hydroxy-1,3-bis-(3-methyl-but-2-enyl)-5-(3-methyl-butyryl)-6-oxo-cyclohexa-2,4-dienyl ester (107)

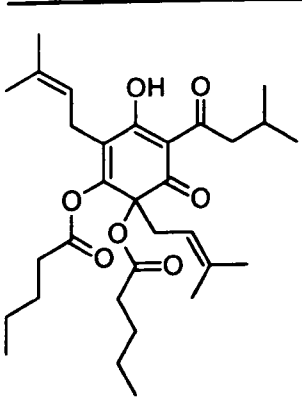


(107)

Chromatography on silica gel with pentane ether (80 ; 20) gave **(107)** as a yellow oil (1.14 g 81 %) $[\alpha]_D^{20°C}$ -33.33 ° (c=0.5 in $CHCl_3$) υ_{max} **(ATR)/cm^{-1}** 2964.55, 2875.06, 1772.25, 1752.84, 1680.09, 1532.50, 1450.54, 1162.67 δ_H **(400MHz $CDCl_3$)** 0.89 – 0.96 (9H m) 1.00 (3H t J 7.52 Hz) 1.47- 1.75 (16H, m) 2.07 (1H, sept, J 6.72 Hz) 2.16 – 2.31 (2H, m) 2.45 (2H, t J 7.52 Hz) 2.57 (1H, dd J 13.43 Hz 7.25 Hz,) 2.68 (1H, dd, J 13.16Hz, 8.19 Hz) 2.75 – 3.18 (4H, m) 4.82 (1H, t J 7.92

Hz) 4.98 (1H, t, J 6.98 Hz) 18.13 (0.17H, s) 18.88 (0.83H, s) . **δ_C (100MHz, $CDCl_3$)** 13.65 (q) 13.72 (q) 17.95 (q) 17.97 (q) 18.09 (t) 18.47 (t) 22.79 (q) 22.82 (q) 23.41 (t) 25.80 (q) 26.00 (q) 26.03 (d) 35.50 (t) 35.89 (t) 36.63 (t) 47.52 (t) 79.94 (s) 110.06 (s) 114.51 (d) 119.75 (d) 127.73 (s) 133.45 (s) 137.72 (s) 155.78 (s) 169.66 (s) 171.71 (s) 189.08 (s) 190.37 (s) 202.78 (s) MS calcd for $C_{29}H_{42}O_7$ 503.3003 $[M+H]^+$ found 503.3005

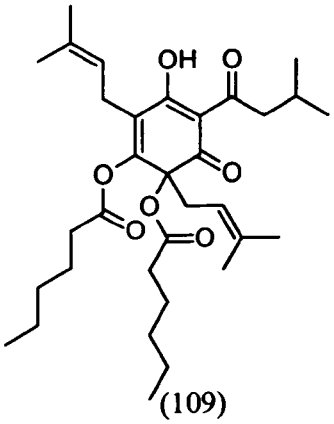
Pentanoic acid (R)-4-hydroxy-1,3-bis-(3-methyl-but-2-enyl)-5-(3-methyl-butyryl)-6-oxo-2-pentanoyloxy-cyclohexa-2,4-dienyl ester (108)



(108)

Chromatography on silica gel with pentane ether (80 : 20) gave **(108)** as a dark yellow oil (1.19 g 79 %) $[\alpha]_D^{20°C}$ -29.81 ° (c=0.5 in $CHCl_3$) υ_{max} **(ATR)/cm^{-1}** 2960.57, 2873.54, 1773.35, 1752.02, 1680.50, 1533.83, 1451.48, 1159.79, 1126.45 **δ_H (400MHz $CDCl_3$)** 0.88 (3H, t, J 7.39 Hz) 0.93 – 0.98 (9H, m) 1.28 – 1.46 (4H, m) 1.50- 1.71 (16H, m) 2.10 (1H, sept, J 6.85 Hz) 2.16 – 2.35 (2H, m) 2.45 (2H td J 7.25 Hz, 0.94 Hz) 2.64 (2H, m) 2.76 – 3.20 (4H, m) 4.84 (1H, tm J 7.92 Hz, 1.34 Hz) 4.98 (1H, tm J 6.85 Hz, 1.34 H) 18.13 (0.17H, s) 18.88 (0.83H, s) . **δ_C (100MHz, $CDCl_3$)** 13.71 (q) 13.77 (q) 17.91(q) 17.92 (q) 22.18 (t) 22.25 (t) 22.76 (q) 22.77 (q) 23.36 (t) 25.74 (q) 25.95 (q) 25.98 (d) 26.59 (t) 26.95 (t) 33.30 (t) 33.71 (t) 36.59 (t) 47.48 (t) 79.91 (s) 110.03 (s) 114.51 (d) 119.74 (d) 127.69 (s) 133.38 (s) 137.65 (s) 155.77 (s) 169.74 (s) 171.80 (s) 189.03 (s) 190.31 (s) 202.73 (s) MS calcd for $C_{31}H_{46}O_7$ 531.3316 $[M+H]^+$ found 531.3313

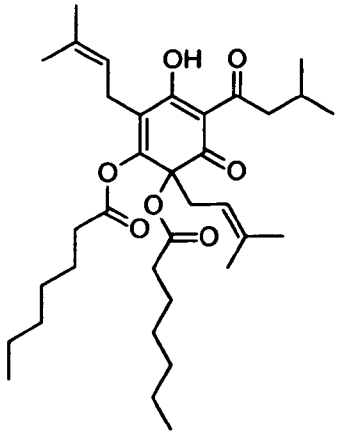
Hexanoic acid (R)-2-hexanoyloxy-4-hydroxy-1,3-bis-(3-methyl-but-2-enyl)-5-(3-methyl-butyryl)-6-oxo-cyclohexa-2,4-dienyl ester (109)



(109)

Chromatography on silica gel with pentane ether (80 : 20) gave **(109)** as an orange oil (1.21 g 77 %) $[\alpha]_D^{20°C}$ -28.11 ° (c=0.5 in $CHCl_3$) υ_{max} **(ATR)/cm^{-1}** 2959.34, 2872.78, 1773.05, 1752.44, 1681.15, 1535.41, 1451.81, 1157.41 δ_H **(400MHz $CDCl_3$)** 0.85 – 0.98 (12H, m) 1.25 – 1.40 (8H, m) 1.50- 1.73 (16H, m) 2.10 (1H, sept, J 6.72 Hz) 2.18 – 2.39 (2H, m) 2.45 (2H, td J 7.52 Hz, 0.94 Hz) 2.64 (2H, m) 2.76 – 3.20 (4H, m) 4.84 (1H, tm J 8.19, Hz, 1.34 Hz) 4.98 (1H, tm, J 6.72 Hz, 1.34 Hz) 18.15 (0.17H, s) 18.89 (0.83H, s) , δ_C **(100MHz, $CDCl_3$)** 13.96 (q) 14.00 (q) 17.96 (q) 17.98 (q) 22.40 (t) 22.43 (t) 22.82 (q) 23.41 (t) 24.24 (t) 24.65 (t) 25.80 (q) 26.01 (q) 26.04 (d) 26.59 (t) 26.95 (t) 31.26 (t) 31.27 3 (t) 33.60 (t) 34.02 (t) 36.59 (t) 47.54 (t) 79.94 (s) 110.06 (s) 114.53 (d) 119.79 (d) 127.75 (s) 133.43 (s) 137.71 (s) 155.82 (s) 169.82 (s) 171.87 (s) 189.09 (s) 190.36 (s) 202.78 (s) MS calcd for $C_{33}H_{50}O_7$ 559.3629 $[M+H]^+$ found 559.3632

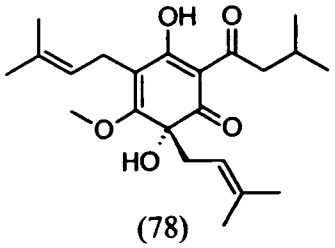
Heptanoic acid (R)-2-heptanoyloxy-4-hydroxy-1,3-bis-(3-methyl-but-2-enyl)-5-(3-methyl-butyryl)-6-oxo-cyclohexa-2,4-dienyl ester (110)



(110)

Chromatography on silica gel with pentane ether (80 : 20) gave **(110)** as an orange oil (1.24 g 75.5 %) $[\alpha]_D^{20°C}$ -32.06 ° (c=0.5 in $CHCl_3$) υ_{max} **(ATR)/cm^{-1}** 2958.92, 2860.14, 1773.29, 1752.35, 1680.80, 1534.60, 1451.76, 1155.36 δ_H **(400MHz $CDCl_3$)** 0.85 (3H, t J 7.12 Hz) 0.88 (3H, t J 6.98 Hz) 0.93 (3H, d J 6.72 Hz) 0.94 (3H, d J 6.72 Hz) 1.22 – 1.41 (12H, m) 1.49- 1.71 (16H, m) 2.09 (1H, sept, J 6.72 Hz) 2.16 – 2.33 (2H, m) 2.48 (2H, td J 7.52 Hz, 0.94 Hz) 2.64 (2H, m) 2.77 – 3.19 (4H, m) 4.84 (1H, tm J = 8.19 Hz, 1.34 Hz) 4.97 (1H, tm J 6.72 Hz, 1.34 H) 18.14 (0.17H, s) 18.88 (0.83H, s) . δ_C **(100MHz, $CDCl_3$)** 14.07 (q) 14.09 (q) 17.93 (q) 17.95 (q) 22.55 (t) 22.79 (q) 23.39 (t) 24.51 (t) 24.93 (t) 25.77 (q) 26.00 (q) 26.59 (t) 28.75 (t) 28.81 (t) 31.50 (t) 31.55 (t) 33.63 (t) 34.04 (t) 36.62 (t) 47.51 (t) 79.93 (s) 110.05 (s) 114.55 (d) 119.80 (d) 127.73 (s) 133.37 (s) 137.65 (s) 155.82 (s) 169.78 (s) 171.83 (s) 189.079 (s) 190.33 (s) 202.73 (s) MS calcd for $C_{35}H_{54}O_7$ 587.3942 $[M+H]^+$ found 587.3939

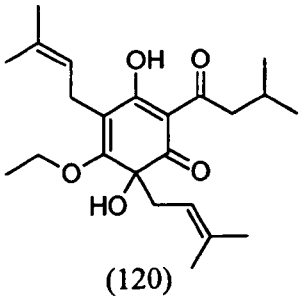
Synthesis of 3,6-Dihydroxy-5-methoxy-4,6-bis-(3-methyl-but-2-enyl)-2-(3-methyl-butyryl)-cyclohexa-2,4-dienone (78)



(78)

To a flask under nitrogen containing potassium carbonate (3 g, 21.7 mmol) and acetone (20 ml) was added humulone **(8)** (1g, 2.8 mmol) this was stirred at ambient temperature for 10 minutes. After this time 18 –crown – 6 (75 mg, 0.28 mmol) was added followed by dimethyl sulphate (1 ml, 7.9 mmol). After the addition the solution was heated to reflux for 3 hours. After this time concentrated ammonia solution (1ml) was added and the mixture stirred for 15 minutes. To this was added dilute hydrochloric acid (20ml). This was then extracted using hexane (3 x 15 ml). The combined organic fractions were washed with water (2 x 10 ml) and brine (2 X 10 ml) and dried over sodium carbonate to give and orange residue (930 mg, 2.4 mmol, 85%) this was chromatographed on silica gel using hexane ethyl acetate (8:2) to give (78) as an orange oil (900 mg 85%) $[\alpha]_D^{20°C}$ -43.40 ° υ_{max} **(ATR)/cm^{-1}** 3444.67, 2961.31, 1721.14,1652.43, 1531.59, 1450.52, 1368.29, 1241.97, 1134.35 δ_H **(400MHz $CDCl_3$)** 0.95 (3H, d, J 6.68 Hz) 0.98 (3H, d, J 7.26 Hz) 1.49 (3H, d, J 1.07 Hz) 1.63 (3H, d, J 0.81 Hz) 1.66 (3H, d, J 1.07 Hz) 1.70 (3H, s) 2.14 (1H, sept J 6.72 Hz) 2.54 (2H, m) 2.78 (2H, m) 3.06 (2H ,m) 4.17 (3H, s) 4.26 (1H, s) 4.81 (1H, tm, J 7.92 Hz, 1.48 Hz) 4.98 (1H, tm, J 6.98 Hz, 1.34 Hz) 18.85 (1H, s) δ_C **(100 MHz, $CDCl_3$)** 17.88 (q) 21.77 (t) 22.71 (q) 22.8 (q) 25.86 (q) 26.02 (q) 26.16 (d) 42.11 (t) 47.05 (t) 60.83 (q) 79.95 (s) 106.20 (s) 116.01 (s) 116.30 (d) 121.59 (d) 132.38 (s) 137.19 (s) 169.14 (s) 190.31 (s) 195.87 (s) 201.19 (s) MS calcd for $C_{22}H_{32}O_5$ 377.2323 $[M+H]^+$ found 377.2328

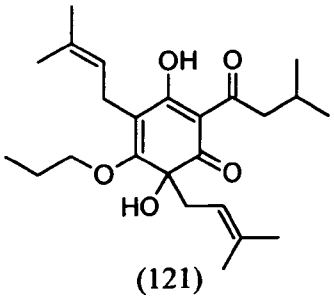
(R)-5-Ethoxy-3,6-dihydroxy-4,6-bis-(3-methyl-but-2-enyl)-2-(3-methyl-butyryl)-cyclohexa-2,4-dienone (120)



(120)

To a flask containing acetone (20 ml) was added potassium carbonate (3 g, 21.7 mmol) and 18 – crown – 6 (75 mg, 0.28 mmol) was added humulone **(8)** (1g, 2.8 mmol) and diethyl sulphate (1 ml, 6.5 mmol) the solution was brought to reflux for 5 hours under nitrogen. After this time it was clear by TLC that the reaction was slow so refluxing was continued overnight. After this time the solution quenched with ammonia (2 ml) and poured into water (20ml) the ammonia was neutralised with 5% HCl and the mixture extracted with hexane (2 x 10 ml) the combined organic extracts were washed with water (10 ml) and brine (10 ml), dried over sodium sulphate and concentrated to give a dark oil. Chromatography over silica gel using ether hexane (15 : 85) gave ethyl humulone **(120)** as an orange oil (0.71g, 1.8 mmol 65 %) $[\alpha]_D^{20°C}$ -63.46 ° (c 0.5 in $CHCl_3$) υ_{max} **(ATR)/cm^{-1}** 3444.13, 2960.89, 2652.6, 1524. 45, 1452.37, 1383.81, 1297.40, 1217.06, 1137.42, 1102.10 δ_H **(400MHz $CDCl_3$)** 0.96 (3H, d, J 6.72 Hz) 0.99 (3H, d, J 7.26 Hz) 1.37 (3H, t J 6.98 Hz) 1.50 (3H, d, J 1.07 Hz) 1.64 (3H, d J 0.94 Hz) 1.68 (3H, d J 1.07 Hz) 1.72 (3H, d J 0.81 Hz) 2.15 (1H, sept, J 6.72 Hz) 2.53 (2H, m) 2.79 (2H, m,) 3.04 (1H, dd J 6.98 Hz, 14.24 Hz) 3.12 (1H, dd J 7.12 Hz, 14.24 Hz) 4.25 (1H, s) 4.48 (1H, dq J 9.94 Hz, 6.98 Hz) 4.60 (1H, dq J 9.94 Hz, 7.12 Hz) 4.89 (1H tm, J7.92 Hz, 1.48 Hz) 5.06 (1H, tm, J 7.12 Hz, 1.48 Hz) 18.86 (1H s) δ_C **(100 MHz, $CDCl_3$)** 16.18 (q) 17.92 (q) 17.97 (q) 21.95 (t) 22.75 (q) 22.92 (q) 25.91 (q) 26.06 (q) 26.18 (d) 42.40 (t) 47.19 (t) 69.37 (t) 80.07 (s) 106.17 (s) 115.87 (s) 116.37 (d) 121.67 (d) 132.23 (s) 137.18 (s) 169.21 (s) 190.26 (s) 196.02 (s) 201.33 (s) MS calcd for $C_{23}H_{34}O_5$ 390 found 390 NO HRMS, sample degraded in transit.

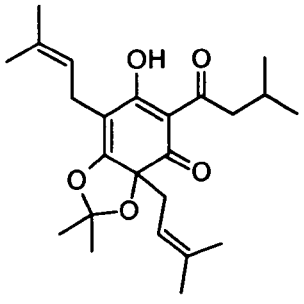
3,6-Dihydroxy-4,6-bis-(3-methyl-but-2-enyl)-2-(3-methyl-butyryl)-5-propoxy-cyclohexa-2,4-dienone (121)



(121)

To a flask containing acetone (20 ml) was added potassium carbonate (3 g, 21.7 mmol) and 18 – crown – 6 (75 mg, 0.28 mmol) was added humulone **(8)** (1g, 2.8 mmol), potassium iodide (0.46 g, 2.8 mmol), and propyl bromide (1 ml, 8.1 mmol) the solution was brought to reflux for 5 hours under nitrogen. After this time it was clear by TLC that the reaction was slow so refluxing was continued for one week. After this time the solution was poured into water (20ml) and the mixture extracted with hexane (2 x 10 ml) the combined organic extracts were washed with water (10 ml) and brine (10 ml), dried over sodium sulphate and concentrated to give a dark oil. Chromatography over silica gel using ether hexane (15 : 85). **(121)** 0.25 g (21.7 %)$[\alpha]_D^{20°C}$ -61.67 ° (c 0.5 in $CHCl_3$) υ_{max} **(ATR)/cm^{-1}** 3444.21, 2961.73, 1729.55, 1649.81, 1523.82, 1462.93, 1377.19, 1269.34, 1216.56, 1128.17, 1101.10 δ_H **(400MHz $CDCl_3$)** 0.96 (3H, d, J 6.58 Hz) 0.99 (3H, d, J 6.72Hz) 1.01 (3H, t J 7.39 Hz)1.50 (3H, d J 0.81 Hz) 1.64 (3H, d J 0.67 Hz) 1.67 (3H, d J 1.07 Hz) 1.71 (3H, d J 0.54 Hz) 1.76 (2H, q J 7.39 Hz) 2.15 (1H, sep,t J =6.72 Hz) 2.53 (2H, m) 2.79 (2H, m,) 3.04 (1H dd J 6.98 Hz, 14.24 Hz) 3.12 (1H, dd J 7.12 Hz, 14.24 Hz) 4.27 (1H, s) 4.38 (1H, dt J 9.81 Hz, 6.58 Hz) 4.52 (1H, dt J 9.81 Hz, 6.45 Hz) 4.89 (1H, tm, J 7.79 Hz, 1.34 Hz) 5.05 (1H, tm, J 7.12 Hz, 1.48 Hz) 18.86 (1H s) δ_C **(100 MHz, $CDCl_3$)** 10.44 (q) 17.93 (q) 17.95 (q) 21.85 (t) 22.74 (q) 22.91 (q) 23.88 (t) 25.90 (q) 26.06 (q) 26.16 (d) 42.39 (t) 47.20 (t) 74.94 (t) 80.04 (s) 106.07 (s) 115.45 (s) 116.37 (d) 121.71 (d) 132.20 (s) 137.14 (s) 169.37 (s) 190.20 (s) 195.98 (s) 201.32 (s) MS calcd for $C_{24}H_{36}O_5$ 405.2636 $[M+H]^+$ found 405.2637

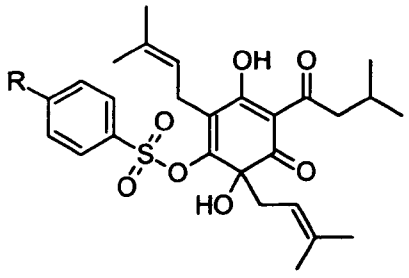
(R)-6-Hydroxy-2,2-dimethyl-3a,7-bis-(3-methyl-but-2-enyl)-5-(3-methyl-butyryl)-3a*H*-1,3-benzodioxol-4-one (126)



(126)

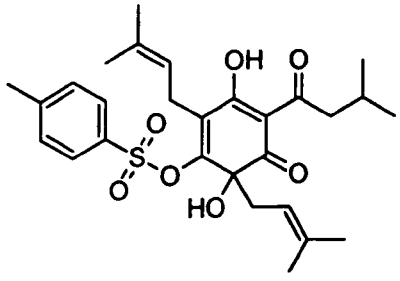
To a flask containing humulone (1.0 g, 2,8 mmol) was added 2-metoxypropene (10 ml, 1.39 mol) and PPTS (0.1 g, 0.4 mmol). This solution was stirred at 0°C for 3 hours. After this time the solution was allowed to return to room temperature and stirred until no starting material could be detected by TLC (hexane : ethyl acetate, 80 : 20). After this time hexane (10 ml) was added and 10 % sodium hydrogen carbonate solution (10 ml). The aqueous phase was extracted with hexane (10ml) and the combined organic fractions were washed with water (2 x 10 ml), dried and concentrated to give a dark orange oil. Chromatogrpahy over silica gel using ether hexane (25 : 75) afforded (126) as a yellow oil 0.89 g (80 %) $[\alpha]_D^{20°C}$ -232 ° (c 0.5 in $CHCl_3$) υ_{max} **(ATR)/cm^{-1}** 2961.00, 1683.73, 1375.25, 1228.09, 1375.83, 1221.88, 109.11 **δH (400MHz CDCl3)** 0.93 (3H, d, J 6.58 Hz) 0.98 (3H, d, J 6.58 Hz) 1.49 (3H, s) 1.50 (3H, d J 0.81 Hz) 1.66 (6H, s) 1.69 (3H, d J 0.54 Hz) 1.71 (3H, s) 1.76 (2H, q J 7.39 Hz) 2.10 (1H, sept, J 6.85 Hz) 2.64 (4H, m) 2.96 (1H, dd J 7.25 Hz, 14.64 Hz) 3.02 (1H, dd J 7.12 Hz, 14.64 Hz) 5.06 (1H, tm, J 7.59 Hz, 1.48 Hz) 5.12 (1H, tm, J 7.25 Hz, 1.34 Hz) 18.30 (1H, s) **δC (100 MHz, $CDCl_3$)** 17.86 (q) 18.09 (q) 22.03 (t) 22.61 (q) 22.94 (q) 25.80 (q) 25.96 (q) 27.09 (d) 27.10 (q) 28.83 (q) 40.77 (t) 44.90 (t) 89.034 (s) 107.62 (s) 107.94 (s) 116.32 (d) 118.50 (s) 120.89 (d) 132.93 (s) 137.04 (s) 166.61 (s) 192.39 (s) 193.16 (s) 196.30 (s) MS calcd for $C_{24}H_{34}O_5$ 403.2479 $[M+H]^+$ found 403.2477

General Procedure for the Synthesis of benzene sulfonates:



To a flask under nitrogen containing humulone (1g, 2.76 mmol) was added pyridine (5 ml) this mixture was stirred for 10 minutes at ambient temperature. After this time the solution was cooled in ice and the substituted benzene sulfonate (8.28 mmol, 3.0eq) in pyridine (5 ml) was added dropwise via syringe. After addition was complete the solution was warmed to room temperature for 5 minutes, after this time it was heated to 60°C and stirring was continued under nitrogen for 3 hours. After three hours, the solution was cooled to room temperature and water (2 ml) was added. Stirring was continued for a further 1 hour. The entire solution was then poured into 5 % HCl (50 ml) and the mixture extracted with hexane (3 x 10 ml) the combined organic fractions were washed with 5% HCl (2 x 10 ml), dried and concentrated. The crude product was chromatographed over silica gel (ether hexane all products were isolated as extremely viscous oils.

Toluene-4-sulfonic acid (R)-3,6-dihydroxy-2,6-bis-(3-methyl-but-2-enyl)-4-(3-methyl-butyryl)-5-oxo-cyclohexa-1,3-dienyl ester (111)

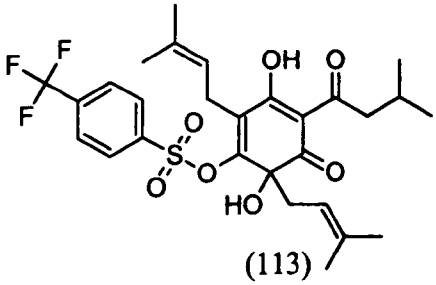


(111)

Ether : Hexane (20 : 80) 1.03 g (71 %) yellow viscous oil $[\alpha]_D^{20°C}$ -24.05 ° (c 0.5 in $CHCl_3$) υ_{max} **(ATR)/cm^{-1}** 3477.69, 2961.98, 1665.24, 1596.94, 1538.82, 1448.50,

1372.33, 1178.92, 1089.18 δ_H **(400MHz $CDCl_3$)** 0.96 (3H, d, J 6.58 Hz) 0.99 (3H, d, J 6.72 Hz) 1.38 (2.7H, s) 1.40 (0.3H, s) 1.52 (0.3H, s) 1.56 (2.7H, s) 1.61 (0.3H, s) 1.64 (0.3H, s) 1.66 (3H, s) 1.68 (3H, s) 2.15 (1H, sept J 6.72 Hz) 2.36 (1H, dd J 8.06 Hz, 13.57 Hz) 2.43 (1H, dd, J 7.92 Hz, 13.70 Hz) 2.45 (3H, s) 2.78 (1,H dd J 7.52 Hz, 13.97 Hz) 2.86 (1H, dd, J 6.45 Hz, 13.97 Hz) 3.13 (1H, dd, J 6.18 Hz, 14.51 Hz) 3.29 (1H, broad s) 3.32 (1H, dd, J 7.52 Hz, 14.51 Hz) 4.67 (0.1H, tm J 6.58 Hz, 1.34 Hz) 4.75 (0.9H, tm, J 7.92 Hz, 1.48 Hz) 5.02 (0.1H, tm J 6.85 Hz, 1.34 Hz) 5.11 (0.9H, tm, J 6.98 Hz, 1.34 Hz) 7.33 (2H, d, J 8.19 Hz) 7.91 (2H d J 8.46 Hz) 18.08 (0.1 H, s) 18.65 (0.9H, s) δ_C **(100 MHz, $CDCl_3$)** 17.73 (q) 18.02 (q) 21.82 (q) 22.62 (q) 22.88 (q) 24.10 (t) 25.88 (q) 25.93 (q) 26.34 (d) 40.47 (t) 46.59 (t) 78.5 (s) 108.21 (s) 116.17 (d) 119.65 (d) 128.49 (d) 128.72 (s) 129.53 (d) 134.07 (s) 134.58 (s) 137.74 (s) 145.28 (s) 157.63 (s) 190.19 (s) 195.21 (s) 201.65 (s) MS calcd for $C_{28}H_{36}O_7S$ = 517.2255 $(M+H)^+$found 517.2252

4-Trifluoromethyl-benzenesulfonic acid 3,6-dihydroxy-2,6-bis-(3-methyl-but-2-enyl)-4-(3-methyl-butyryl)-5-oxo-cyclohexa-1,3-dienyl ester (113)

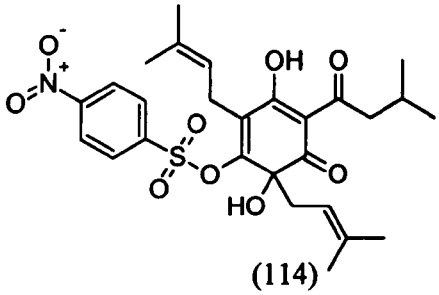


(113)

Humulone **(8)** (0.5g) gave **(113)** (0.46 g 57 %) ether hexane (20 : 80) red viscous oil $[\alpha]_D^{20°C}$ -23.95 ° (c 0.5 in $CHCl_3$) υ_{max} **(ATR)/cm^{-1}** 3478.57, 2963.09, 1667.38, 1538.65, 1451.65, 1407.06, 1380.19, 1302.78, 1172.68, 1135.20, 1062.52 δ_H **(400MHz $CDCl_3$)** 0.96 (3H ,d, J 6.72 Hz) 1.09 (3H, d, J 6.72 Hz) 1.36 (2.7H, d J 1.07 Hz) 1.38 (0.3H, d J 0.94 Hz) 1.54 (0.3H, s) 1.57 (2.7H, s) 1.66 (0.3H, s) 1.67 (0.3H, s) 1.71 (6H, s) 2.15 (1H, sept J 6.72 Hz) 2.29 (1H, dd J 7.92 Hz, 13.57 Hz) 2.37 (1H, dd, J 7.92 Hz, 13.57 Hz) 2.78 (1H, dd J 7.39 Hz, 13.97 Hz) 2.86 (1H, dd, J 6.58 Hz, 13.97 Hz) 3.18 (1H, dd J 6.18 Hz, 14.37 Hz) 3.35 (1H, dd, J 7.66 Hz,

14.37 Hz) 3.53 (1H, s) 4.66 (0.1H, tm J 8.19 Hz, 1.484 Hz) 4.72 (0.9H, tm, J 8.06 Hz, 1.34 Hz) 5.03 (0.1H, tm, J 6.87 Hz, 1.48 Hz) 5.12 (0.9H, tm, J 6.85 Hz, 1.34 Hz) 7.81 (2H, d J 8.87 Hz) 8.15 (2H, d J 8.19 Hz) 17.97 (0.1H, s) 18.63 (0.9H, s) δ_C **(100 MHz, $CDCl_3$)** 17.7 (q) 18.08 (q) 22.62 (q) 22.90 (q) 24.11 (t) 25.92 (q) 25.96 (q) 26.44 (d) 41.50 (t) 46.52 (t) 78.52 (s) 108.12 (s) 115.88 (d) 119.27 (d) 125.96 (CF_3) 129.06 (d) 129.19 (s) 134.47 (s) 138.13 (s) 141.05 (s) 157.11 (s) 190.03 (s) 194.94 (s) 201.77 (s) MS calcd for $C_{28}H_{33}F_3O_7S$ = 571.1971 $(M+H)^+$ found 571.1966

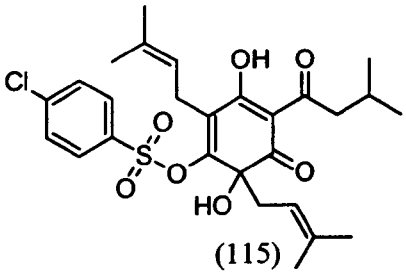
4-Nitro-benzenesulfonic acid 3,6-dihydroxy-2,6-bis-(3-methyl-but-2-enyl)-4-(3-methyl-butyryl)-5-oxo-cyclohexa-1,3-dienyl ester (114)



(114)

Ether : Hexane : acetic acid (30 : 69 :1) brown glass 0.87 g (57 %) $[\alpha]_D^{20°C}$ -35.93 ° (c 0.5 in $CHCl_3$) υ_{max} **(ATR)/cm^{-1}** 3443.55, 2961.77, 1712.72, 1609.03, 1532.36, 1434.34, 1382.59, 1347.95, 1189.63, 1088.13 δ_H **(400MHz $CDCl_3$)** 0.96 (3H, d, J 6.59 Hz) 1.00 (3H, d, J 6.59 Hz) 1.37 (3H, d, J 0.92 Hz) 1.58 (3H, s) 1.71 (3H, s) 1.74 (3H, s) 2.14 (1H, sept J 6.96 Hz) 2.26 (1H, dd, J 8.06 Hz, 13.55 Hz) 2.33 (1H, dd, J 8.06 Hz, 13.73 Hz) 2.77 (1H, dd, J 7.51 Hz, 14.10 Hz) 2.86 (1H, dd, J 6.59 Hz, 14.10 Hz) 3.20 (1H, dd, J 6.22 Hz, 14.46 Hz) 3.67 (1H, dd, J 7.51 Hz, 14.46 Hz) 3.52 (1H, s) 4.65 (0.1H, broad t, J 8.06 Hz) 4.70 (0.9H, tm, J 8.06 Hz, 1.28 Hz) 5.05 (0.1H, broad t, J 6.41 Hz, 1.34 Hz) 5.12 (0.9H, tm, J 6.96 Hz, 1.46 Hz) 8.20 (2H, d, J 8.60 Hz) 8.38 (2H, d, J 8.79 Hz) 18.08 (0.1H, s) 18.65 (0.9H, s) δ_C **(100 MHz, $CDCl_3$)** 17.76 (q) 18.15 (q) 21.63 (q) 22.92 (q) 24.12 (t) 25.99 (q) 26.45 (d) 41.59 (t) 46.48 (t) 78.5 (s) 108.05 (s) 115.79 (d) 119.07 (d) 123.98 (d) 129.41 (s) 129.83 (d) 134.67 (s) 138.21 (s) 150.78 (s) 156.70 (s) 189.98 (s) 194.79 (s) 201.77 (s) LRMS calcd for $C_{27}H_{33}NO_9S$ = 548.1949 $(M+H)^+$ found 548.1951

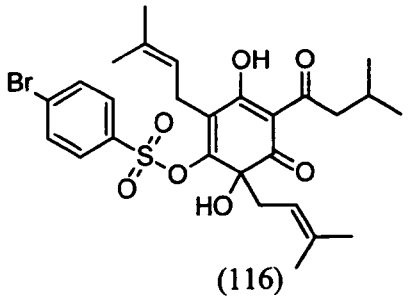
4-Chloro-benzenesulfonic acid 3,6-dihydroxy-2,6-bis-(3-methyl-but-2-enyl)-4-(3-methyl-butyryl)-5-oxo-cyclohexa-1,3-dienyl ester (115)



(115)

Ether : Hexane (20 : 80) 1.21 g (80.5 %) Brown viscous oil $[\alpha]_D^{20°C}$ -45.82 ° (c 0.5 in $CHCl_3$) υ_{max} **(ATR)/cm^{-1}** 3467.93, 2962.57, 1665.68, 1538.99, 1450.09, 1376.54, 1279.09, 1191.18, 1156.40, 1085.49 δ_H **(400MHz $CDCl_3$)** 0.96 (3H, d, J 6.72 Hz) 0.99 (3H, d, J 6.72 Hz) 1.37 (2.7H, d, J 1.07 Hz) 1.39 (0.3H, d, J 1.07 Hz) 1.52 (0.3H, s) 1.57 (2.7H, s) 1.66 (0.6H, s) 1.69 (2.7H, d J 0.94 Hz) 1.70 (2.7H, s) 2.14 (1H, sept, J 6.72 Hz) 2.31 (1H, dd, J 7.92 Hz, 13.70 Hz) 2.38 (1H, dd, J 8.06 Hz, 13.70 Hz) 2.77 (1H, dd, J 7.52 Hz, 14.10 Hz) 2.85 (1H, dd, J 6.58 Hz, 14.10 Hz) 3.16 (1H, dd, J 6.18 Hz, 14.51 Hz) 3.34 (1H, dd, J 7.66 Hz, 14.51 Hz) 3.57 (1H, s) 4.65 (0.1H, tm J 8.06 Hz, 1.21 Hz) 4.73 (2.9H, tm J 8.06 Hz, 1.34 Hz) 5.02 (0.1H, tm J 6.92 Hz, 1.48 Hz) 5.11 (2.9H, tm J 6.98 Hz,1.34 Hz) 7.51 (2H, dm, J 9.00 Hz) 7.96 (2H, dm, J 8.87 Hz) 18.01 (0.1H, s) 18.64 (0.9H, s) δ_C **(100 MHz, $CDCl_3$)** 17.72 (q) 18.07 (q) 22.61 (q) 22.88 (q) 24.09 (t) 25.90 (q) 25.95 (q) 26.37 (d) 41.51 (t) 46.53 (t) 78.52 (s) 108.13 (s) 115.96 (d) 119.39 (d) 123.98 (d)) 129.01 (s) 129.16 (d) 129.96 (d) 134.32 (s) 135.94 (s) 137.95 (s) 140.80 (s) 157.24 (s) 190.07 (s) 195.04 (s) 201.71 (s) MS calcd for $C_{27}H_{33}{}^{35}ClO_7S$ = 536.1708 $(M+H)^+$ found 536.1705

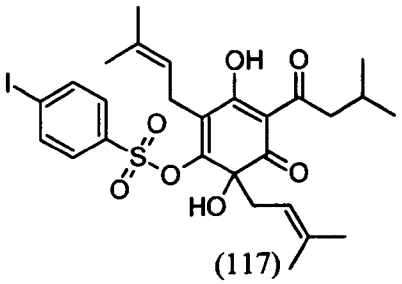
4-Bromo-benzenesulfonic acid 3,6-dihydroxy-2,6-bis-(3-methyl-but-2-enyl)-4-(3-methyl-butyryl)-5-oxo-cyclohexa-1,3-dienyl ester (116)



(116)

Ether : Hexane (20 : 80) 1.25 g (78.6 %) Brown viscous oil $[\alpha]_D^{20°C}$ -37.04 ° (c 0.5 in $CHCl_3$) υ_{max} **(ATR)/cm^{-1}** 3469.16, 2962.12, 1665.42, 1574.92, 1538.81, 1449.72, 1376.32, 1191.54, 1155.61, 1010.44 **δ_H (400MHz $CDCl_3$)** 0.96 (3H, d, J 6.72 Hz) 1.00 (3H, d, J 6.72 Hz) 1.38 (2.7H, d, J 1.07 Hz) 1.40 (0.3H, d J 1.07 Hz) 1.54 (0.3H, s) 1.57 (2.7H, s) 1.66 (0.6H, s) 1.70 (5.4H, s) 2.15 (1H, sept, J 6.72 Hz) 2.31 (1H, dd J 7.92 Hz, 13.70 Hz) 2.39 (1H, dd J 8.06 Hz, 13.70 Hz) 2.77 (1H, dd J 7.52 Hz, 13.9713.97 Hz) 2.85 (1H, dd J 6.58 Hz, 13.83 Hz) 3.16 (1H, dd, J 6.18 Hz, 14.51 Hz) 3.34 (1H, dd, J 7.66 Hz, 14.51 Hz) 3.55 (1H, s) 4.66 (0.1H, tm J 8.06 Hz, 1.21 Hz) 4.73 (2.9H, tm, J 7.92 Hz, 1.34 Hz) 5.02 (0.1H, tm, J 6.78 Hz, 1.48 Hz) 5.11 (2.9H, tm, J 6.98 Hz, 1.34 Hz) 7.68 (2H, dm, J 8.87 Hz) 7.88 (2H, dm, J 8.87 Hz) 18.00 (0.1H, s) 18.64 (0.9H, s) **δ_C (100 MHz, $CDCl_3$)** 17.74 (q) 18.10 (q) 22.63 (q) 22.90 (q) 24.11 (t) 25.93 (q) 25.97 (q) 26.39 (d) 41.53 (t) 46.55 (t) 78.54 (s) 108.15 (s) 115.97 (d) 119.39 (d) 123.98 (d)) 129.02 (s) 129.42 (s) 130.00 (d)132.17 (d) 134.36 (s) 136.52 (s) 137.99 (s) 140.80 (s) 157.24 (s) 190.08 (s) 195.04 (s) 201.72 (s) MS calcd for $C_{27}H_{33}{}^{79}BrO_7S$ = 581.1203 $(M+H)^+$ found 581.1202

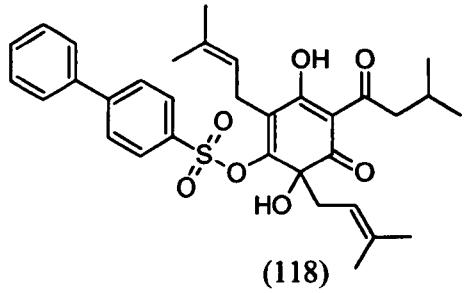
4-Iodo-benzenesulfonic acid 3,6-dihydroxy-2,6-bis-(3-methyl-but-2-enyl)-4-(3-methyl-butyryl)-5-oxo-cyclohexa-1,3-dienyl ester (117)



(117)

Ether : Hexane (20 : 80) 1.24 g (70.5 %) $[\alpha]_D^{20°C}$ -43.10 ° Dark brown viscous oil (c 0.5 in $CHCl_3$) υ_{max} **(ATR)/cm^{-1}** 3476.76, 2961.32, 1665.58, 1568.44, 1538.89 1449.75, 1375.70, 1191.95, 1038.48, 1006.08 δ_H **(400MHz $CDCl_3$)** 0.96 (3H, d, J 6.58 Hz) 1.00 (3H, d, J 6.58 Hz) 1.38 (2.7H, s) 1.39 (0.3H, s) 1.54 (0.3H, s) 1.57 (2.7H, s) 1.65 (0.3H, s) 1.66 (0.3H, s) 1.69 (5.4H, d J 0.94 Hz) 2.15 (1H, sept, J 6.98 Hz) 2.31 (1H, dd, J 8.06 Hz, 13.57 Hz) 2.39 (1H, dd, J 8.06 Hz, 13.57 Hz) 2.77 (1H, dd, J 7.39 Hz, 14.10 Hz) 2.86 (1H, dd, J 6.58 Hz, 14.10 Hz) 3.15 (1H, dd, J 6.18 Hz, 14.51 Hz) 3.33 (1H, dd, J 7.66 Hz,14.51 Hz) 3.55 (1H, broad s) 4.65 (0.1H, tm J 8.19 Hz, 1.21 Hz) 4.73 (2.9H, tm, J 7.92 Hz, 1.21 Hz) 5.02 (0.1H, tm, J 6.85 Hz,1.34 Hz) 5.10 (2.9H, tm, J 6.85 Hz, 1.34 Hz) 7.72 (2H, dm, J 8.60 Hz) 7.90 (2H, dm, J 8.46 Hz) 18.01 (0.1H, s) 18.64 (0.9H, s) δ_C **(100 MHz, $CDCl_3$)** 17.75 (q) 18.10 (q) 22.64 (q) 22.91 (q) 24.11 (t) 25.93 (q) 25.99 (q) 26.38 (d) 41.53 (t) 46.55 (t) 78.53 (s) 102.07 (s) 108.14 (s) 115.97 (d) 119.38 (d) 123.98 (d) 129.01 (s) 129.74 (d) 134.36 (s) 137.19 (s) 138.01 (s) 138.14 (d) 157.22 (s) 190.08 (s) 195.04 (s) 201.71 (s) MS calcd for $C_{27}H_{33}IO_7S$ = 629.1064 $(M+H)^+$ found 629.1064

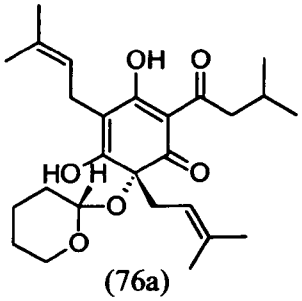
Biphenyl-4-sulfonic acid 3,6-dihydroxy-2,6-bis-(3-methyl-but-2-enyl)-4-(3-methyl-butyryl)-5-oxo-cyclohexa-1,3-dienyl ester (118)



(118)

Ether : Hexane (20 : 80) 0.5 g (31 %) incomplete conversion, brown viscous oil $[\alpha]_D^{20°C}$ -43.91 ° (c 0.5 in $CHCl_3$) υ_{max} **(ATR)/cm^{-1}** 3460.04, 2961.94, 1665.64, 1593.67, 1538.81, 1449.23, 1371.81, 1333.53, 1182.61, 1156.59, 1092.73 δ_H **(400MHz $CDCl_3$)** 0.97 (3H, d, J 6.72 Hz) 1.01 (3H, d, J 6.72 Hz) 1.40 (2.7H, d, J 0.94 Hz) 1.42 (0.3H, d, J, 0.94 Hz) 1.54 (0.3H, s) 1.58 (2.7H, s) 1.63 (0.3H, s) 1.67 (0.3H, s) 1.68 (2.7H, s) 1.70 (2.7H, s) 2.16 (1H sept, J 6.72 Hz) 2.38 (1H, dd, J 7.92 Hz, 13.57 Hz) 2.45 (1H, dd, J 7.92 Hz, 13.57 Hz) 2.79 (1H, dd J 7.52 Hz, 13.97 Hz) 2.88 (1H, dd, J 6.58 Hz, 13.97 Hz) 3.18 (1H, dd, J 6.18 Hz, 14.37 Hz) 3.29 (0.1H, s) 3.36 (1H, dd, J 7.66 Hz, 14.36 Hz) 3.61 (0.9H, sharp s) 4.69 (0.1H, tm J 8.06 Hz, 1.34 Hz) 4.77 (2.9H, tm J 7.92 Hz, 1.34 Hz) 5.05 (0.1H, tm, J 6.04 Hz, 1.48 Hz) 5.13 (2.9H, tm, J 6.92 Hz, 1.48 Hz) 7.42 – 7.52 (3H, m) 7.61 – 7.64 (2H, m) 7.75 (2H, dm, J 8.73 Hz) 8.10 (2H, dm J 8.73 Hz) 18.10 (0.1H, s) 18.68 (0.9H, s) δ_C **(100 MHz, $CDCl_3$)** 17.75 (q) 18.06 (q) 22.65 (q) 22.91 (q) 24.13 (t) 25.93 (q) 25.99 (q) 26.37 (d) 41.49 (t) 46.60 (t) 78.60 (s) 108.20 (s) 116.08 (d) 119.58 (d) 127.46 (d) 127.50 (d) 129.01 (d) 129.23 (d) 134.21 (s) 135.99 (s) 137.92 (s) 139.14 (s) 147.08 (s) 157.56 (s) 190.17 (s) 195.16 (s) 201.70 (s) MS calcd for $C_{33}H_{38}O_7S$ = 579.2411 $(M+H)^+$ found 579.2412

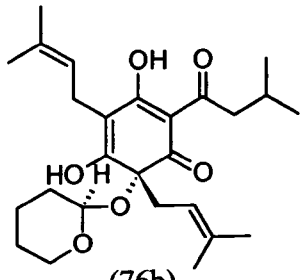
(R)-3,5-Dihydroxy-4,6-bis-(3-methyl-but-2-enyl)-2-(3-methyl-butyryl)-6-[(S)-(tetrahydro-pyran-2-yl)oxy]-cyclohexa-2,4-dienone (76a)



(76a)

To a flask under nitrogen containing dry DCM (10 ml) and humulone **(8)** (1g, 2.8 mmol) was added triphenylphosphine hydrobromide (0.095 g 0.28mmol). The flask was sealed and stirred under nitrogen. To this was added dihydro-*2H*-pyran (1 ml, 11.9 mmol) *via* syringe. This solution was stirred for 3 hours, after this time, 5 % sodium hydrogen carbonate solution (5 ml) was added and the organic fraction was removed and washed with brine (2 x 10 ml) After drying over sodium sulfate the solvent was removed under vacuum and the yellow residue was chromatographer over silica gel using a gradient elution. Hexane : Ether (90 : 10) gave **(76_a)** as white crystals (0.52 g, 1.2 mmol, 46%) Required C, 69.93; H 8.72 % Found: C, 69.76; H, 8.72% MP 100.1 – 102.3 $[\alpha]_D^{20°C}$ -127.78° (c 1.25 in $CHCl_3$) υ_{max} **(ATR)/cm^{-1}** 3175.32, 2965.33, 1656.76, 1623.86, 1514.50m 1455.15, 1418.55, 1363.53, 1221.38, 1131.78, 1103.68, 1078.39 **δ_H (400MHz $CDCl_3$)** 0.95 (3H, d, J 6.58 Hz) 0.98 (3H, d, J 6.72 Hz) 1.43 – 1.55 (2H, m) 1.49 (3H, J 0.81) 1.63 (5H, s) 1.68 (3H, d, J 1.07 Hz) 1.73 (3H, s) 1.86 (1H, m) 2.00 (1H, m) 2.04 (1H, m) 2.13 (1H, sept, J 6.72 Hz) 1.26 (2H, m) 2.74 (1H, dd J 6.58 Hz, 14.24 Hz) 2.92 (1H, dd, J 7.52 Hz, 14.24 Hz) 3.06 (1H, dd, J 6.58 Hz, J 14.37 Hz) 3.13 (1H, dd, J 7.39 Hz, 14.37 Hz) 3.40 (1H, m) 3.95 (1H, m) 4.38 (1H, dd , J 2.42 Hz, 8.06 Hz) 4.95 (1H, tm, J 7.79 Hz, 1.34 Hz) 5.12 (1H, tm, J 7.25 Hz, 1.34 Hz) 7.68 (1H, s) 19.04 (1H, s) **δ_C (100 MHz, $CDCl_3$)** 17.79 (q) 17.94 (q) 21.44 (t) 21.54 (t) 22.81 (q) 22.90 (q) 24.92 (t) 25.92 (q) 26.03 (q) 26.05 (d) 31.66 (t) 41.48 (t) 47.31 (t) 66.36 (t) 86.61 (s) 100.17 (d) 108.47 (s) 113.57 (s) 115.98 (d) 121.54 (d) 132.58 (s) 137.58 (s) 167.27 (s) 190.33 (s) 192.68 (s) 201.32 (s) MS calcd for $C_{26}H_{38}O_6$ 447.2741 $[M+H]^+$ found 447.2738

(R)-3,5-Dihydroxy-4,6-bis-(3-methyl-but-2-enyl)-2-(3-methyl-butyryl)-6-[(R)-(tetrahydro-pyran-2-yl)oxy]-cyclohexa-2,4-dienone (76b)

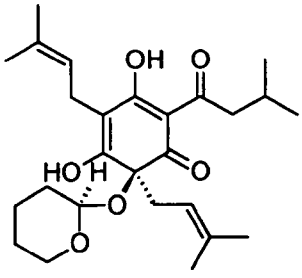


(76b)

Hexane : ether (80 : 20) gave **(76_b)** as white crystals (0.25 g, 0.6 mmol 20%)

MP116.2 -117.1 $[\alpha]_D^{20°C}$ -148.81° (c 0.5 in $CHCl_3$) υ_{max} **(ATR)/cm^{-1}** 3300, 2927.52, 1657.15, 1515.12, 1464.32, 1353.75, 1307.13, 1209.76, 1221.68, 1176.27,1132.31, 1078.82 δ_H **(400MHz $CDCl_3$)** 0.94 (3H, d, J 6.72 Hz) 0.95 (3H ,d, J 6.72 Hz) 1.51 (3H, d, J 0.94 Hz) 1.52 – 1.56 (4H, m) 1.58 (3H, d, J 0.67 Hz) 1.67 (3H, d, J 1.07 Hz) 1.72 (3H, d, J 0.67 Hz) 1.92 (2H, broad m) 2.12 (1H, sept, J 6.72 Hz) 2.71 (2H, m) 2.78 (1H, dd, J 6.85 Hz, 13.83 Hz) 2.89 (1H, dd, J 7.25 Hz, 13.83 Hz) 3.09 (2H, m) 3.52 (1H, m) 4.06 (1H, dt, J 11.42 Hz, 3.76 Hz) 4.81 (1H, tm, J 7.79 Hz, 1.48 Hz) 4.88 (1H, m) 5.08 (1H, tm, J 7.12 Hz, 1.48 Hz) 8.90 (1H, s) 19.15 (1H, s) δ_C **(100 MHz, $CDCl_3$)** 17.94 (q) 17.97 (q) 21.23 (t) 21.31 (t) 22.80 (q) 22.86 (q) 24.83 (t) 25.87 (q) 26.02 (d) 31.13 (t) 35.19 (t) 47.34 (t) 65.67 (t) 81.89 (s) 96.35 (d) 101.38 (s) 112.19 (s) 115.01 (d) 121.56 (d) 132.61 (s) 137.82 (s) 165.85 (s) 190.60 (s) 191.91 (s) 201.52 (s) MS calcd for $C_{26}H_{38}O_6$ 447.2741 $[M+H]^+$ found 447.2738

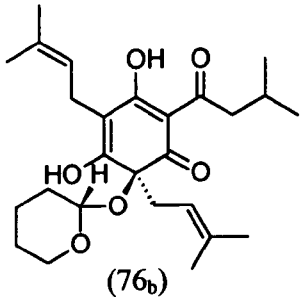
(S)-3,5-Dihydroxy-4,6-bis-(3-methyl-but-2-enyl)-2-(3-methyl-butyryl)-6-[(R)-(tetrahydro-pyran-2-yl)oxy]-cyclohexa-2,4-dienone (76a)



(76_a)

Compound (**76_a**) was prepared using the same method as above using unnatural (S) – (+) – Humulone (8). All spectral data were in agreement with the previously collected data Required C, 69.93; H 8.72 % Found: C, 70.21; H, 8.92% MP 96.6 – 99.4 $[\alpha]_D^{20°C}$ +129.05° (c 0.5 in $CHCl_3$)

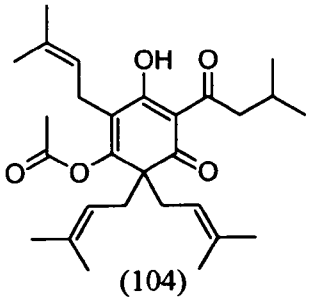
(S)-3,5-Dihydroxy-4,6-bis-(3-methyl-but-2-enyl)-2-(3-methyl-butyryl)-6-[(S)-(tetrahydro-pyran-2-yl)oxy]-cyclohexa-2,4-dienone



(76_b)

Compound (**76_b**) was prepared using the same method as above using unnatural (S) – (+) – Humulone (8). All spectral data were in agreement with the previously collected data Required MP = 115.3 -116.9 $[\alpha]_D^{20°C}$ +147.36° (c 0.5 in $CHCl_3$)

Acetic acid 3-hydroxy-2,6,6-tris-(3-methyl-but-2-enyl)-4-(3-methyl-butyryl)-5-oxo-cyclohexa-1,3-dienyl ester (104)

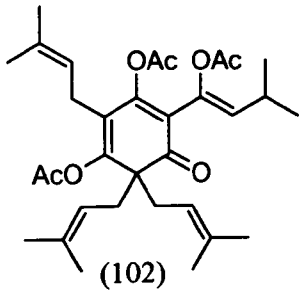


(104)

To a flask containing lupulone (1g, 2.4 mmol) was added acetic anhydride (2.5 ml, 24.5 mmol) the flask was stoppered and purged with nitrogen with stirring. After all the solids had dissolved, pyridine (3 drops) was added. Stirring was continued for 2 hours after which time the mixture was poured into saturated sodium hydrogen carbonate (10 ml) with vigorous stirring. One all of the acetic anhydride had been hydrolysed the solution was extracted with hexane (2 x 10 ml). The combined organic phases were washed with 5 % sodium hydrogen carbonate (2 x 10ml), 5 % HCl (2 x 10ml) and water (2 x 10 ml), dried over sodium sulphate and concentrated to give crude lupulone acetate. The crude material was purified by chromatography over silica gel with ether, hexane (20 : 80) to yield **(104)** as a pale yellow oil (0.94 g, 2.1 mmol, 86%).υ_{max} **(ATR)/cm^{-1}** 2962.61, 1778.23, 1665.21, 1641.55, 1532.29, 1448.22, 1366.81, 1186.48, 1100.52, 1079.15 δ_C **(100 MHz, $CDCl_3$)** 0.91 – 0.94 (6H, m)1.54 (12H, s) 1.62 – 1.67 (6H m) 2.00 – 2.16 (1H m) 2.20 (1.6H, s) 2.22 (1.4H,s) 2.24 (1H, m) 2.33 (1H, m) 2.70 (2H, m) 2.92 (4H, m) 4.85 (2H, m) 4.93 (1H, m) 18.32 (0.55H s) 19.01 (0.45H s) δ_H **(400MHz $CDCl_3$)** 17.84 (q) 17.86 (q) 17.95 (q) 18.02 (q) 20.73 (q) 22.65 (q) 22.79 (q) 23.62 (t) 23.92 (t) 25.78 (q) 25.83 (q) 25.87 (q) 25.09 (q) 25.04 (d) 25.99 (d) 36.13 (t) 37.32 (t) 48.18 (t) 48.22 (t) 49.11 (t) 49.42 (t) 53.29 (q) (57.91 (q) 110.39 (s) 112.15 (s) 112.25 (s) 117.68 (d) 117.70 (d) 118.32 (d) 120.58 (d) 121.26 (d) 126.34 (s) 130.50 (s) 132.13 (s) 132.91 (s) 134.75 (s) 135.45 (s) 156.30 (s) 161.83 (s) 161.93 (s) 166.07 (s) 166.12 (s) 183.85 (s) 183.92 (s) 188.96 (s) 189.52 (s) 195.13 (s) 195.57 (s) 195.69 (s) 196.42 (s) 203.13 (s) 103.72 (s) 205.72 (s) 205.93 (s)

MS calcd for $C_{28}H_{40}O_5$ 457.2949 $[M+H]^+$ found 457.2945

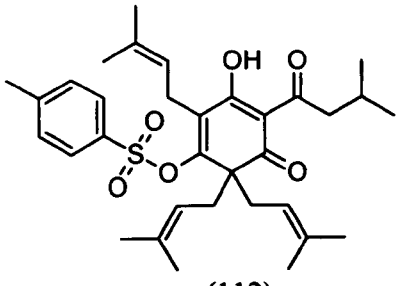
Acetic acid 3-acetoxy-4-((Z)-1-acetoxy-3-methyl-but-1-enyl)-2,6,6-tris-(3-methyl-but-2-enyl)-5-oxo-cyclohexa-1,3-dienyl ester (102)



(102)

To a flask containing lupulone (1g, 2.4 mmol) was added pyridine (10 ml) and the flask was purged with nitrogen. The flask was stirred until all of the solids had dissolved, acetic anhydride (0.8g, 7.8 mmol) was added in one portion. The solution was stirred at ambient temperature for 2 hours. After this time water (20 ml) was added and stirring was continued for 10 minutes. The solution was extracted with ether (2 x 20 ml), the combined organic fractions were washed with 5 % sodium hydrogen carbonate (10 ml), 5 % HCl (2 x 10 ml) and brine (10 ml). The solution was dried over magnesium sulphate and concentrated to give a dark oil. This oil was crystallised from hexane to give white needles (1.17 g, 2.2 mmol, 89.7 %) calculated (71.08 %) H (8.20%) found C(71.12%) H (8.26) %) υ_{max} **(ATR)/cm^{-1}** 2964.60, 1759.29, 1667.56, 1637.91, 1449.53, 1364.03, 1207.98, 1175.97, 1042.43 δ_H **(400MHz $CDCl_3$)** 0.99 (2H, d, J 6.72 Hz) 1.60 (3H, d, J 0.81 Hz) 1.62 (9H s) 1.65 (3H, d, J 1.21 Hz) 2.08 (3H,s) 2.18 (3H, s) 2.22 (3H, s) 2.40 (2H, d, J 7.25 Hz) 2.49 (1H, d sept, J 9.81, 6.72 Hz) 2.96 (2H, d, J 6.72 Hz) 4.84 (1H, tm, J 7.72 Hz, 1.34 Hz) 4.94 (2H, tm, J 7.25 Hz, 1.34 Hz) 5.21 (1H, d, J 9.94 Hz)) δ_C **(100 MHz, $CDCl_3$)** 17.81 (q) 18.06 (q) 20.70 (q) 20.72 (q) 21.02 (q) 22.37 (q) 23.87 (t) 25.86 (q) 25.93 (q) 26.16 (d) 34.82 (t) 51.52 (s) 118.07 (d) 121.10 (d) 126.74 (s) 130.24 (s) 132.00 (s) 132.36 (d) 134.60 (s) 135.08 (s) 159.90 (s) 162.25 (s) 165.55 (s) 166.12 (s) 167.82 (s) 184.46 (s)

Toluene-4-sulfonic acid 3-hydroxy-4-(1-hydroxy-3-methyl-butyl)-2,6,6-tris-(3-methyl-but-2-enyl)-5-oxo-cyclohexa-1,3-dienyl ester (112)



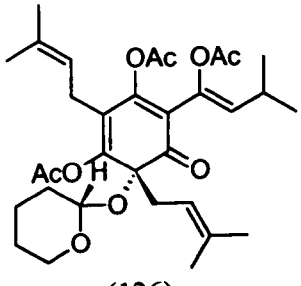
(112)

To a flask containing lupulone (1g, 2.4 mmol) in was added pyridine (10 ml) under nitrogen, to this solution was added toluensulfonyl chloride (0.92 g, 5.3 mmol), and the mixture was heated to 50°C. After 3 hours the reaction was deemed complete by the absence of starting material by TLC. The reaction mixture was quenched by the addition of water (1ml) and the solution was stirred at room temperature for 1 hour. After this time the solution was poured into water (50 ml) and the mixture was extracted using hexane (2 x 20 ml). The combined organic fractions were washed with 5% HCl (2 x 10ml) and brine (20 ml). The organic phase was dried over sodium sulphate and concentrated to give a brown oil. Chromatography over silica gel using hexane : ether (80 : 20) gave **(112)** as a pale yellow oil (1.06 g , 1.9 mmol 77.7 %) υ_{max} **(ATR)/cm^{-1}** 2962.35, 1644.83, 1536.02, 1447.93, 1368.53, 1192.36, 1035.28 δ_H **(400MHz $CDCl_3$)** 0.94 (6H, d, J 6.58 Hz) 1.46 – 1.62 (18H, m) 2.10 (1H, m, J 6.72 Hz) 2.20 (1H, dd, J 14.24 Hz, 6.18 Hz) 2.42 (1H, dd, J 14.24 Hz, 6.18 Hz) 2.47 (3H, s) 2.59 – 2.73 (2H, m) 2.87 (0.8H, d, J 9.98 Hz) 2.98 (1.2 H, d, 6.98 Hz) 3.15 (1.2H, d, J 6.58 Hz) 3.25 (0.8H, d, J 6.72 Hz) 4.82 – 4.89 (2H, m) 4.93 (0.6H, tm, J 6.58 Hz, 1.34 Hz) 5.03 (0.4H, tm, 6.58 Hz, 1.48 Hz) 7.36 (2H, dm, J 7.79 Hz) 7.86 (2H dm, J 8.33 Hz) 18.37 (0.6H, s) 18.93 (0.4H, s) δ_C **(100 MHz, $CDCl_3$)** 17.85 (q) 17.87 (q) 18.03 (q) 18.14 (q) 21.82 (q) 21.83 (q) 22.66 (q) 22.81 (q) 24.56 (t) 24.85 (t) 25.73 (q) 25.83 (q) 25.89 (q) 25.91 (d) 26.06 (d) 35.87 (t) 36.92 (t) 48.15 (t) 48.93 (t) 54.43 (s) 58.72 (s) 110.71 (s) 113.23 (s) 117.50 (d) 118.06 (d) 120.20 (d) 120.72 (d) 127.66 (d) 127.69 (d) 129.37 (q) 129.97 (d) 130.02 (d) 133.07 (s) 133.62 (s) 133.75 (s) 134.29 (s) 134.48 (s) 134.89 (s) 135.69 (s) 145.51 (s) 145.68 (s) 155.94 (s) 160.63 (s) 183.97 (s) 189.38 (s) 195.30 (s)

196.80 (s) 203.92 (s) 205.65 (s) MS calcd for $C_{33}H_{44}O_6S$ 569.2931 $[M+H]^+$ found 569.2936

The following compounds were not stable, consequently the characterisation was limited to ^1H and ^{13}C NMR and IR only.

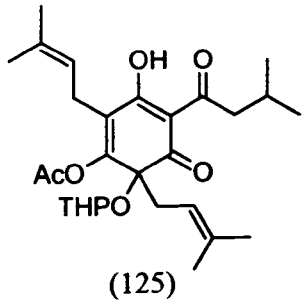
Acetic acid (R)-3-acetoxy-4-((Z)-1-acetoxy-3-methyl-but-1-enyl)-2,6-bis-(3-methyl-but-2-enyl)-5-oxo-6-[(S)-(tetrahydro-pyran-2-yl)oxy]-cyclohexa-1,3-dienyl ester (126)



(126)

To a flask containing humulone THP ether **(74_a)** (100 mg, 0.22 mmol) was added pyridine (1.5 ml) and the flask was purged with nitrogen. The flask was stirred until all of the solids had dissolved acetic anhydride (0.7 g, 0.7 mmol) was added in one portion. The solution was stirred at ambient temperature for 2 hours. After this time water (2 ml) was added and stirring was continued for 10 minutes. The solution was extracted with ether (2 x 5 ml), the combined organic fractions were washed with 5 % sodium hydrogen carbonate (2 ml), 5 % HCl (2 x 3 ml) and brine (5 ml). The solution was dried over magnesium sulphate and concentrated to give **(126)** as a pale oil. (92 mg , 0.16 mmol 72 %) υ_{max} **(ATR)/cm^{-1}** δ_H 2931.88, 1753.44, 1682.49, 1540.87, 1450.94, 1368.37, 1226.08, 1186.00, 1154.52, 1013.81**(400MHz $CDCl_3$)**, δ_C **(100 MHz, $CDCl_3$)** 17.81 (q) 18.17 (q) 20.10 (t) 20.67 (q) 20.93 (q) 21.06 (q) 22.28 (q) 22.47 (q) 24.48 (t) 25.25 (t) 25.89 (q) 26.07 (q) 26.11 (d) 63.58 (t) 78.25 (s) 96.40 (d) 116.26 (d) 120.44 (d) 127.17 (s) 129.59 (s) 132.32 (s) 132.92 (d) 134.57 (s) 135.70 (s) 156.49 (s) 156.98 (s) 165.34 (s) 165.96 (s) 167.79 (s) 183.61 (s)

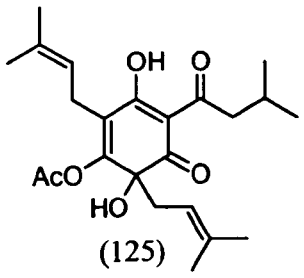
Acetic acid (R)-3-hydroxy-2,6-bis-(3-methyl-but-2-enyl)-4-(3-methyl-butyryl)-5-oxo-6-(tetrahydro-pyran-2-yloxy)-cyclohexa-1,3-dienyl ester (123)



(125)

To a flask containing acetic anhydride (250 mg, 2.45 mmol) was added humulone THP ether **(74)** (mixture of diastereoisomers) (100 mg, 0.22 mmol) once all of the solids had dissolved, pyridine (4 drops) was added. This mixture was stirred at room temperature for 1 hour. After this time water (10 ml) was added and sodium carbonate (1 g) was added cautiously. This was stirred for 1 hour. After this time hexane (2 X 3 ml) was added and the aqueous layer discarded. The combined organic layers were washed with water (3 X 2 ml) and dried over sodium sulphate. Concentration gave a (125) as a brown oil. Chromatography on silica gel with pentane / ether)80 : 20 gave **(125)** as a pale yellow oil (102 mg, 93 %) υ_{max} **(ATR)/cm^{-1}** 2957.70, 1778.26, 1651.86, 1538.73, 1451.85, 1367.89, 1236.23, 1184.97, 1156.00, 1074.41 δ_H **(400MHz $CDCl_3$)** 0.93 – 1.00 (6H, m) 1.39 – 1.87 (18H m) 1.95 – 2.19 (2H, m) 2.21 (0.7H, s) 2.23 (1.8H, s) 2.28 (0.5H, s) 2.44 – 2.64 (1H, m) 2.69 – 2.88 (2H, m) 2.93 – 3.01 (2H, m) 3.11 – 3.36 (1H, m) 3.82 – 3.97 (1H, m) 4.40 (0.59H, m) 4,48 (0.27H, m) 4.64 (0.14H, m) 4.79 – 4.89 (1H, m) 4.94 – 5.03 (1H, m) 17.98 (0.03H, s) 18.21 (0.26H, s) 18.60 (0.15H, s) 18.97 (0.56H, s) δ_C **(100 MHz, $CDCl_3$)** ^{13}C collected but due to presence of 2 diastereomers each in two tautomeric forms the data was complex.

Acetic acid (R)-3,6-dihydroxy-2,6-bis-(3-methyl-but-2-enyl)-4-(3-methyl-butyryl)-5-oxo-cyclohexa-1,3-dienyl ester (125)



(125)

To a flask containing **(125)** (100 mg, 0.20 mmol) as a mixture of diastereoisomers was added THF (2 ml) acetic acid (4 ml) and water (1 ml) this mixture was stirred at ambient temperature for 3 hours, after this time hexane (5 ml) was added. The organic phase was removed and the aqueous fraction extracted with hexane (5 ml) the combined organic fractions were extracted washed with water (2 x 3 ml) and brine (5 ml) dried over sodium sulphate and concentrated in *vacuo* to yield (125) as pale yellow oil (75 mg, 90 %) υ_{max} **(ATR)/cm^{-1}** 3340.26, 2963.41, 1765.32, 1662.36, 1641.84, 1531.09, 1447.44, 1370.92, 1203.53 δ_H **(400MHz $CDCl_3$)** 0.94 (3H, d, J 6.72 Hz) 0.96 (3H, d, J 6.85 Hz) 1.54 (3H, d, J 1.07 Hz) 1.65 (3H, d, J 0.94 Hz) 1.74 (3H, d, J 1.21 Hz) 1.75 (3H, d, J 0.94 Hz) 2.10 (1H, sept, J 6.72 Hz) 2.14 (3H, s) 2.63 (2H, d, J 7.92 Hz) 2.76 (1H, dd, J 13.97 Hz, 6.58 Hz) 2.90 (1H, dd, J 13.97 Hz, 7.39 Hz) 3.15 (2H, d, 7.52 Hz) 4.96 (1H, tm, J 7.92 Hz, 1.48 Hz) 5.20 (1H, tm, J 7.25 Hz, 1.48 Hz) 19.11 (1H, s) δ_C **(100 MHz, $CDCl_3$)** 18.00 (q) 18.06 (q) 20.64 (q) 21.58 (t) 22.79 (q) 22.88 (q) 25.92 (q) 26.07 (q) 26.09 (d) 37.64 (t) 47.51 (t) 81.37 (s) 107.77 (s) 109.61 (s) 114.81 (d) 120.67 (d) 136.67 (s) 138.46 (s) 166.20 (s) 169.51 (s) 189.75 (s) 190.01 (s) 201.83 (s)

6.4 Manganese Chelation.

Mn^{2+} Solution solution A

To a 2 L volumetric flask was added manganese chloride (0.360178 g, 1.82 mmol), this was made up to 2L using distilled water to give a Mn^{2+} concentration of 50 ppm, 0.91mM.

Ligand solutions.

10 molar equivalents, solution B

To a 10 ml volumetric flask was added substrate (0.091 mmol) and made up to 10 ml with water saturated octanol.

15 molar equivalents, Solution C

To a 10 ml volumetric flask was added substrate (0.1365 mmol) and made up to 10 ml with water saturated octanol

1 molar equivalent Solution D

To a 10 ml flask was added an aliquot from solution A (1ml) using an auto pipette and this was made up to 10 ml using water saturated octanol.

The experiments were set up in triplicate as follows. To a 15 ml sample vial with a polythene cap was added manganese solution (2ml) and ligand solution (2ml) the solutions were mixed for a period of 15 hours on a roller mixer. After the mixing period was complete an aliquot of the aqueous phase was removed (1ml) using an auto pipette and made up to 10 ml in a volumetric flask with 5% HCl.

All results were recorded against a blank, no ligand in the octanol phase, and a standard calibration curve consisting of the following concentrations 1.75, 2.5, 5 and 10 ppm. All standard solutions were made by serial dilution from the 50 ppm stock solution.

Dilutions were analysed using AAS.

Chapter 7
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Chapter 8
Appendix

Table 1. Crystal data and structure refinement for lupulone (22).

Identification code	lupac	
Empirical formula	C26 H38 O4	
Formula weight	414.56	
Temperature	150(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2(1)/n	
Unit cell dimensions	a = 13.354(5) Å	α= 90°.
	b = 10.130(4) Å	β= 97.084(7)°.
	c = 18.473(7) Å	γ = 90°.
Volume	2479.7(17) $Å^3$	
Z	4	
Density (calculated)	1.110 Mg/m^3	
Absorption coefficient	0.073 mm^{-1}	
F(000)	904	
Crystal size	0.40 x 0.26 x 0.22 mm^3	
Theta range for data collection	1.78 to 25.00°.	
Index ranges	-15<=h<=15, -12<=k<=12, -21<=l<=21	
Reflections collected	17265	
Independent reflections	4356 [R(int) = 0.0981]	
Completeness to theta = 25.00°	100.0 %	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4356 / 0 / 281	
Goodness-of-fit on F^2	1.046	
Final R indices [I>2sigma(I)]	R1 = 0.0720, wR2 = 0.1884	
R indices (all data)	R1 = 0.0808, wR2 = 0.1955	
Largest diff. peak and hole	1.239 and -0.346 $e.Å^{-3}$	

Table 2. Hydrogen bonds for lupulone (22) [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(1)-H(1)...O(4)	0.84	1.63	2.397(3)	150.7

O(3)-H(3)...O(2)#1	0.84	1.91	2.701(2)	157.1

Symmetry transformations used to generate equivalent atoms:

#1 -x+3/2,y-1/2,-z+1/2

Table 3. Crystal data and structure refinement for colupulone.

Identification code	colacnr	
Empirical formula	C25 H36 O4	
Formula weight	400.54	
Temperature	150(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	Pbca	
Unit cell dimensions	a = 20.331(3) Å	α= 90°.
	b = 10.9190(18) Å	β= 90°.
	c = 21.327(4) Å	γ = 90°.
Volume	4734.4(13) $Å^3$	
Z	8	
Density (calculated)	1.124 Mg/m^3	
Absorption coefficient	0.074 mm^{-1}	
F(000)	1744	
Crystal size	0.31 x 0.23 x 0.19 mm^3	
Theta range for data collection	1.91 to 25.00°.	
Index ranges	-24<=h<=24, -12<=k<=12, -25<=l<=25	
Reflections collected	32137	
Independent reflections	4169 [R(int) = 0.0829]	
Completeness to theta = 25.00°	100.0 %	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4169 / 0 / 272	
Goodness-of-fit on F^2	1.089	
Final R indices [I>2sigma(I)]	R1 = 0.0549, wR2 = 0.1215	
R indices (all data)	R1 = 0.0745, wR2 = 0.1306	
Largest diff. peak and hole	0.237 and -0.173 $e.Å^{-3}$	

Table 4. Hydrogen bonds for colupulone [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(3)-H(3)...O(2)#1	0.84	1.94	2.6846(19)	146.9
O(1)-H(1)...O(4)	0.84	1.63	2.398(2)	151.0

Symmetry transformations used to generate equivalent atoms:

#1 -x+1/2,y+1/2,z

Table 5. Crystal data and structure refinement for natural humulone THP ether (76a).

Identification code	PC042	
Empirical formula	C26 H38 O6	
Formula weight	446.56	
Temperature	150(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 9.676(4) Å	α= 90°.
	b = 13.348(5) Å	β= 90°.
	c = 19.285(7) Å	γ = 90°.
Volume	2490.7(16) Å^3	
Z	4	
Density (calculated)	1.191 Mg/m^3	
Absorption coefficient	0.083 mm^{-1}	
F(000)	968	
Crystal size	0.29 x 0.20 x 0.17 mm^3	
Theta range for data collection	1.86 to 25.00°.	
Index ranges	-11<=h<=11, -15<=k<=15, -22<=l<=22	
Reflections collected	18012	
Independent reflections	2502 [R(int) = 0.0872]	
Completeness to theta = 25.00°	100.0 %	
Absorption correction	None	

Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2502 / 0 / 298
Goodness-of-fit on F^2	1.078
Final R indices [I>2sigma(I)]	R1 = 0.0424, wR2 = 0.0939
R indices (all data)	R1 = 0.0499, wR2 = 0.0967
Absolute structure parameter	0(10)
Largest diff. peak and hole	0.206 and -0.212 e.$Å^{-3}$

Table 6. Hydrogen bonds for 76a [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(1)-H(1)...O(4)	0.84	1.63	2.400(3)	151.9
O(3)-H(3)...O(2)#1	0.84	2.00	2.777(2)	153.2
O(3)-H(3)...O(5)	0.84	2.29	2.760(3)	115.3

Symmetry transformations used to generate equivalent atoms:

#1 x-1/2,-y+1/2,-z+2

Table 7. Crystal data and structure refinement for unnatural humulone THP ether (76a)

Identification code	pc049a	
Empirical formula	C26 H38 O6	
Formula weight	446.56	
Temperature	150(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 9.6889(16) Å	α= 90°.
	b = 13.365(2) Å	β= 90°.
	c = 19.318(3) Å	γ = 90°.
Volume	2501.4(7) $Å^3$	
Z	4	
Density (calculated)	1.186 Mg/m^3	
Absorption coefficient	0.083 mm^{-1}	
F(000)	968	

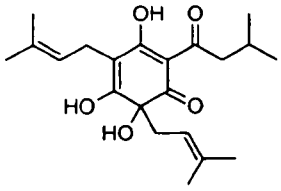
Crystal size	0.26 x 0.22 x 0.18 mm^3
Theta range for data collection	1.85 to 24.99°.
Index ranges	-11<=h<=11, -15<=k<=15, -22<=l<=22
Reflections collected	18137
Independent reflections	2508 [R(int) = 0.0642]
Completeness to theta = 24.99°	100.0 %
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2508 / 0 / 298
Goodness-of-fit on F^2	1.079
Final R indices [I>2sigma(I)]	R1 = 0.0384, wR2 = 0.0846
R indices (all data)	R1 = 0.0435, wR2 = 0.0866
Absolute structure parameter	0(10)
Largest diff. peak and hole	0.173 and -0.139 $e.Å^{-3}$

Table 8. Hydrogen bonds for 76a [Å and °].

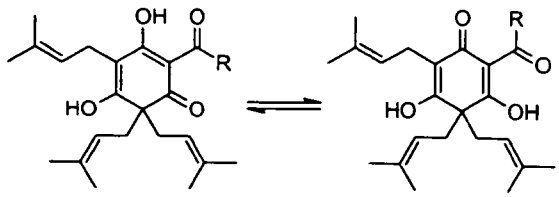
D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(1)-H(1)...O(4)	0.84	1.63	2.405(2)	151.8
O(3)-H(3)...O(2)#1	0.84	2.00	2.781(2)	153.9
O(3)-H(3)...O(5)	0.84	2.30	2.763(2)	115.4

Symmetry transformations used to generate equivalent atoms:

#1 x+1/2,-y+3/2,-z+2

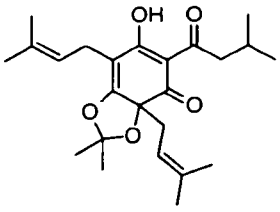


Humulone (8)

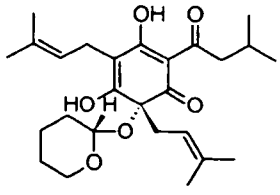


Lupulone (22) R = $CH_2CH(CH_3)_2$

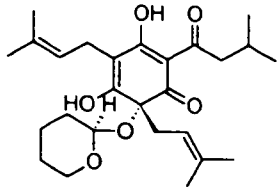
Colupulone (50) R = $CH(CH_3)_2$



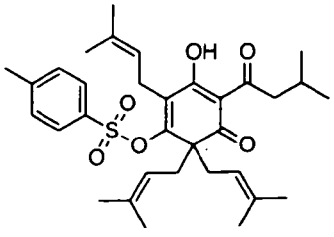
Humulone Acetonide (126)



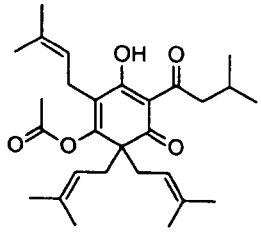
Humulone THP ether (76_a)



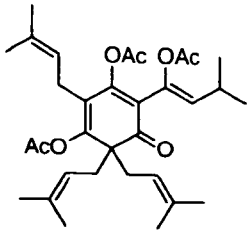
Humulone THP ether (76_b)



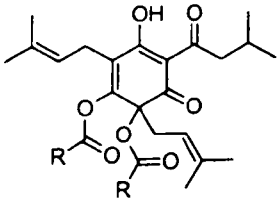
Lupulone tosylate (112)



Lupulone monoacetate (104)



Lupulone triacetate (102)



R = CH_3 (77)

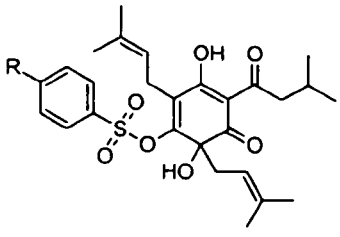
= C_2H_5 (106)

= C_3H_7 (107)

= C_4H_9 (108)

= C_5H_{11} (109)

= C_6H_{13} (110)



R = CH_3 (111)

= CF_3 (113)

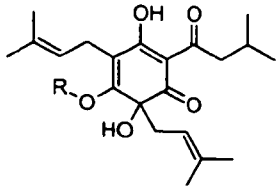
= NO_2 (114)

= Cl (115)

= Br (116)

= I (117)

=Ph (118)



R = CH_3 (76)

= C_2H_5 (120)

= (C_3H_7) (121)