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CHEMISTRY OF HUMULENE EPOXIDES

A Thesis

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Doctor of Philosophy

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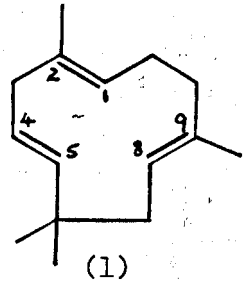
CONTENTS

INTRODUCTION

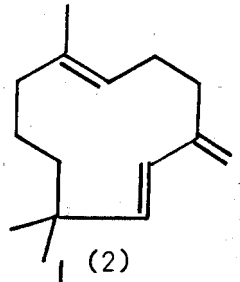
During the latter half of the 19th century there was considerable interest amongst chemists in those compounds derived from natural sources. In particular, the essential oils of a number of plants and related species provided a rich source of such compounds, principally of the terpene type. Within this group of naturally-occurring compounds the C_{15} sesquiterpenes were of particular interest largely as a result of their relatively high abundance. In 1895, Chapman[1] presented evidence of a new sesquiterpene, humulene, which he isolated from oil of hops. By the preparation of various derivatives, he was able to distinguish this hydrocarbon, previously called α-caryophyllene, from caryophyllene. However, largely because of the lack of spectroscopic techniques, it was not until much later that the structure of humulene was solved. Although it was commonly accepted in the early 1950's that humulene embodied an eleven-membered ring with three double bonds, the exact juxtaposition of these olefinic linkages was the subject of much debate.[2-4]

In 1960, Dev[5], on analysis of the nmr spectrum of humulene, concluded that the characteristic resonance expected for an exo-methylene group was absent and therefore it was proposed that the olefinic linkages in humulene were all endocyclic. In an attempt to purify humulene, the bis-silver nitrate adduct was isolated and recrystallised[6] and pure humulene was regenerated by treatment of the adduct with aqueous ammonia solution. Careful

chemical degradation of the hydrocarbon[7] led to the proposal that humulene had an all-trans double bond system and this was finally substantiated by X-ray analysis[8] of the bis-silver nitrate adduct which revealed the correct structure (1). It is now clear that although oil of hops is one of the richest sources of this hydrocarbon, humulene (1) is, in fact, a ubiquitous sesquiterpene in Nature and has been isolated from a wide variety of plant sources. More recently Bohlmann et al., in particular, have demonstrated the widespread occurrence of an isomer of humulene (1), namely γ-humulene (2).[9] Many functionalised compounds related to humulene (1) have also been found in natural



(1)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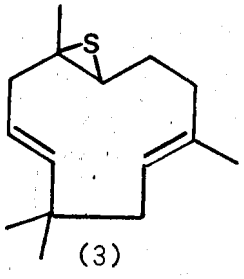

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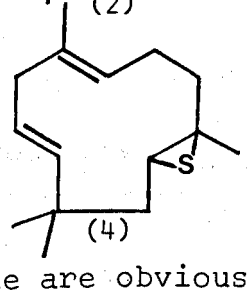
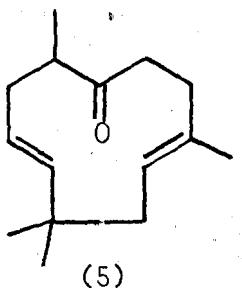


(4)

sources and while some are obviously artefacts produced by external influences, others are genuine plant metabolites. Two episulphides of humulene (1) have been discovered on careful investigation of hop oil and these have been assigned the structures (3) and (4).[10] It is thought that these arise as a direct result of spraying the hops with preparations containing flowers of sulphur. Steam distillation of hop oil[11] and also extraction of the water soluble compounds obtained from boiling hops[12] produced oxygenated compounds such as humuladienone (5)

and humulenol-I (6). Other oxygenated humulene derivatives



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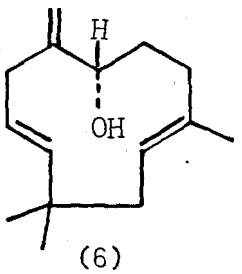

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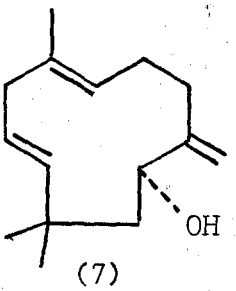

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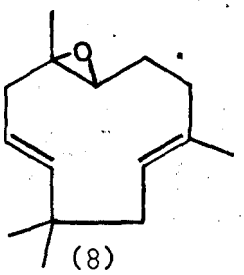

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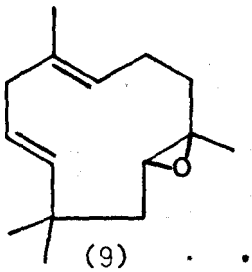

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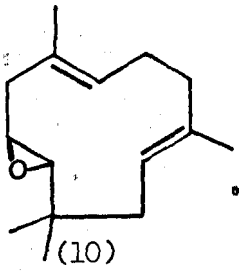

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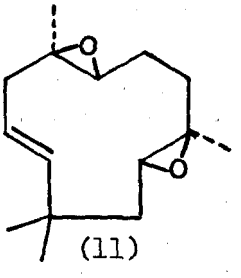
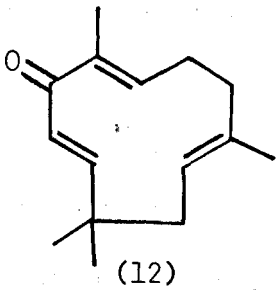


(11)

have been isolated from Zingiber zerumbet Smith[13] and some of these are illustrated by structures (7) - (11).

Zerumbone[5,14] (12) was also isolated from the same source and Chhabra et al.[15,16] have isolated the zerumbone epoxides (13) - (15) and zerumbol (16), all of which display plant growth regulation properties.

A Russian group have also isolated a series of naturally-occurring humulene derivatives although the structural evidence for these compounds is not entirely convincing. The sesquiterpenes juniferin (17a) and juniferinin (17b) were isolated from Ferula juniperina,[17] ferocin (18a), ferocinin (18b),



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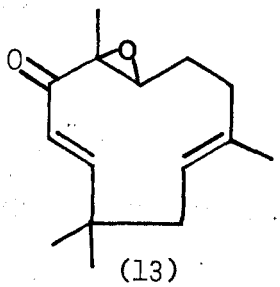

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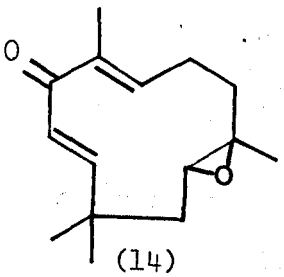

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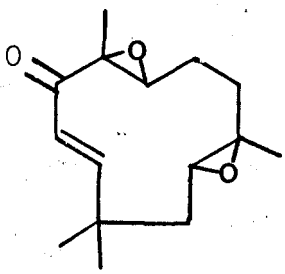

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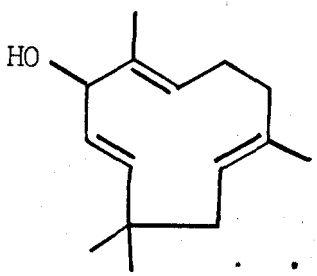

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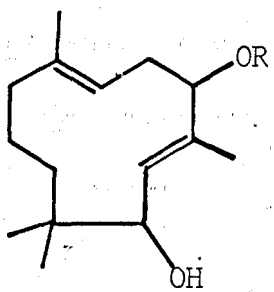
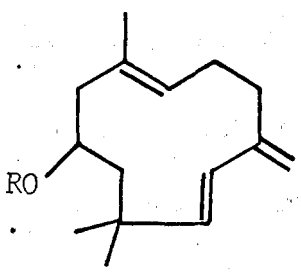
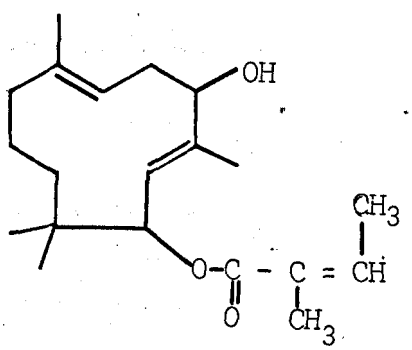
and fecerol (18c) from Ferula ceratophylla[18] and fekserin (19) from Ferula xeromorpha.[19]



(17a) R=vanilloyl
(17b) R=p-HOC_6H_4CO



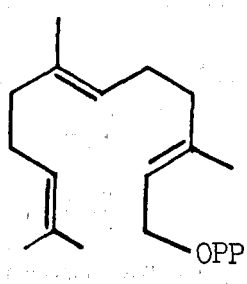
(18a) R=vanilloyl
(18b) R=p-HOC_6H_4CO
(18c) R=H



(19)

The biogenesis of humulene (1) was proposed initially in terms of the Biogenetic Isoprene Rule[20] and subsequent

embellishments were added later.[21] Thus, the cyclisation of farnesyl pyrophosphate (20) via the intermediate cation (21) to give humulene (1) after deprotonation and subsequently to give caryophyllene (22) by a further cyclisation and deprotonation was postulated as the probable route to these compounds based on the fact that both compounds usually co-exist in Nature.



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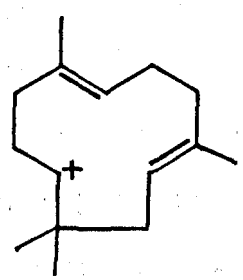

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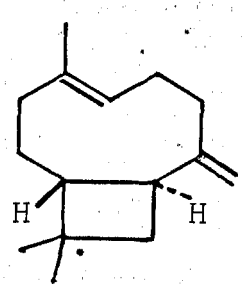
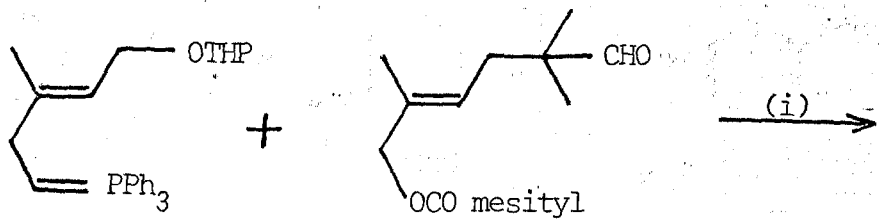


(22)

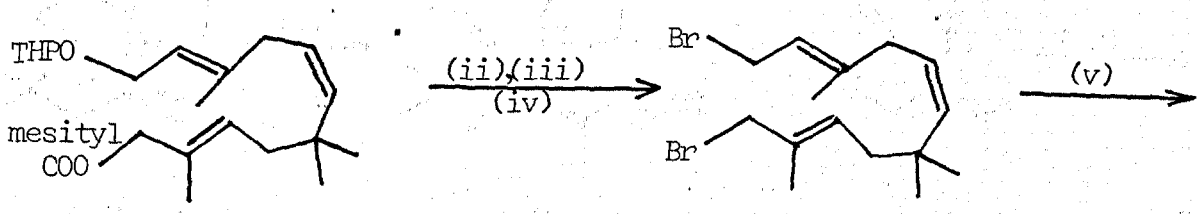
Before proceeding to the chemistry of humulene (1) it should be noted that three syntheses of the hydrocarbon have been recorded. The motivation behind these syntheses has been the challenge inherent in constructing a large monocyclic compound containing three trans-double bonds and some very elegant work and new methodology have arisen from these studies. The earliest synthesis, reported in 1967,[22] is shown in Figure 1. The two compounds (23) and (24), prepared by a number of previous steps, were coupled by a standard Wittig reaction to give, after subsequent reaction, the dibromide (26). The key step in the synthesis was the cyclisation of the dibromide using nickel carbonyl, a method which had been devised by Corey for the cyclisation of allylic dibromides. The major product obtained

was the cis-isomer (27) of humulene (1) which on irradiation in the presence of diphenyl disulphide gave the required product (1), separable from unchanged (27) by preparative glc. In 1976, an Indian research team[23] published a synthesis of humulene (1) employing Corey's nickel carbonyl cyclisation step but initially introducing the trans-disubstituted double bond before achieving ring closure. The reaction sequence can be seen in Figure 2. In going from the keto-ester (33) to the diester (34) no separation of the all-trans-diester from the cis-trans-isomer was carried out because at the cyclisation stage, both yield the same product. It would appear from the experimental details that the reaction sequence proceeded smoothly and in good yield.

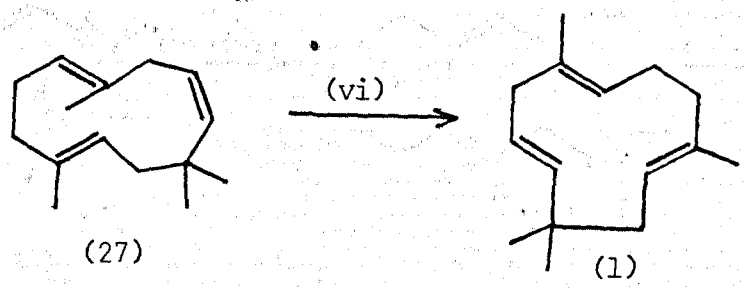
The third synthesis, carried out by a group of Japanese workers,[24] embodies a highly stereoselective approach and is shown in Figure 3. There are several interesting points which arise from this sequence. The use of a π-allyl palladium complex to trigger intramolecular anionic cyclisation neatly forms the eleven-membered ring, this method having been extensively developed by Trost.[25] The key intermediate (36) possesses the two trisubstituted double bonds necessary to prepare humulene (1) and the method of introducing the disubstituted trans-double bond via an oxetane (37) involved the development of new methodology using diethylaluminium-N-methylanilide. Previous observations by the same group had shown that oxetanes opened in regio- and stereospecific manner to form the corresponding allylic alcohols. This was explained in terms of a cyclic syn-elimination mechanism where the aluminium coordinates to the oxygen of the oxetane and the nitrogen binds to a proton on the adjacent carbon. As a result, very high stereoselectivity (∿ 99%) was observed in the formation of the olefin.



(23) (24)



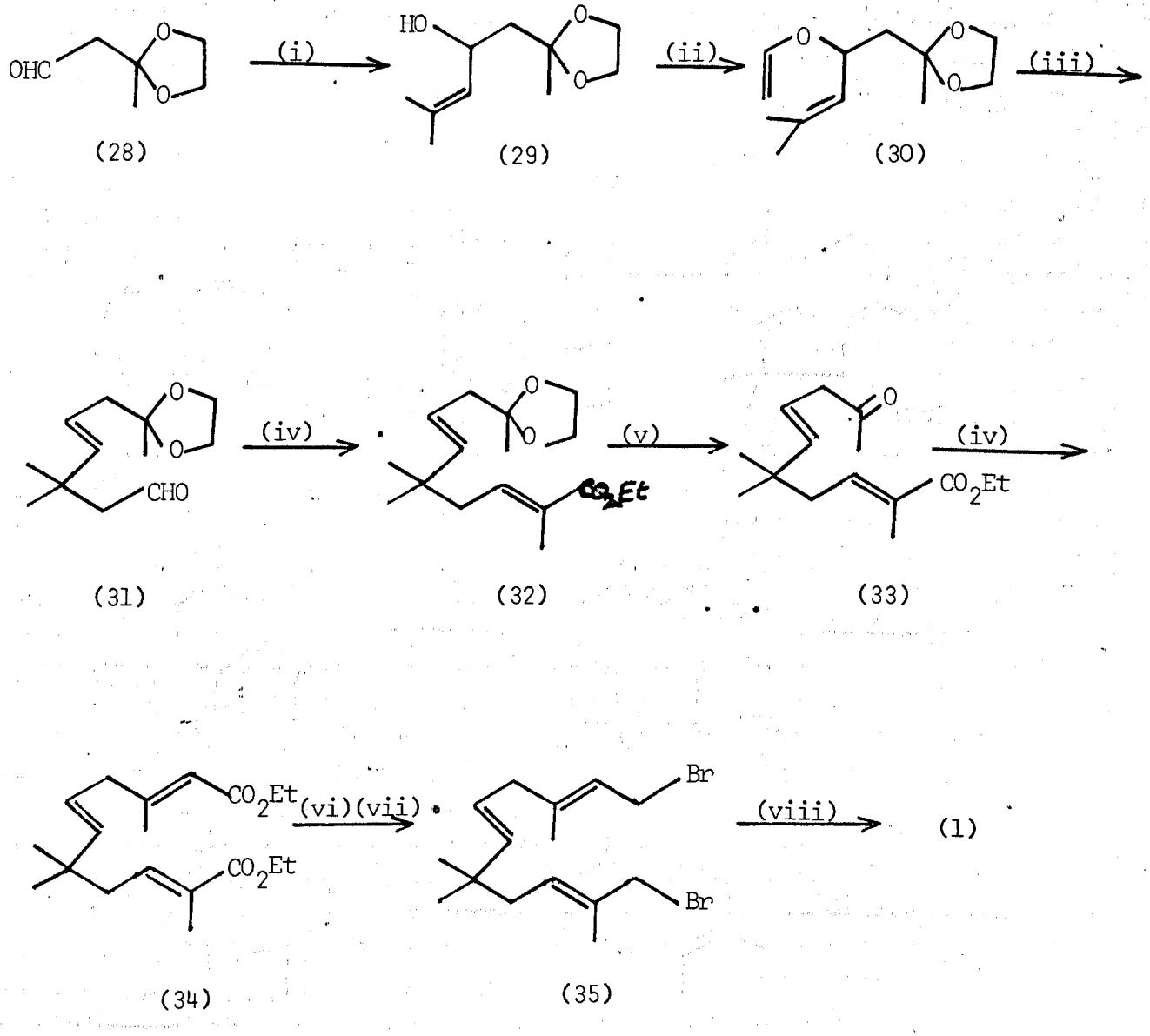
(25) (26)

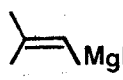
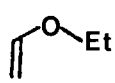


(27) (1)

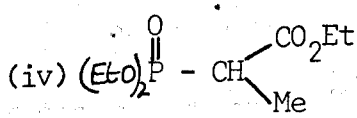
Reagents: (i) Base
(ii) $LiAlH_4/Et_2O$;
(iii) $MeOH/H^+$;
(iv) PBr_3;
(v) $Ni(CO)_4$/N-methylpyrrolidone/50°C;
(vi) hν/PhSSPh/C_6H_{12}.

Figure 1



Reagents: (i)  /THF; (ii)  /$Hg(OAc)_2$;

(iii) 190-195°C/N_2;

(iv)  /NaH/diglyme;

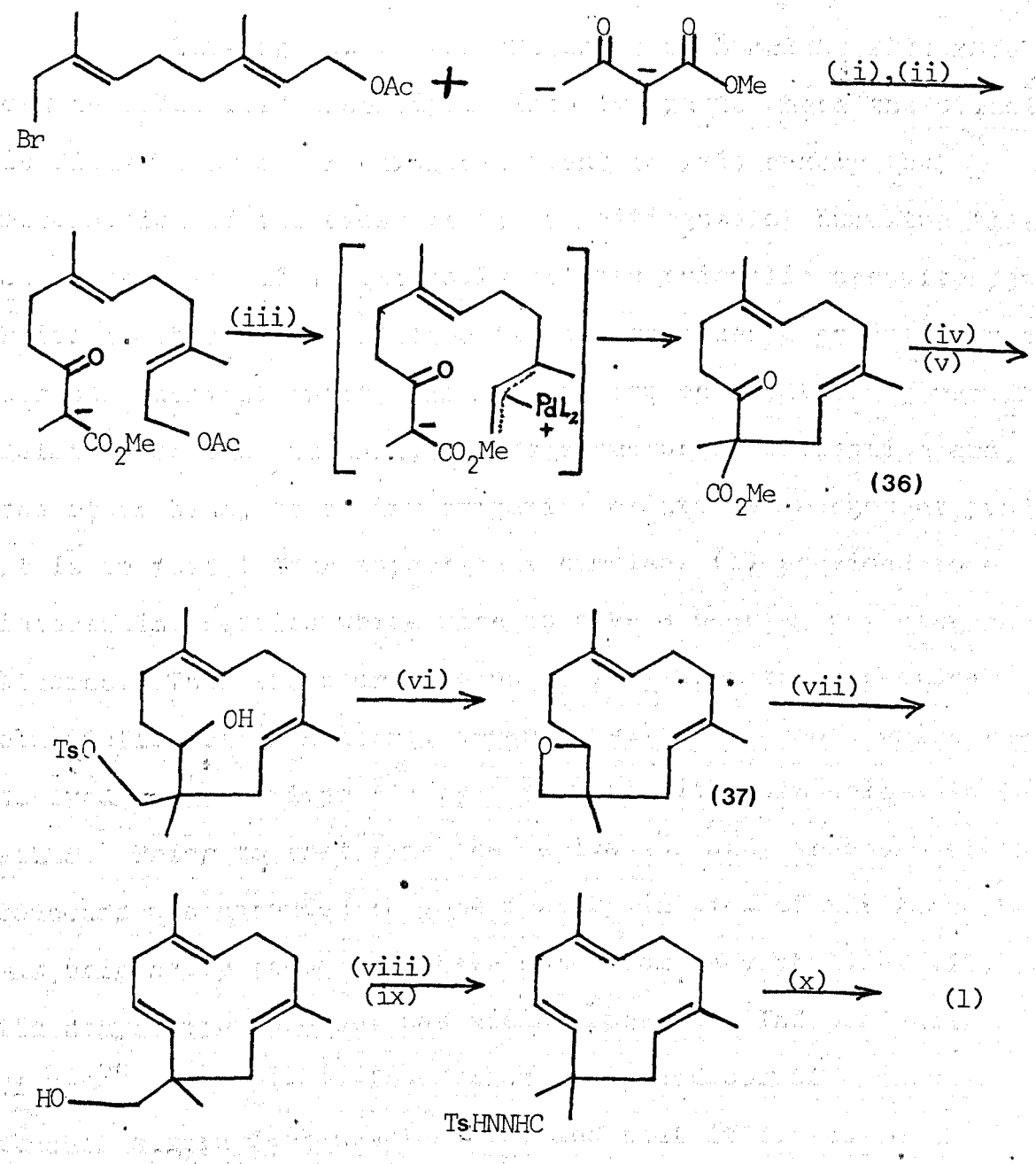
(v). 10% HCl/acetone;

(vi) $LiAlH_4$;

(vii) PBr_3/pyr./Et_2O;

(viii) $Ni(CO)_4$/dry DMF

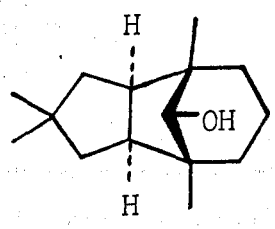
Figure 2



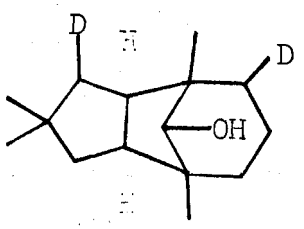
Reagents: (i) THF/0°C; (ii) Ac_2O/pyr.; (iii) $(Ph_3P)_4Pd/Ph_2P$⌒PPh_2/ HMPA/THF/reflux; (iv) $LiAlH_4$; (v) TsCl/pyr.; (vi) K-OBu^t/THF; (vii) $Et_2AlNMePh/C_6H_6$/reflux; (viii) Me_2S/NCS/Et_3N; (ix) $TsNHNH_2$/EtOH/reflux; (x) $LiAlH_4$/dioxane.

Figure 3

Turning now to the chemistry of humulene (1), this can be subdivided essentially into two parts where the division is dictated by a chronological event in 1965 namely the recognition of the biosynthetic significance of humulene (1) as a precursor of structurally related tricyclic sesquiterpenoids. Prior to this date, humulene (1) was considered principally as a curious chemical entity and much of its chemical study was related, on the one hand, to its structural elucidation and, on the other hand, to an investigation of its rearrangement products. It is on this latter aspect that humulene (1) provided some interesting results which were to have a bearing on later chemical studies. Thus the story begins in 1964 with the structural elucidation of an alcohol, α-caryophyllene alcohol, which was derived from humulene (1) by treatment with sulphuric acid in ether. Prior to that time the derivation and structure of this compound was shrouded in some mystery in view of the fact that it was originally thought to have come from caryophyllene (22) and its dehydration product was misinterpreted. Independently Nickon[26] and Parker[27] found that the precursor of α-caryophyllene alcohol was in fact humulene (1) and that it possessed the beautifully symmetrical structure (38). Later on Nickon[28] coined



(38)



(39)

the name apollan-11-ol for this compound on the grounds that it avoided confusion about its genesis and secondly it commemorated the historic Apollo-11 moon shot (structure (38) should be turned 90° in an anticlockwise manner to appreciate its resemblance to a three-stage rocket). The mechanism proposed[2] for the formation of apollan-11-ol (38) from humulene (1) is outlined in Figure 4. This was later investigated by deuterium labelling, coupled with ^{13}C nmr spectroscopy.[29] It was found

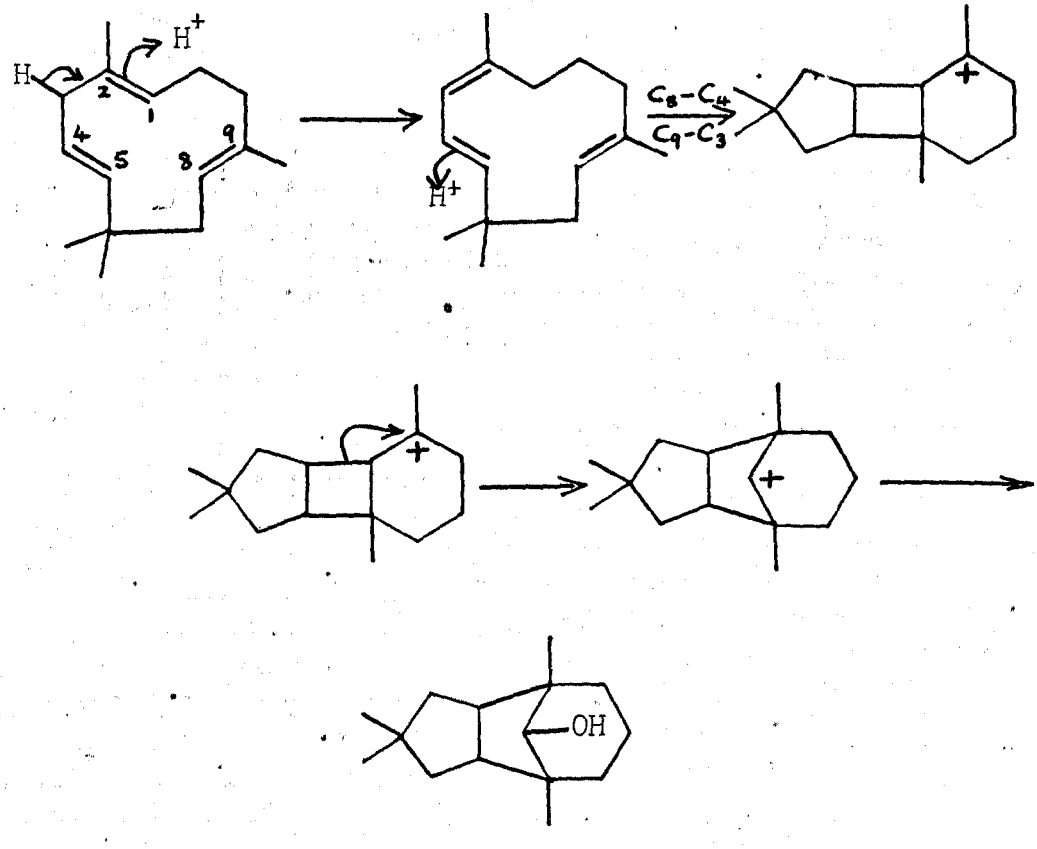
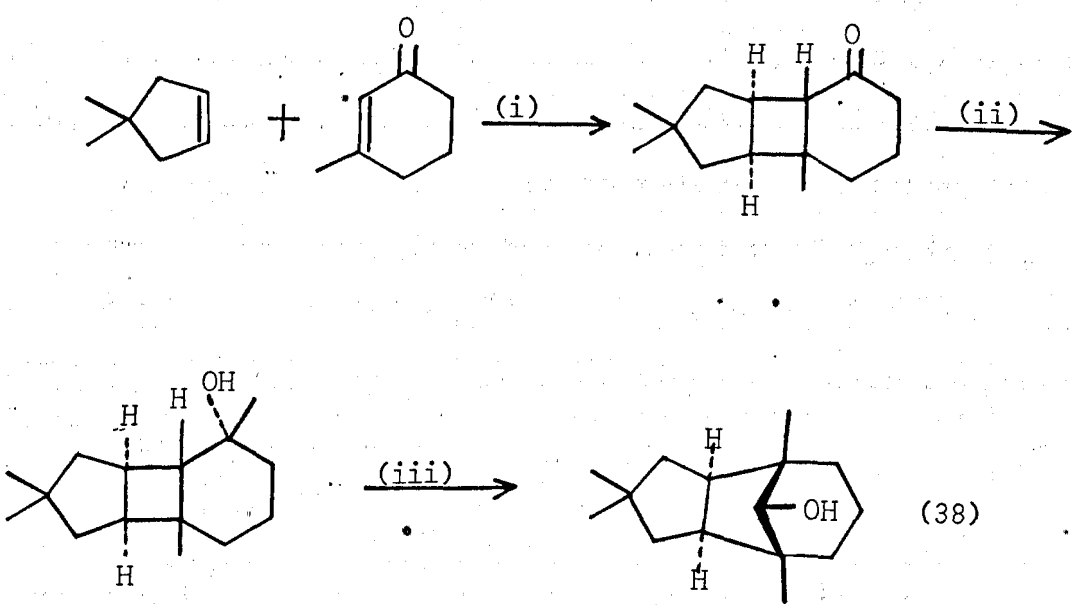


Figure 4

that the labelled alcohol (39) was formed, the labelling pattern being consistent with initial deuterium incorporation at C-1 of humulene (1), the second label being introduced at C-5 and the subsequent cyclisations and rearrangement occurring as depicted

in Figure 4. Further evidence, although not absolute, was obtained when Corey[30] carried out a simple synthesis of apollon-11-ol (38) as shown in Figure 5. The synthesis followed the proposed mechanistic pathway in Figure 4 and gave the product with the correct stereochemistry.

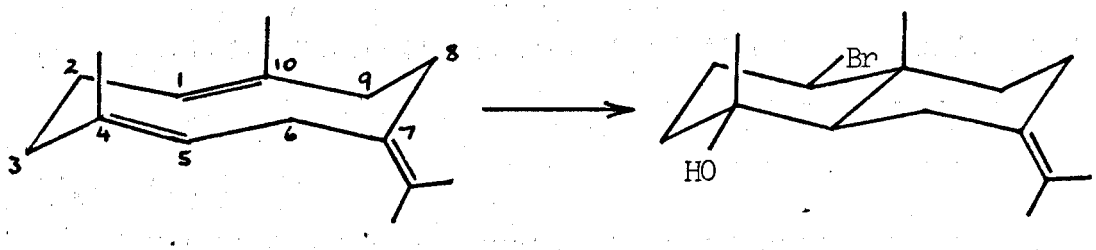


Reagents: (i) hν; (ii) MeLi; (iii) H_2SO_4

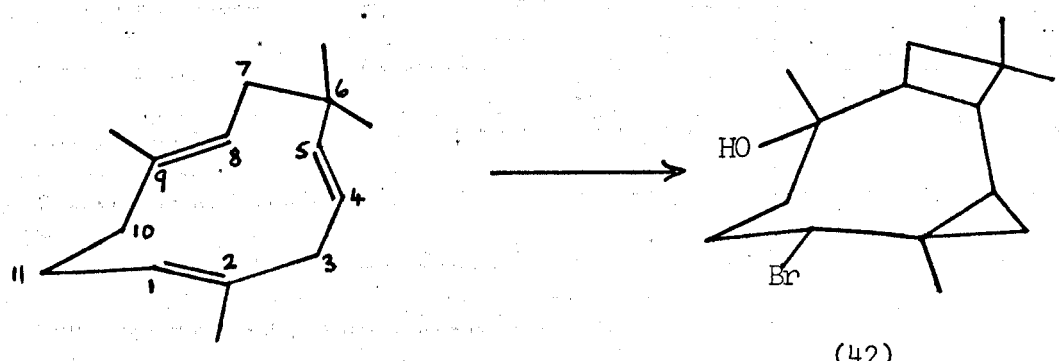
Figure 5

Pertinent to the chemistry of humulene (1) is the work of Sutherland *et al.* who studied the stereoselectivity in the cyclisation of medium ring 1,5-dienes on the basis of preferred conformations according to a classification dependent

on whether cyclisation between C-1 and C-6 of the diene would give a chair cyclohexane (C) or a twist-boat (T) ring conformation.[31] They rationalised the regiospecificity of the reactions on the basis of studies of the X-ray structures of the silver nitrate adducts of germacratriene (40)[32] and humulene (1)[8] from which the relative strain energies of the double bonds within each compound were calculated.[33] The preferred reactivity of any particular double bond was expressed in terms of sp^3-sp^2 and sp^2-sp^2 torsional strain, the double bond having the highest torsional strain being the most reactive i.e. it reacts to release the inherent strain. Both germacratriene (40) and humulene (1) were cyclised with aqueous N-bromosuccinimide,[34,35] the products (41) and (42) maintaining the anticipated conformations of their respective starting compounds.



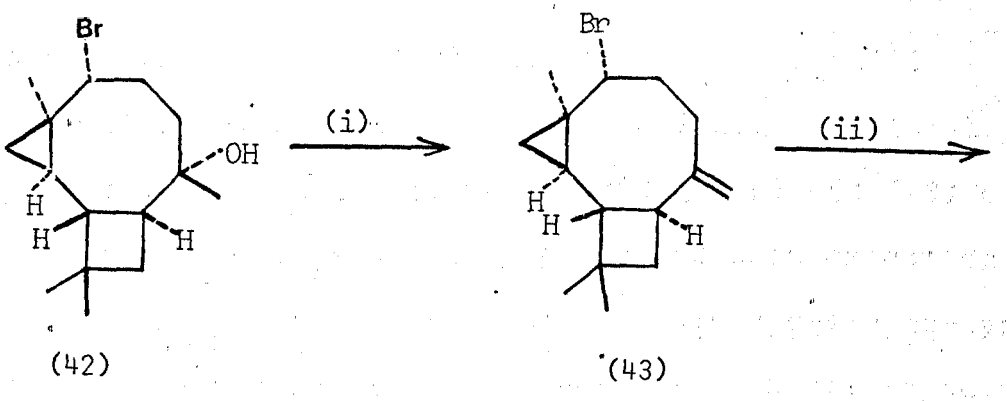
(40) (41)



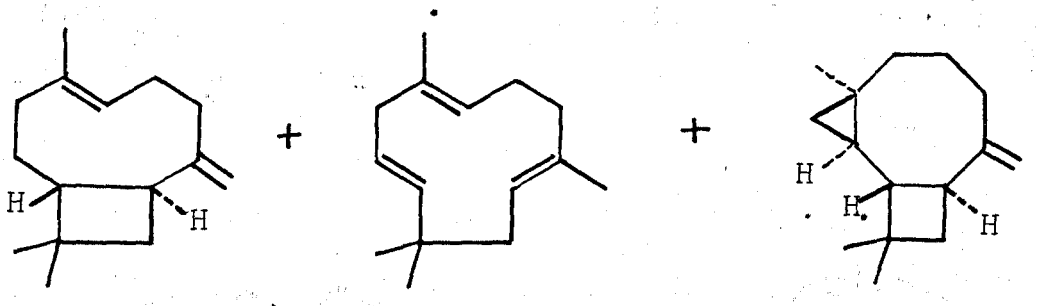
(1) (42)

Cyclisation therefore is initiated by electrophilic attack on the $\Delta^{1,10}$ double bond of germacratriene (40) and on the $\Delta^{1,2}$ double bond of humulene (1) followed by participation of the neighbouring π bonds. The strain calculations from the X-ray studies corresponded to this order of reactivity. Regarding stereochemistry, the theory concerning the conformation classification states that if a cyclisation of a C conformation occurs then the ring-junction will be trans- and for T the cis-fused ring is produced. In the case of germacratriene (40), this is confirmed as it has CC conformation.[36] By notation, this means that after cyclisation to form the two cyclohexane rings in (41), the first letter denotes the conformation of the ring from C-10 to C-5 and the second letter denotes the conformation of the ring from C-5 to C-10. Humulene (1) also cyclises with high stereoselectivity and gives a trans-fused and cis-fused ring in accord with its then accepted CT conformation. Noteworthy is the fact that further chemical treatment of the bromohydrin (42) yields, amongst other products, caryophyllene (22). This is shown in Figure 6.

The other products observed were humulene (1) and the tricyclic compound (44). It was proposed that the bicyclobutonium ion (45) was the intermediate, the hydride ion either attacking C-2 with the subsequent cleavage of the C-2 - C-4 bond to give caryophyllene (22) or alternatively the hydride ion attacking the exo-methylene double bond with subsequent opening of the cyclobutyl and cyclopropyl rings to give humulene (1). Whatever the mechanism, the formation of both (1) and (22) is stereospecific, and this sequence effects a conversion of humulene (1) to caryophyllene (22).



(42) (43)

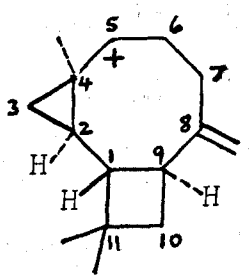


(22) (1) (44)

Reagents: (i) $POCl_3$/pyr;

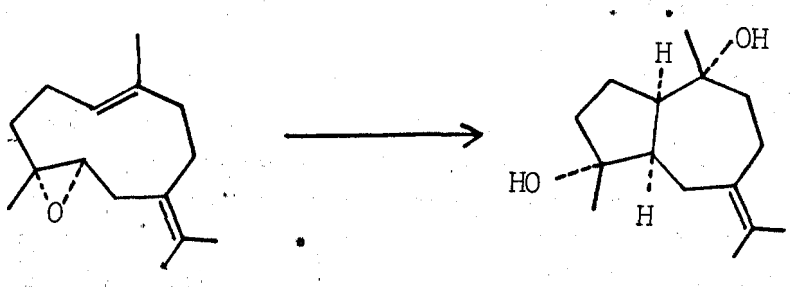
(ii) $LiAlH_4$/THF.

Figure 6

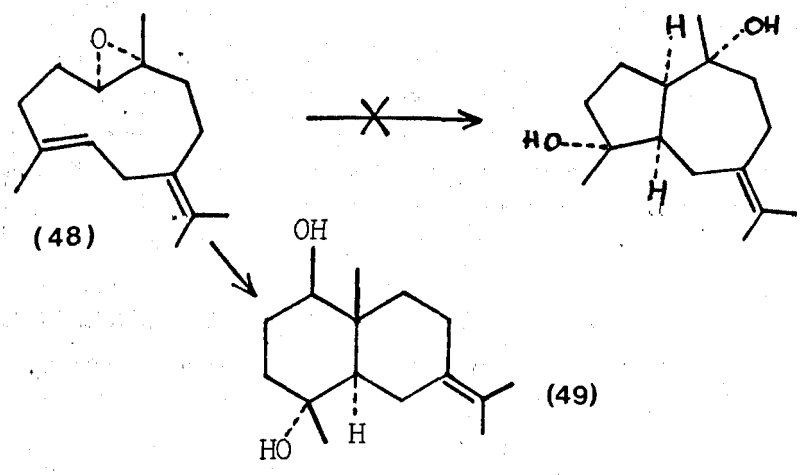


(45)

The same group then extended their investigations by cyclising germacratriene (40) with other electrophilic and radical reagents affording selinane-type derivatives.[37] Sutherland[38] attempted the cyclisation of the epoxides (46) and (48) of germacratriene (40), produced by peracetic acid oxidation, epoxide (46) being the major isomer. Again a selinane-type derivative (49) was realised on treatment with dilute sulphuric acid but only from the 1,10-epoxide (48) whereas the 4,5-epoxide (46) gave a guaiane-type derivative (47). These results were in



(46) (47)

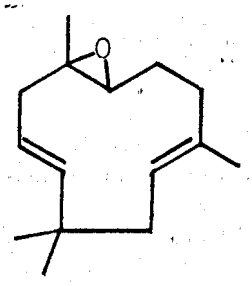


(49)

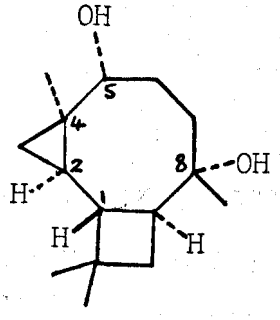
keeping with the epoxides reacting in the crown or CC conformation, the direction of ring closure being considered in terms of the steric strain of the transition state generated in Markovnikov

and/or anti-Markovnikov additions.

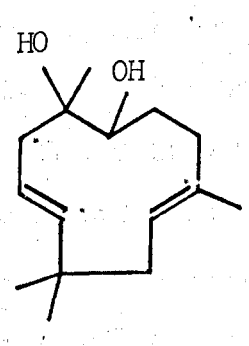
Although displaced from its chronological subdivision of humulene chemistry, the results of the acid catalysed rearrangement of humulene-1,2-epoxide (8) are pertinent at this point since they relate closely to the study by Sutherland et al. on the reactions of humulene (1) with hypobromous acid. In 1970 it was reported that the acid-catalysed cyclisation of humulene-1,2-epoxide (8), the major product of peracid monoepoxidation of humulene (1), produced the tricyclic diol (50) along with humulenol (6) and the diol (51).[39] Recently a group of Japanese workers[40,41] have reinvestigated this reaction and isolated many



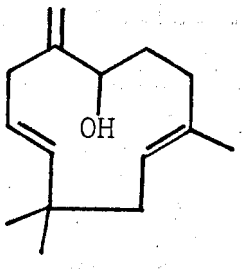
(8)



(50)



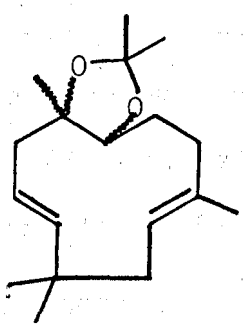
(51)



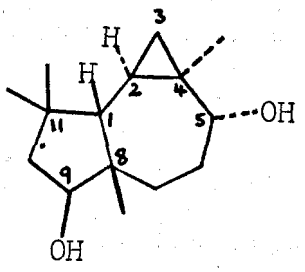
(6)

more compounds. A time-dependent product analysis using glc

indicated that the epoxide (8) initially cyclised to the tricyclohumuladiol (50) which then decyclised or rearranged to give the products (6) and (51) - (53). This study was carried out by isolating the tricyclohumuladiol (50) and then treating it with 1.8M sulphuric acid in acetone. As can be seen, a complex mixture of compounds is produced in this reaction. The novel compound (53) is thought to be produced by loss of the hydroxyl group at C-8 in diol (50) with subsequent rearrangement



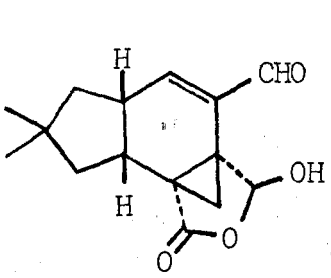
(52)



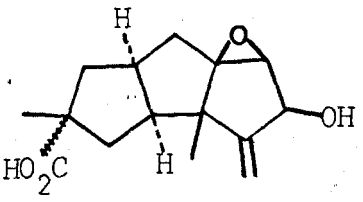
(53)

to give the product (53). Tricyclohumuladiol (50) has been isolated from hop oil[42] but it is felt that the tricyclohumuladiol (50) is an artefact of the isolation procedure since it was not isolated as an optically active material. It is known that the three humulene monoepoxides are found optically active in Nature.[13]

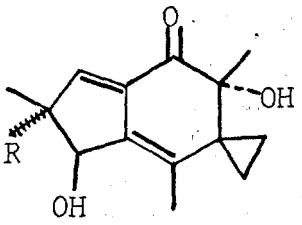
The year 1965 saw a turning point in the study of humulene chemistry since that year heralded the structural elucidation of four fungal metabolites, marasmic acid (54),[43] hirsutic acid (55)[44] and illudins M (56)[45] and S (57)[45]. The tricyclic carbon skeletons of all of these could be derived formally from humulene (1) by intramolecular cyclisations and subsequent rearrangements. Since that time the isolation and structural determination of an ever increasing number of naturally-occurring compounds, largely of



(54)



(55)

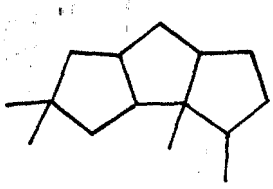


(56) R = Me
(57) R = CH_2OH

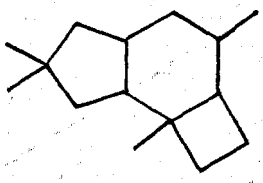
fungal origin, have served to highlight the importance of humulene (1) as a key sesquiterpenoid building block.

The present range of compounds considered to be derived from humulene (1) can be classified under six general skeletal types i.e. hirsutane (58), protoilludane (59), marasmane (60), illudane (61), africane (62) and pentalane (63), the majority of which are found as fungal metabolites. Mechanistically these skeletal types can be derived from humulene (1) by initial protonation of the $\Delta^{4,5}$ double bond followed by subsequent cyclisation involving the remaining double bonds within the molecule.[46] The modes of cyclisation necessary to produce the various tricyclic structural types are summarised in Figure 7.

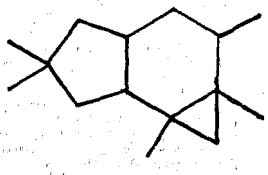
An important observation on studying the cyclisation pathways is that the proposed protoilludyl cation (59) plays a major rôle in most of the biogenetic schemes. Five of the six skeletons can be derived via this intermediate although alternative routes to the hirsutane (58) and pentalane (63) types can be postulated. Research using radiolabelled precursors has been carried out extensively in an effort to assess the feasibility of the biogenetic proposals and an overview of this work follows.



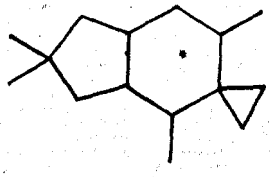
(58)



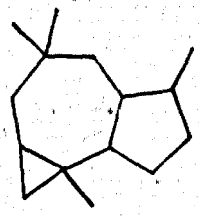
(59)



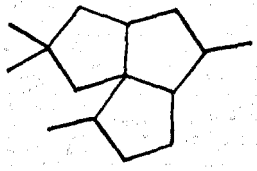
(60)



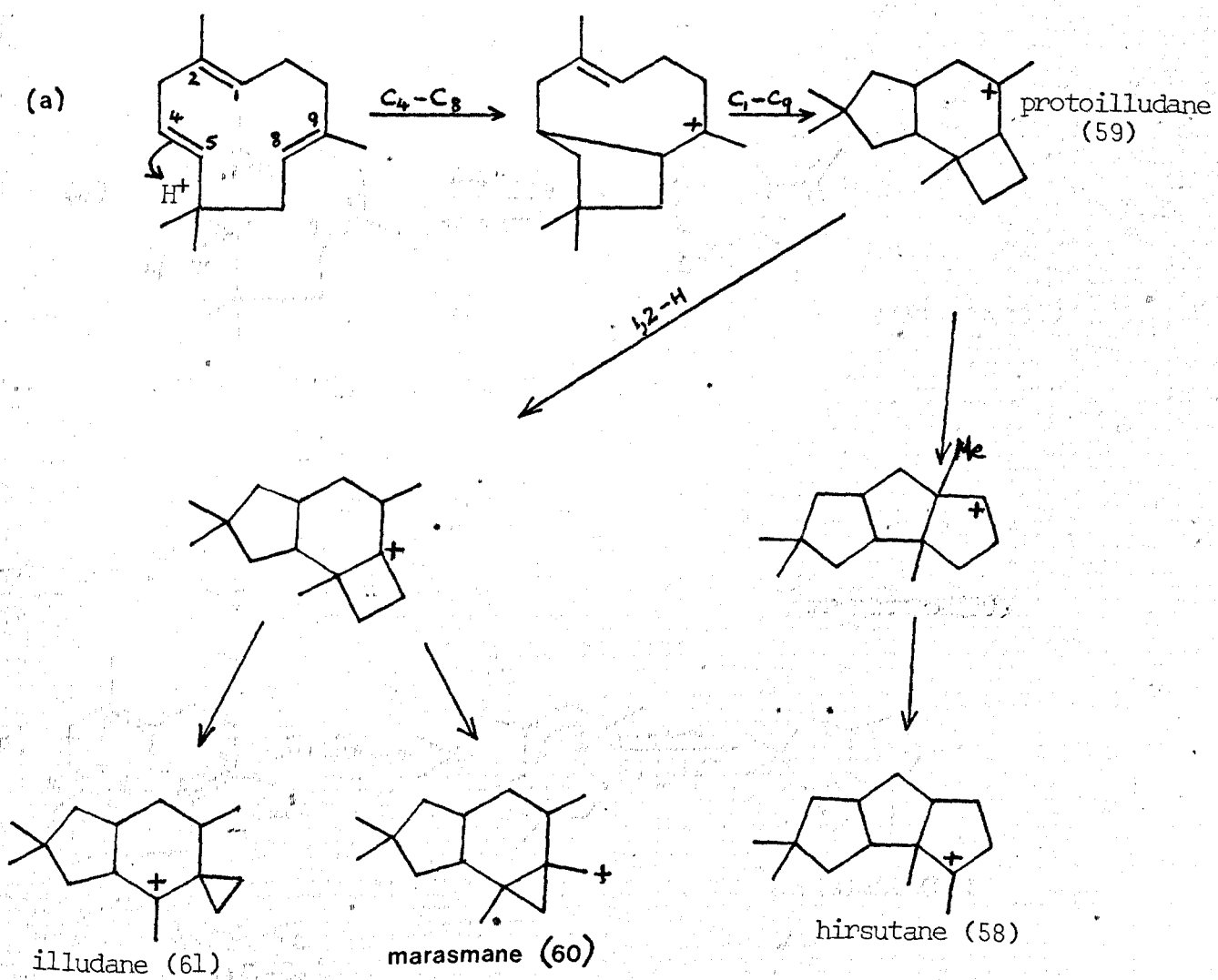
(61)

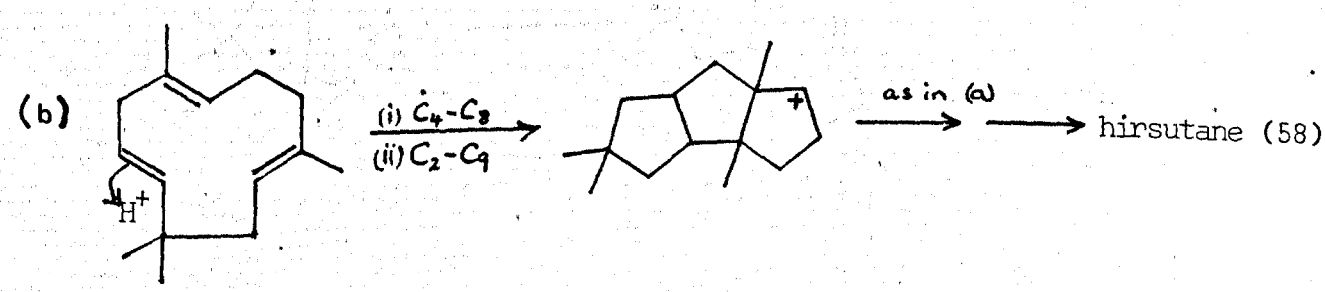


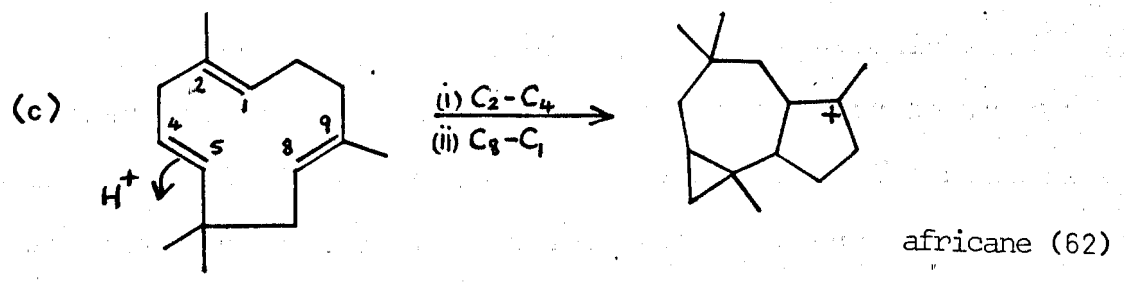
(62)



(63)


(a)
C_4-C_8
C_1-C_9
protoilludane (59)
1,2-H
Me
illudane (61)
marasmane (60)
hirsutane (58)


(b)
(i) C_4-C_8
(ii) C_2-C_9
as in (a)
hirsutane (58)


(c)
(i) C_2-C_4
(ii) C_8-C_1
africane (62)

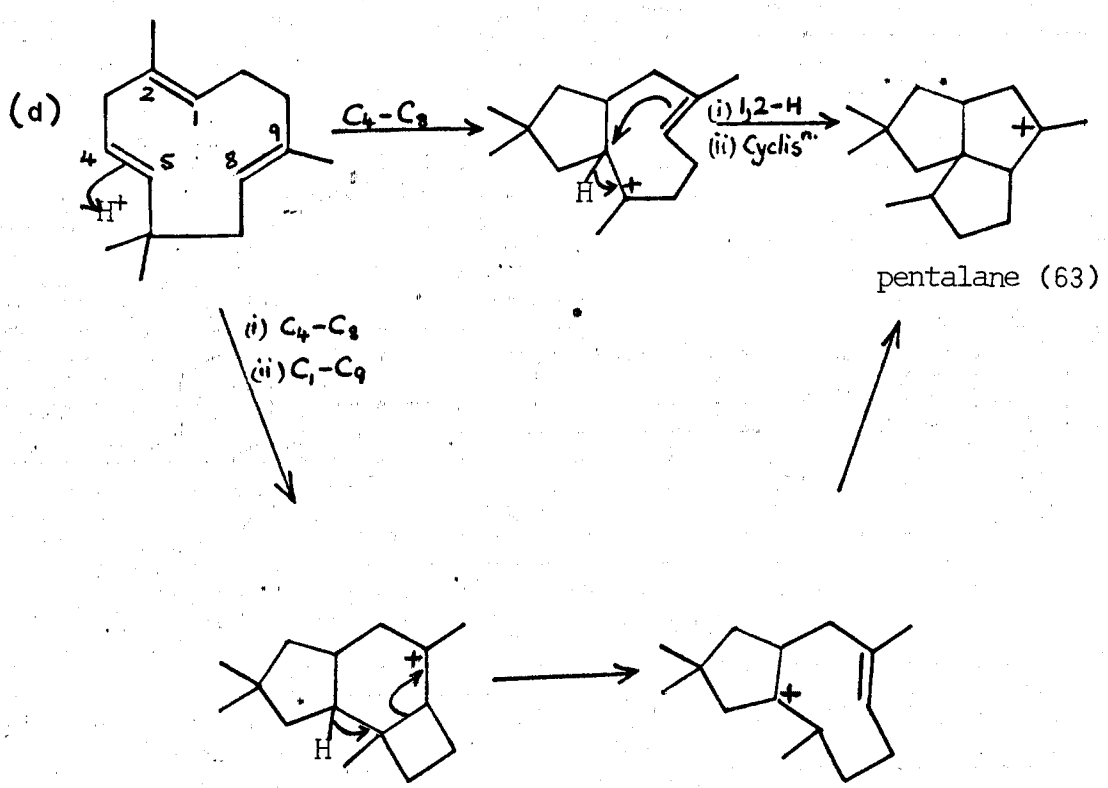
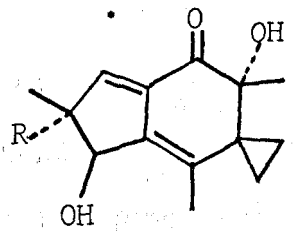
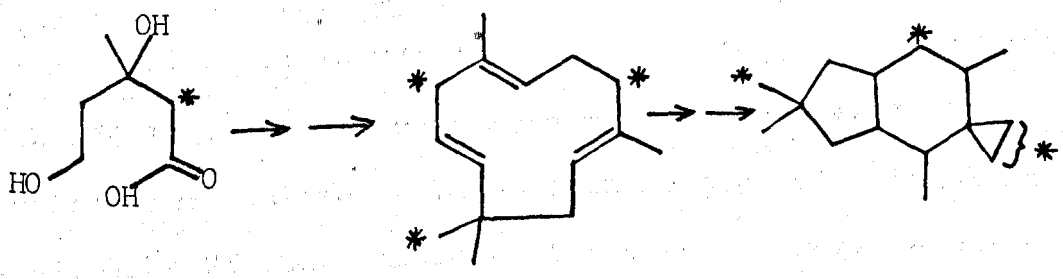

(d)
C_4-C_8
(i) 1,2-H
(ii) Cyclisn.
pentalane (63)
(i) C_4-C_8
(ii) C_1-C_9

Figure 7

Illudin M (56) and S (57) were isolated from cultures of Clitocybe illudens fed with [2-^{14}C] mevalonic acid[47] (64), degradative experiments showing the labels to be in the anticipated sites based on a humulene type intermediate (65) as shown in Figure 8. The absolute configuration of the illudins was established by X-ray analysis.[48]



(56) R = Me
(57) R = CH_2OH



(64) (65)

Figure 8

Further studies[49,50] on illudin M (56) using double labelling techniques incorporating [2-$^3H_2$, 2-^{14}C] and [4(R)-4-^{3}H] mevalonate confirmed the results of Anchel et al.[47] but also raised some questions as to the precise nature of the proposed mechanistic route. The conclusion of this group was that the cyclisation of humulene may be non-concerted perhaps

with a partial mechanism as seen in Figure 9.

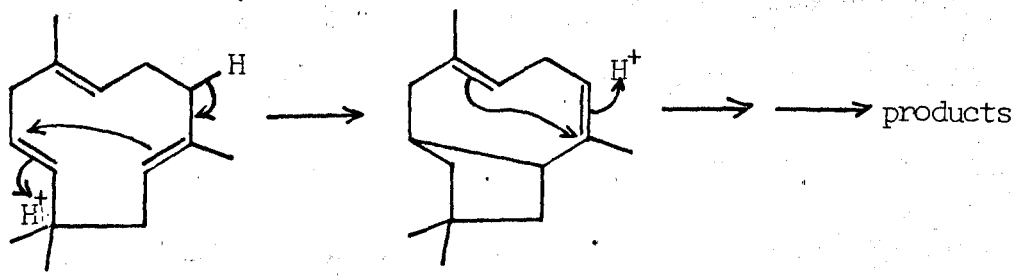
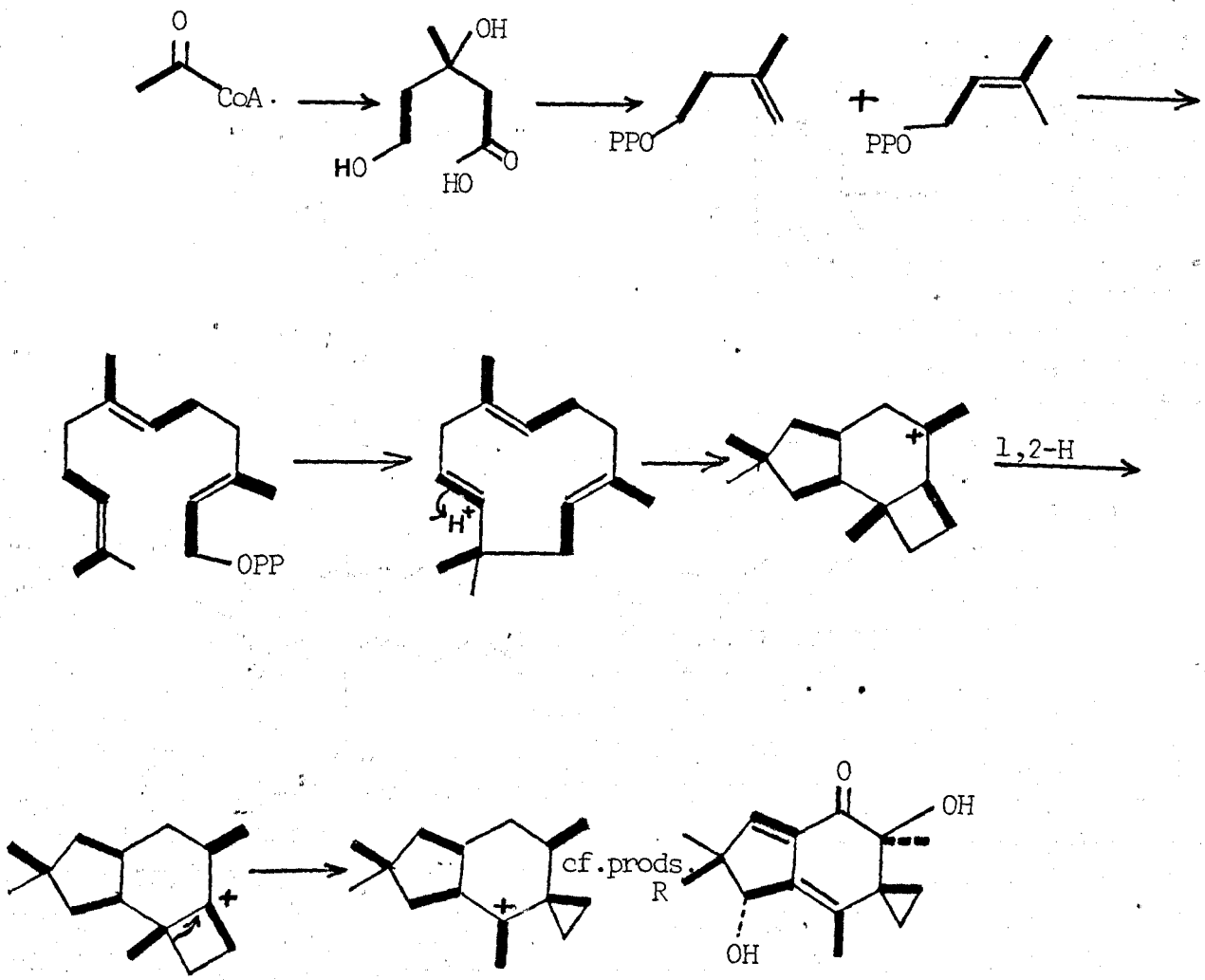


Figure 9

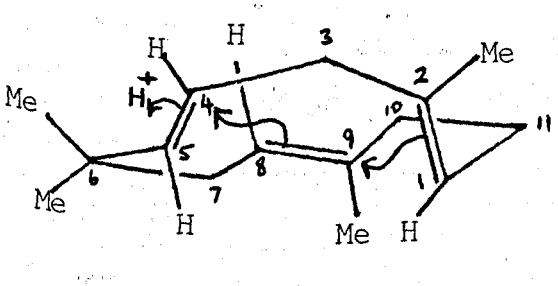
Using [1,2-$^{13}C_2$] acetate and ^{13}C nmr techniques the same group[51] extended the labelling studies to show the compatibility of the proposed rearrangements with the observed results. Figure 10 outlines the theoretical pathway and compares the resultant labelled skeleton (66) with the isolated products.

McMorris et al.[52] isolated illudol (68), the structure and stereochemistry[53,54] being determined by chemical and physical means. It was found that illudol (68) has a cis-fused hydrindane skeleton. This is in keeping with humulene being held in a CT conformation[31] (67), the hydrogen atoms on C-4 and C-8 already being held in a cis-arrangement to each other before cyclisation. Δ^6-Protoilludene (69) and the related alcohol (70) have been isolated from Fomitopsis insularis[55] and these also can be rationalised biogenetically by quenching of the protoilludyl cation (59). Closely related to illudol (68) are the pterosins H(71), I(72) and Z(73) isolated from the fern Hypolepsis punctata Mett.[56] along with hypacrone (74). The pterosides related to the pterosins were isolated from Pteridium aquilinum var. latiusculum[57] and labelling experiments using [2-^{14}C] mevalonate indicated that the biosynthesis of these molecules proceeded through the accepted



(66)

Figure 10



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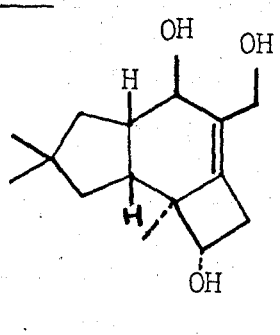
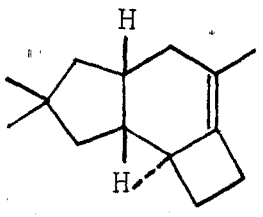
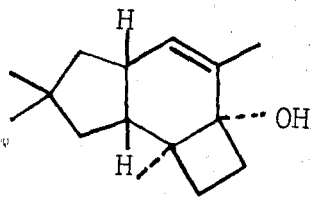


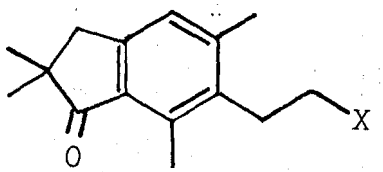
(68)



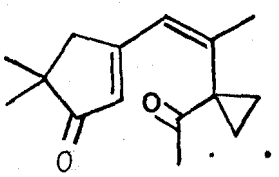
(69)



(70)



(71) X = Cl
(72) X = OMe
(73) X = OH

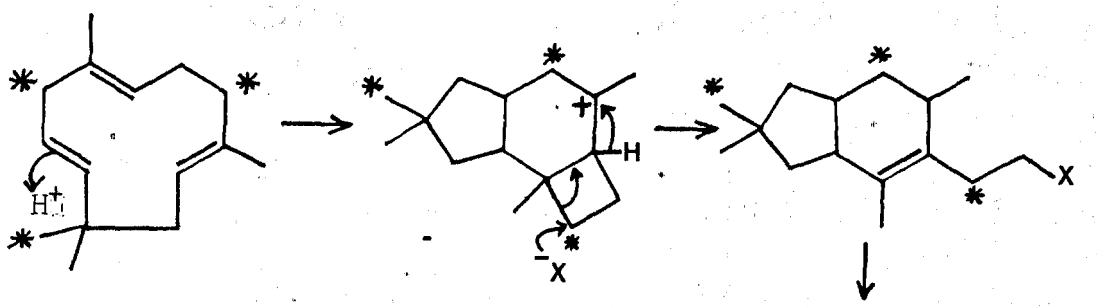


(74)

pathway to the sesquiterpenoids. Figure 11 outlines the proposed mechanism commencing with labelled humulene (65) derived from the labelled mevalonate.

The labelling pattern, whilst not totally conclusive, would suggest that there was incorporation in one of the gem-dimethyl groups and degradative oxidation to give acetic acid provided supporting evidence because the expected radioactivity ratio of 9:1 parent compound:acetic acid was observed. Because aromatic methyls are slower to oxidise than aliphatic, it was concluded that one of the gem-dimethyls was labelled. This

provides evidence that the pathway outlined in Figure 11 is possible.



(65)

products

Figure 11

Also shown was the synthetic conversion of hypacrone (74), itself thought to arise by cleavage of the protoilludyl cation (59), to the pterosins H(71) and Z(73).[56] Figure 12 shows the proposed mechanism. This transformation suggests a common biosynthetic process in the plant for the pterosins and hypacrone (74).

Fomannosin (75), which is isolated from the same fungus as Δ^6-protoilludene (69),[55] was identified[58] and the absolute configuration determined[59] as [7S,9R]. It was proposed that fomannosin (75) was also formed from the protoilludyl cation (59) by oxidative cleavage of the bond shown below, substantial evidence being obtained by feeding experiments with [1,2-$^{13}C_2$] acetate.[60,61] The labelling pattern was investigated using a combination of ^{13}C and ^{1}H nmr techniques, and it was shown that the observed pattern was in accord with that expected from the cyclisation of a humulene-type intermediate

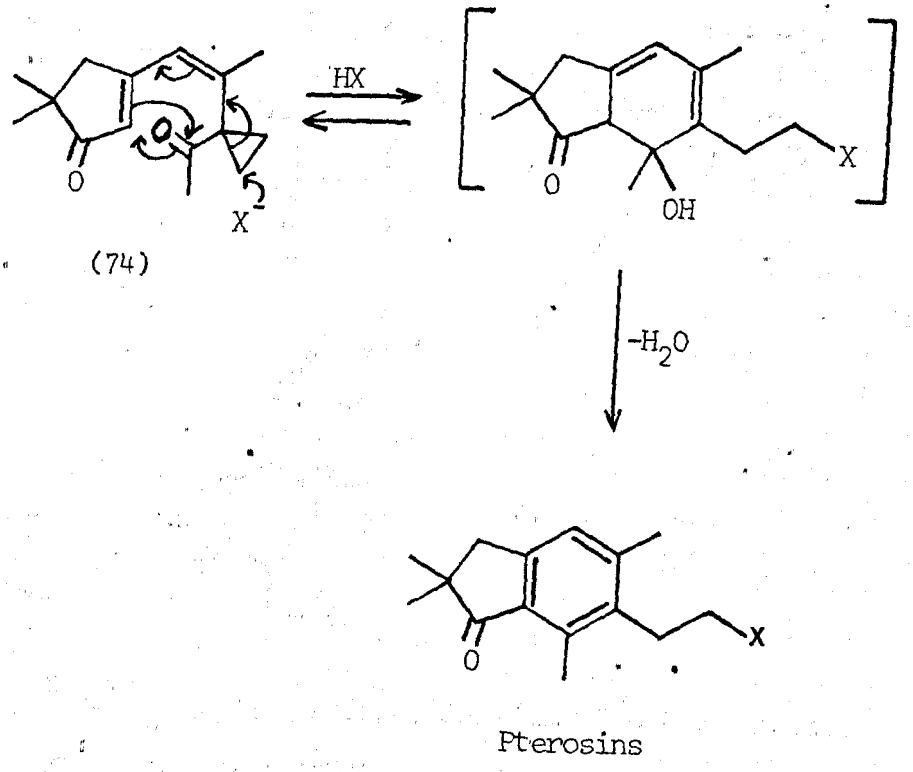
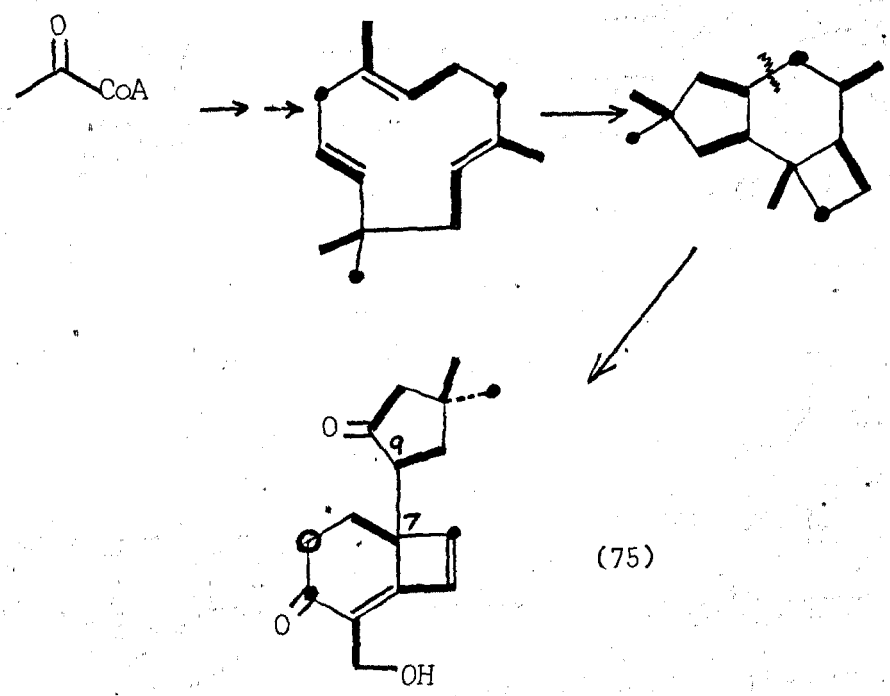


Figure 12

via the labelled protoilludyl cation as shown in Figure 13. Closer examination of the ^{13}C nmr spectral data enabled differentiation between which of the two gem-dimethyl groups was biosynthetically derived from C-2 of mevalonate. This gives an insight into the stereochemistry of cyclisation of farnesylpyrophosphate (20) and humulene (1) to give the correct stereochemical configuration observed in fomannosin (75). By nmr analysis it was deduced that the methyl of the cyclopentanone ring which is cis- with respect to the cyclobutyl substituent is in fact the methyl group which is derived from C-2 of mevalonate. The stereochemistry of cyclisation has been described as shown in Figure 14.



(75)

— ^{13}C-^{13}C coupling arising from [1,2-$^{13}C_2$] of acetate

• Enhanced signal (^{13}C) due to C-2 of mevalonate

Figure 13

Initial cyclisation of trans,trans-farnesyl pyrophosphate (76) occurs by electrophilic attack by the C-1 carbinyl carbon at C-11 on the si-face of the distal double bond thus causing the methyl group derived from C-2 of mevalonate to become the pro-R methyl of humulene (67). Protonation on the re-face of the 4,5 double bond of humulene (67) followed by intramolecular cyclisation gives a cis-fused cyclopentane ring and subsequent cleavage of the six-membered ring leads to fomannosin (75) in which the C-15 methyl is cis- to the cyclobutyl substituent and the C-9 proton has the R configuration. This stereochemical analysis was applied to other labelled compounds such as illudol (68),

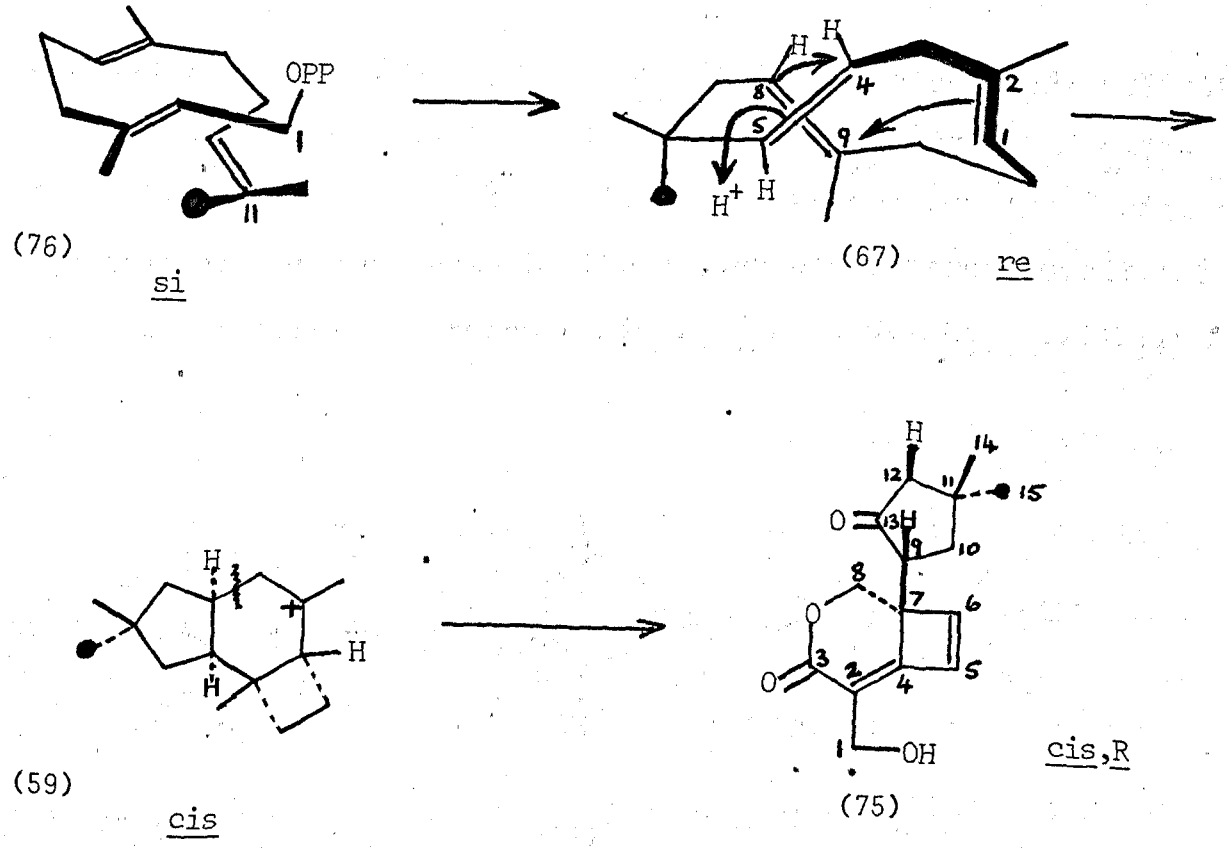
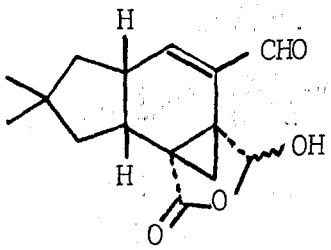


Figure 14

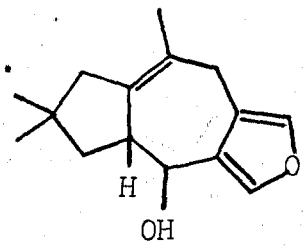
marasmic acid (54) and hirsutic acid (55), the latter two to be discussed later. It was seen that with illudol (68) and marasmic acid (54), the same methyl group was derived from C-2 of mevalonate but in the case of hirsutic acid (55), the opposite methyl group bore the label. The implication then is that hirsutic acid (55) arises from a different conformation of humulene (67) and/or via a different mechanistic pathway.

Marasmic acid (54), first isolated in 1949,[62] the structure determined by spectral analysis and chemical degradation[63] and confirmed by X-ray studies,[54] proved to have the same cis-fused hydrindane skeleton as illudol (68). Feeding the parent organism with [2-^{14}C] mevalonic acid followed by

degradative analysis,[63] whilst not totally unambiguous, certainly indicated the probability that marasmic acid (54) was derived from the protoilludyl cation (59) as shown previously in Figure 7 and this is substantiated by the evidence discussed immediately above concerning the stereochemical aspects of the cyclisation process.



(54)



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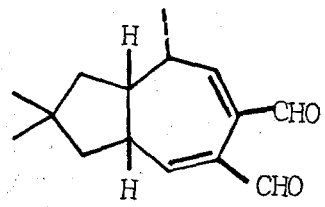
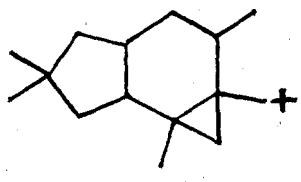


(78)

Interest was also raised in the vellerane type skeleton, examples of which can be seen in the compounds (77) and (78). Parker *et al.*[64] suggested that the cation (79), derived from the protoilludyl cation (59), could be a common precursor for both marasmanes and velleranes, but experimental proof seemed to be lacking until the interrelationship between



(79)

the marasmane and vellerane skeletons was shown when isovelleral (80) underwent thermal rearrangement to give a vellerane-type compound (81).[65] The mechanism proposed for the rearrangement is shown in Figure 15 and an alternative pathway leading to velleral (78) is depicted.

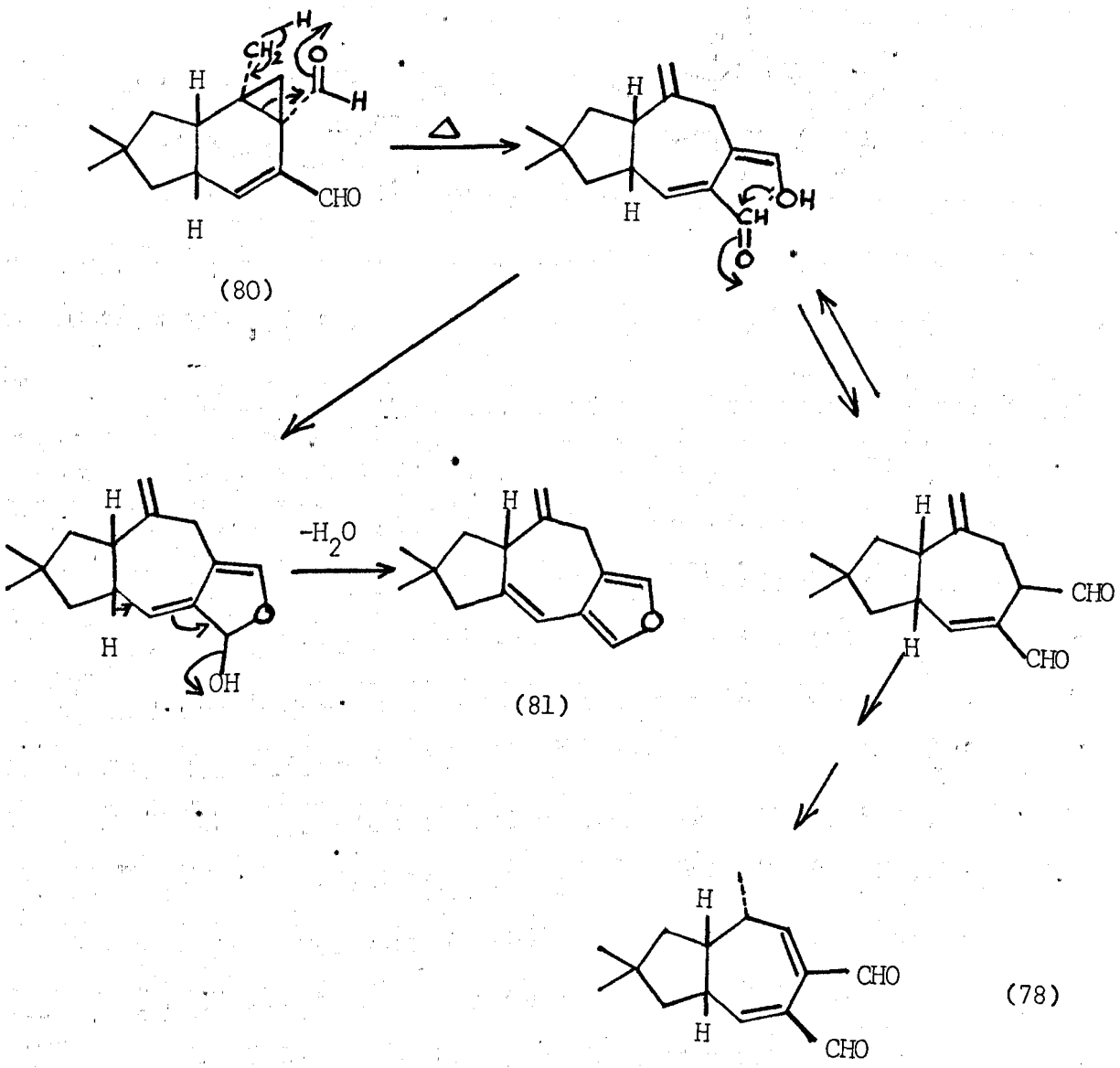
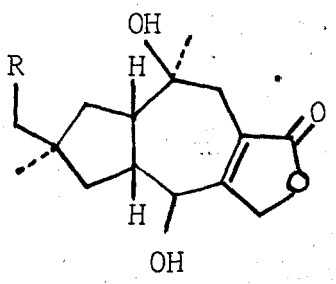


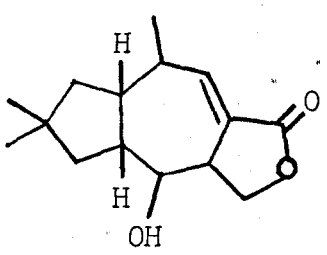
Figure 15

From this rearrangement it would appear that isovelleral (80) is a key intermediate in the biosynthesis of vellerane sesquiterpenes. Closely associated with the velleranes are the lactarorufins A (82), B (83) and N (84), the structures eventually being correctly assigned as those shown.[66]



(82) R = H

(83) R = OH

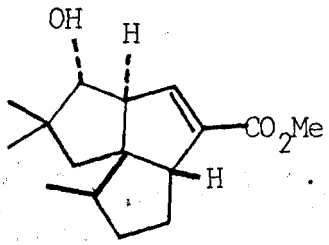


(84)

Pentalenic acid, isolated as its methyl ester (85)[67], pentalenolactone G (86)[67], pentalenolactone H (87)[67] and pentalenolactone E (88)[68] are also considered to be derived from the protoilludyl cation (59) by the mechanism[69] (a) shown in Figure 16. However a much simpler mechanism (b) can also be proposed which does not necessitate the intermediacy of the protoilludyl cation (59).

Another closely related compound isolated from the fermentation broth of Streptomyces UC 5319[70] is pentalenolactone (89) which is in effect a dehydrated pentalenolactone which has undergone a methyl rearrangement.

In 1969 a new sesquiterpene antibiotic was isolated from Coriolus consors[71] but it was not until 1971 that the correct structure was assigned.[72] Later the absolute configuration was determined on a hexahydrocoriolin derivative.[73] Coriolin (90)



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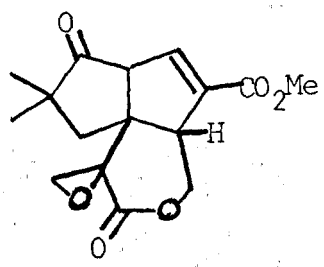

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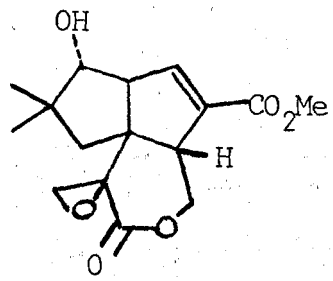

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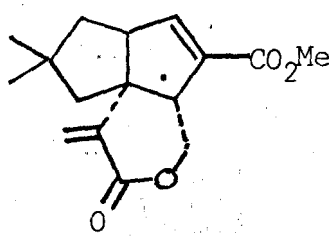

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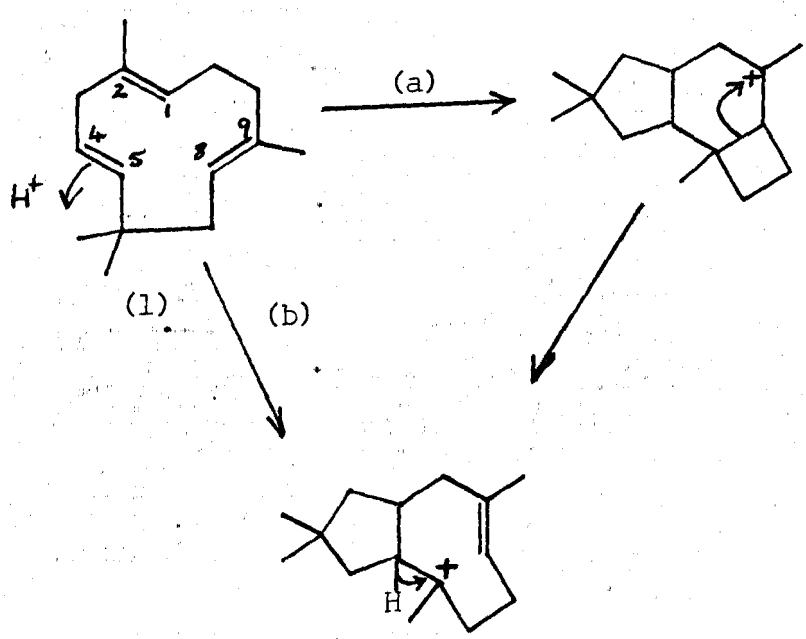


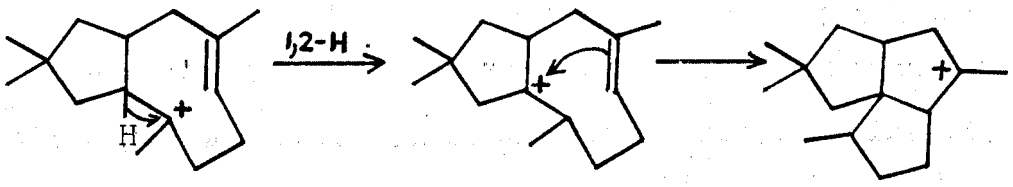
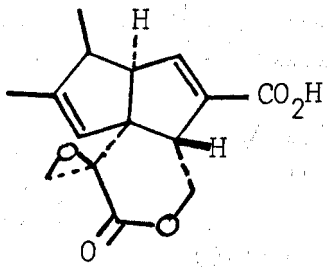
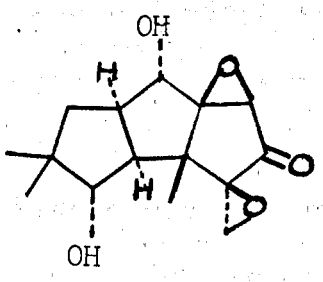


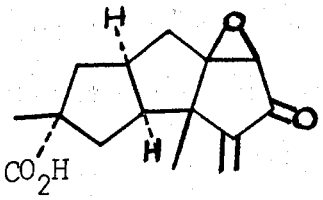
Figure 16



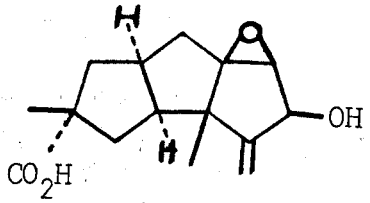
(89)



(90)

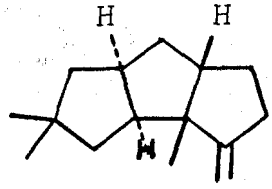


(91)



(55)

was shown to have the cis-fused hydrindane skeleton but it was observed that the ring-fusions were antipodal to those found in the previously discussed hydrindane-type compounds. A similar situation was noted in complicatic (91) and hirsutic (55) acids, isolated from Stereum complicatum,[74] and on the basis of the structural similarities of the coriolins, and the hirsutanes, a common precursor, hirsutene (92) was proposed. This theory was



(92)

enhanced by the isolation of hirsutene (92) from the extract of Coriolus consors[75], previously shown to produce coriolin (90)[71]. Also found in the hydrocarbon mixture were traces of humulene (1) and caryophyllene (22) giving strong support for the eleven-membered ring precursor theory. However, because of the antipodal relationship between the skeletons derived from the protoilludyl cation (59) and the hirsutane group, it was thought that the latter was not derived from the protoilludyl cation (59) although Figure 7 showed two possible routes to the hirsutane group. Studies were carried out in an attempt to determine exactly the pathway followed in the biosynthesis of these compounds. Tanabe and Suzuki[76] fed [1,2-$^{13}C_2$] acetate as a precursor to Coriolus consors and analysed the coriolin (90) produced by ^{13}C-nmr spectroscopy. In theory the labelled coriolin can be derived

from labelled humulene by any one of four pathways. These are outlined in Figure 17.

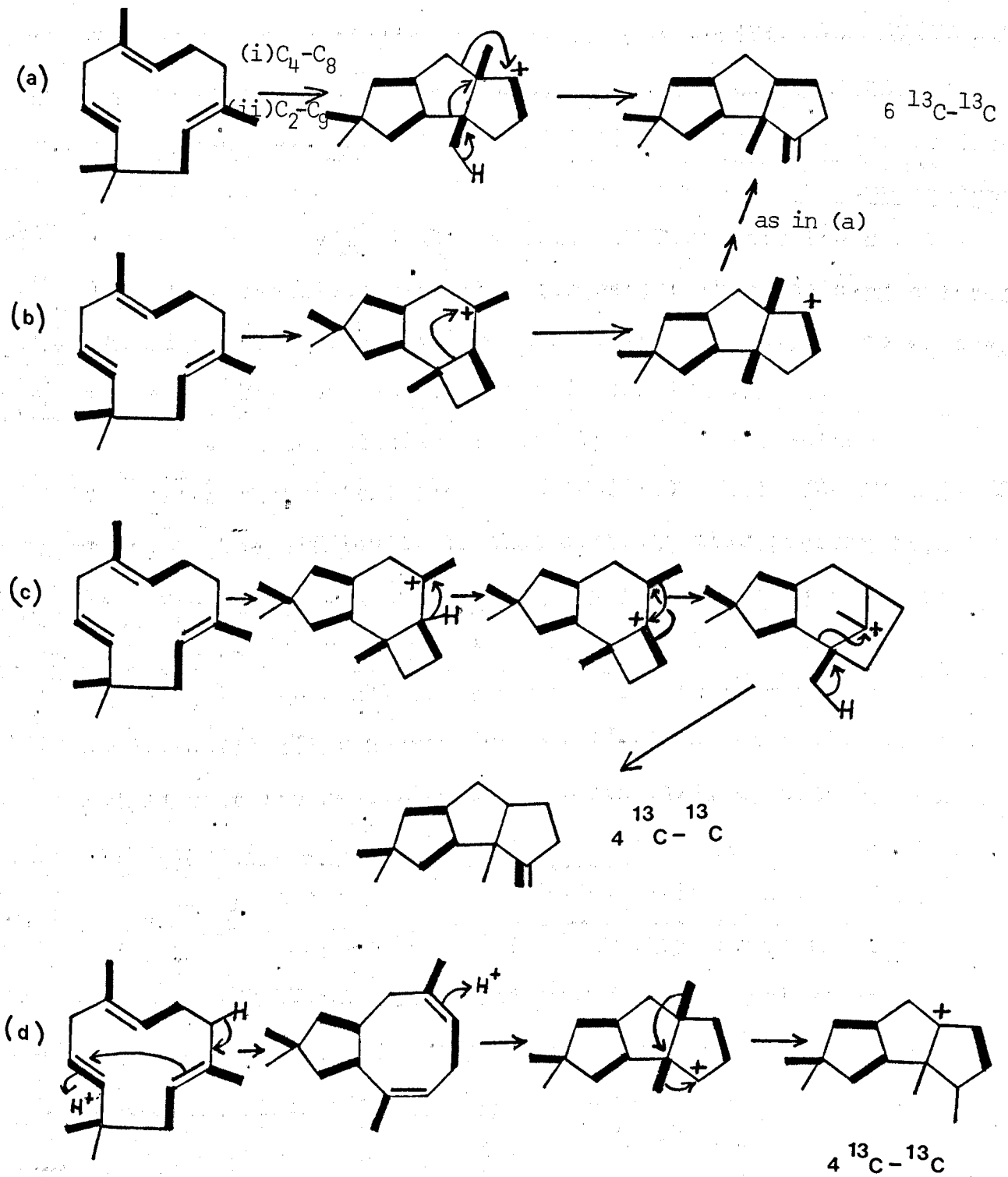


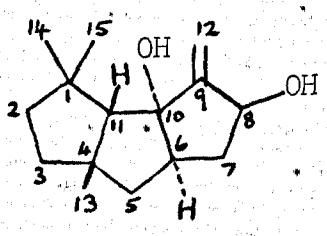
Figure 17

The ^{13}C nmr study of a dihydrocoriolin derivative showed labelling compatible with either pathway (a) or (b), the observed labelling pattern displaying six ^{13}C-^{13}C couplings with the expected multiplicities. This work verified proposals made by Feline et al.[77] who had used parallel single labelling experiments with [1-^{13}C] and [2-^{13}C] acetate and who postulated that the hirsutic acid (55) obtained from Stereum complicatum (Fr.)Fr. arose via similar pathways. Certainly the double labelling technique can probe far deeper than the single label technique but there still remains some ambiguity as to whether or not a protoilludyl intermediate is involved.

In the studies previously discussed, Sutherland suggested that in solution, humulene (1) adopts the CT conformation.[31] The difficulty is that although this conformation is observed in the silver nitrate adduct of humulene (1),[8] it does not necessarily follow that this will be the case in solution. However, Sutherland's experimental results from the cyclisation of humulene (1) using N-bromosuccinimide in aqueous acetone[35] clearly showed that the resulting bromohydrin (42), upon X-ray analysis,[78] maintained the CT conformation and so it can be assumed that humulene (1) is also in the CT conformation. Low temperature nmr studies carried out in 1968[79] clearly showed the existence of more than one major conformation of humulene (1) although the most stable conformation in solution could not be assessed due to resolution difficulties. Recent molecular mechanics calculations carried out by Matsumoto and co-workers[80] involved the energy minimisation of the four principal conformers of humulene (1) by computer methods.[81] From the results obtained, these workers

proposed that the major conformers in solution were CT and CC and that a separate pathway to the hirsutane group could be shown *via* the CC conformer whilst the CT conformer produced the protoilludyl cation (59). Figure 18 outlines these pathways and it can be seen that the TT and TC conformers are excluded because they would cyclise to form *trans*-fused bicyclic compounds which so far have not been found as natural products.

A number of compounds isolated from the soft coral *Capnella imbricata*[82,83] were identified as having a new skeleton, similar to the hirsutane-type compounds but with different positioning of the methyl groups. This group was given the name capnellane and typical compounds are seen in $\Delta^{9(12)}$-capnellane-8β, 10α-diol (93) and $\Delta^{9(12)}$-capnellene (94). It has been proposed that these compounds are derived from an isomer (95) of farnesyl pyrophosphate (20) which after cyclisation undergoes a methyl migration.



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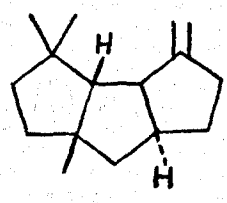

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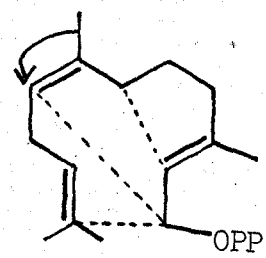

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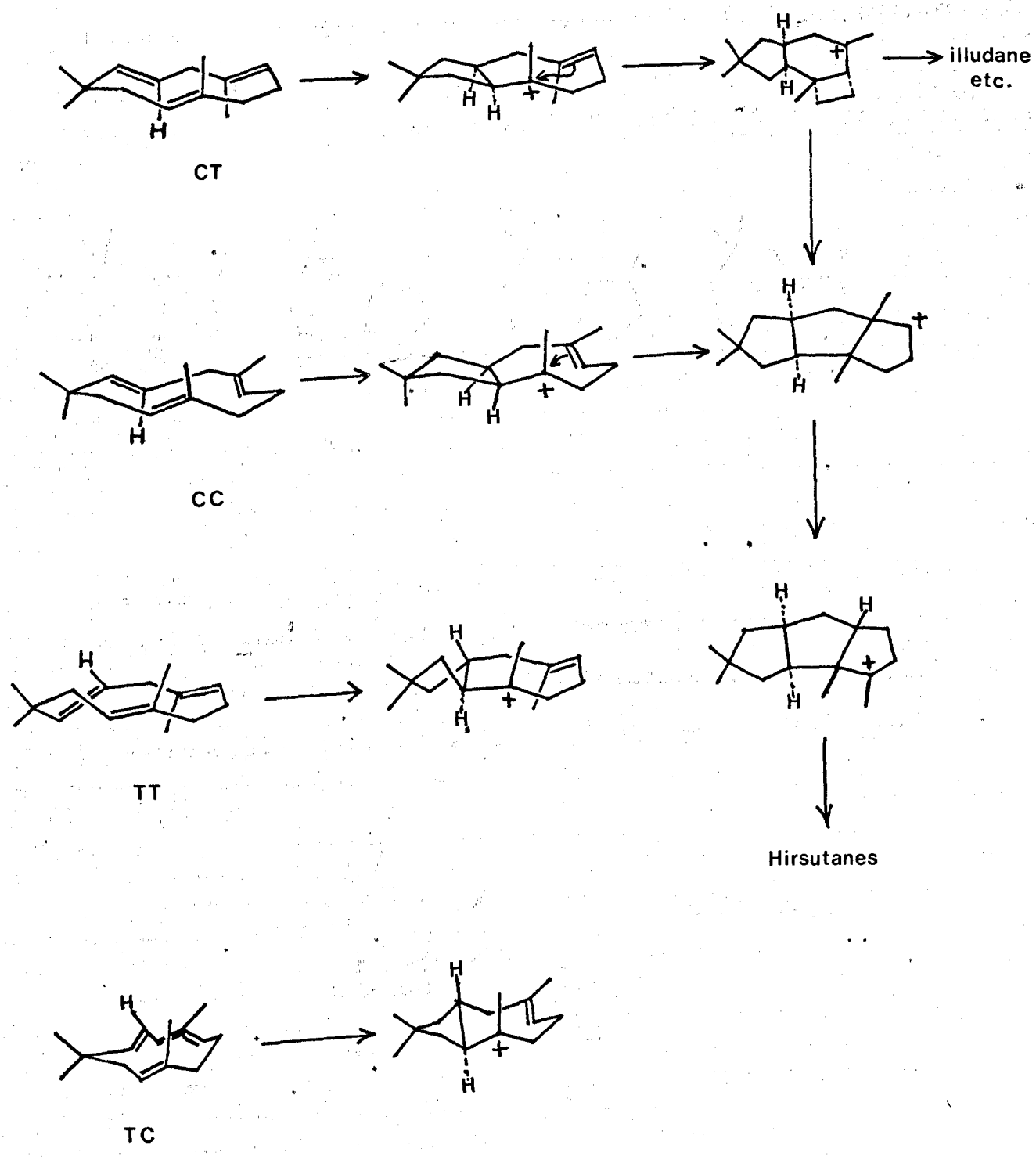
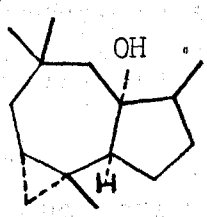


Figure 18

The first africane-type compound, africanol (96), was isolated along with the hydrocarbons (97), (98) and (99) from Lemnalia africana, a marine sponge,[84] (96) being identified by X-ray studies. As shown above in Figure 7, it was proposed that



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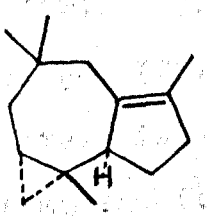

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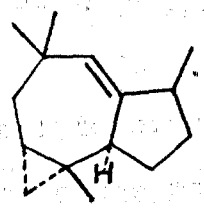

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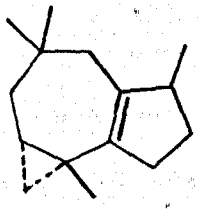
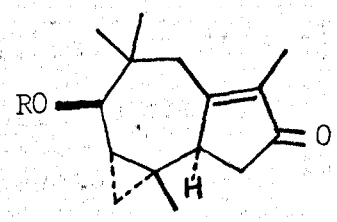


(99)

the africane skeleton arose by a different mode of cyclisation than the other skeletal types. Recently Bohlmann and Zdero[85] isolated the keto-angelate (100), elucidated the structure by spectroscopic means and proposed that humulene-8,9-epoxide (9)



(100) R = Ang.

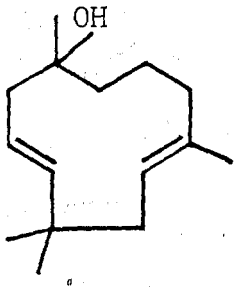
was the precursor. However it seems particularly difficult to visualise a mechanism which could embody the epoxide (9) as a starting material and it would seem much more acceptable to propose humulene-4,5-epoxide (10) as the precursor.

Before discussing the attempts to synthesise some of

these tricyclic compounds by biomimetic strategies, it is important to note that a significant number of them have been obtained by total syntheses, many of which have been extremely elegant in design. These include illudins S (57)[86] and M (56)[87], marasmic acid (54)[88-90], hirsutic acid (55)[91], pentalenolactone (89)[92] and velleral (78).[93]

The stage is now set to discuss the more recent chemistry of humulene (1) where the emphasis has been directed towards two goals, namely methods of inducing humulene (1) to undergo the postulated ring closures and secondly investigations of the *in vitro* reactivity of protoilludyl cation equivalents. In considering humulene (1) first of all, Naya and Hirose[94] found that treatment of humulene (1) with 80% aqueous acetic acid at 100°C gave a variety of compounds, the ratio of these being dependent upon time and acid/substrate ratios. An independent study by Parker and co-workers[64] using sulphuric acid as the proton source produced identical results but proved that humulol (101) was the initial hydration product and as the reaction continued the alcohol was converted into the range of bicyclic products such as (102) - (105). It should also be pointed out that apollan-11-ol (38) was also a product of these reaction conditions. Deuterium incorporation experiments[95] showed that two deuterium labels were introduced in each cyclisation and the mechanisms are as shown in Figure 19 for bicyclic products and Figure 4 for tricyclic products.

Dauben *et al.*[96] concurred with these findings by cyclisation of humulene (1) using perchloric acid to produce α-caryophyllene alcohol (38), the alcohol (102) and the associated hydrocarbons. It is interesting to note that



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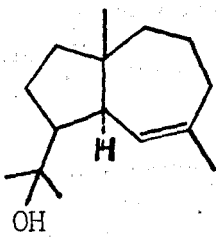

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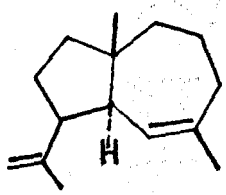

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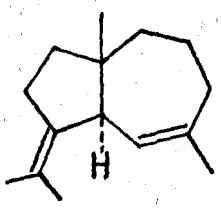

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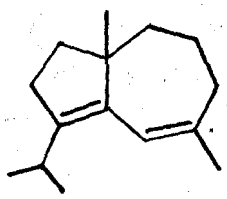


(105)

α-caryophyllene alcohol (38) has been isolated from a natural source, Cedrus atlantica Manet.[97] The authors proposed that it was unlikely that the conditions of steam distillation of the wood or chromatography of the oil on silica would be acidic enough to induce rearrangement of humulene (1) to the alcohol (38); the alcohol (38) is present in the wood rather than being formed from humulene (1) during isolation.

Mehta and Singh[98] reported that humulene (1) rearranges to give δ-selinene (106), a eudesmane-type skeleton, by treatment with concentrated sulphuric acid in dichloromethane. It was thought that the hydrocarbon (106) did not arise by direct

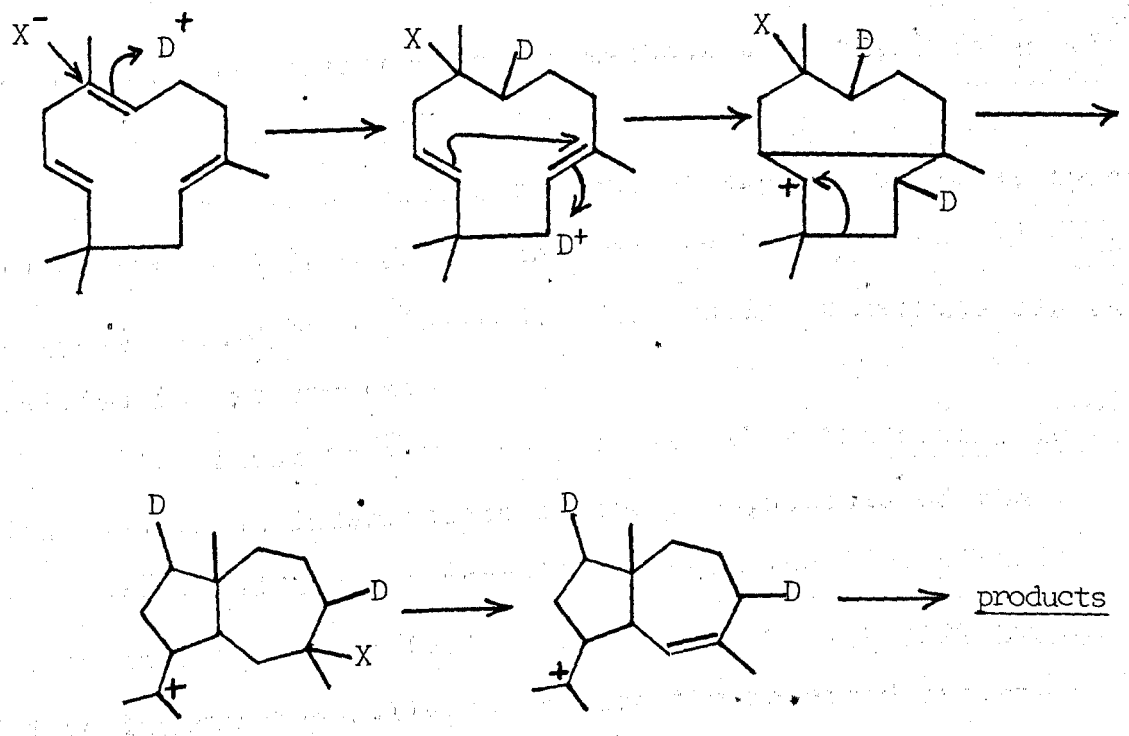
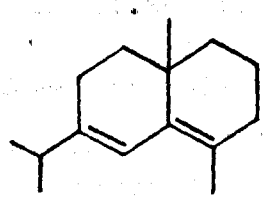


Figure 19

cyclisation of humulene (1) but instead came about via the rearrangement of the intermediate hydrocarbon (105) previously discussed.



(106)

None of these products were in any way related to the desired compounds therefore other methods were tried in an effort to induce the desired cyclisations.

It became obvious that none of the six skeletal types would be obtained from experiments on humulene *per se* and so new approaches had to be engineered. The resultant methods can be classified in two groups.

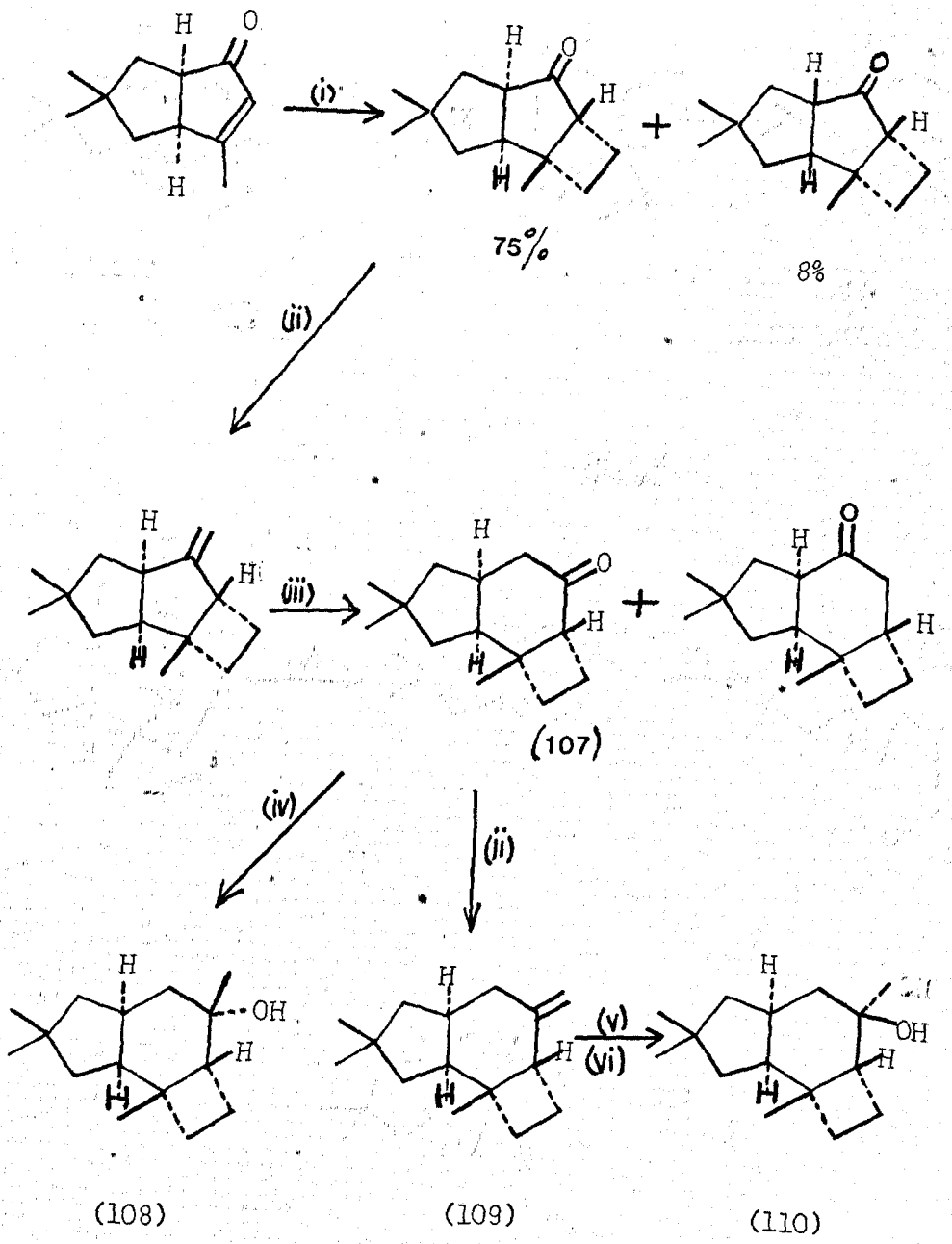
The first follows the proposal that the protoilludyl cation (59) is an intermediate in the biosynthesis of the marasmanes, illudanes and protoilludanes. Thus by synthesising the intermediate cation (59) or its equivalent, rearrangement could be induced hopefully to produce the required skeletal types. Matsumoto and co-workers have carried out some very elegant synthetic work and certainly have moved much closer to a biomimetic approach than any previous attempts. Their approach[99] to give the cationic equivalents (108), (109) and (110) is outlined in Figure 20.

The ketone precursor (107) was then utilised in a biogenetic-like synthesis of d,l-hirsutene[100] (92) *via* the *endo*-isomer (113) as shown in Figure 21.

Formolysis[69] of the cationic equivalents (108), (109) and (110) produced the bridged compound (114) and the pentalene-type hydrocarbon (115).

The different products were explained in terms of two possible conformations (116) and (117) of the protoilludyl cation.

In (116) the vacant p-orbital on C-7 and the C-5-C-6 bond are parallel. Therefore the C-5 - C-6 bond cleaves and rearranges to give (118). In (117), the C-2-H, C-3-C-6 bonds and the vacant p-orbital on C-7 are parallel leading to cleavage of



(108) (109) (110)

Reagents: (i) C_2H_4/n-hexane; (ii) Ph_3PCH_3Br/t-AmONa/C_6H_6;

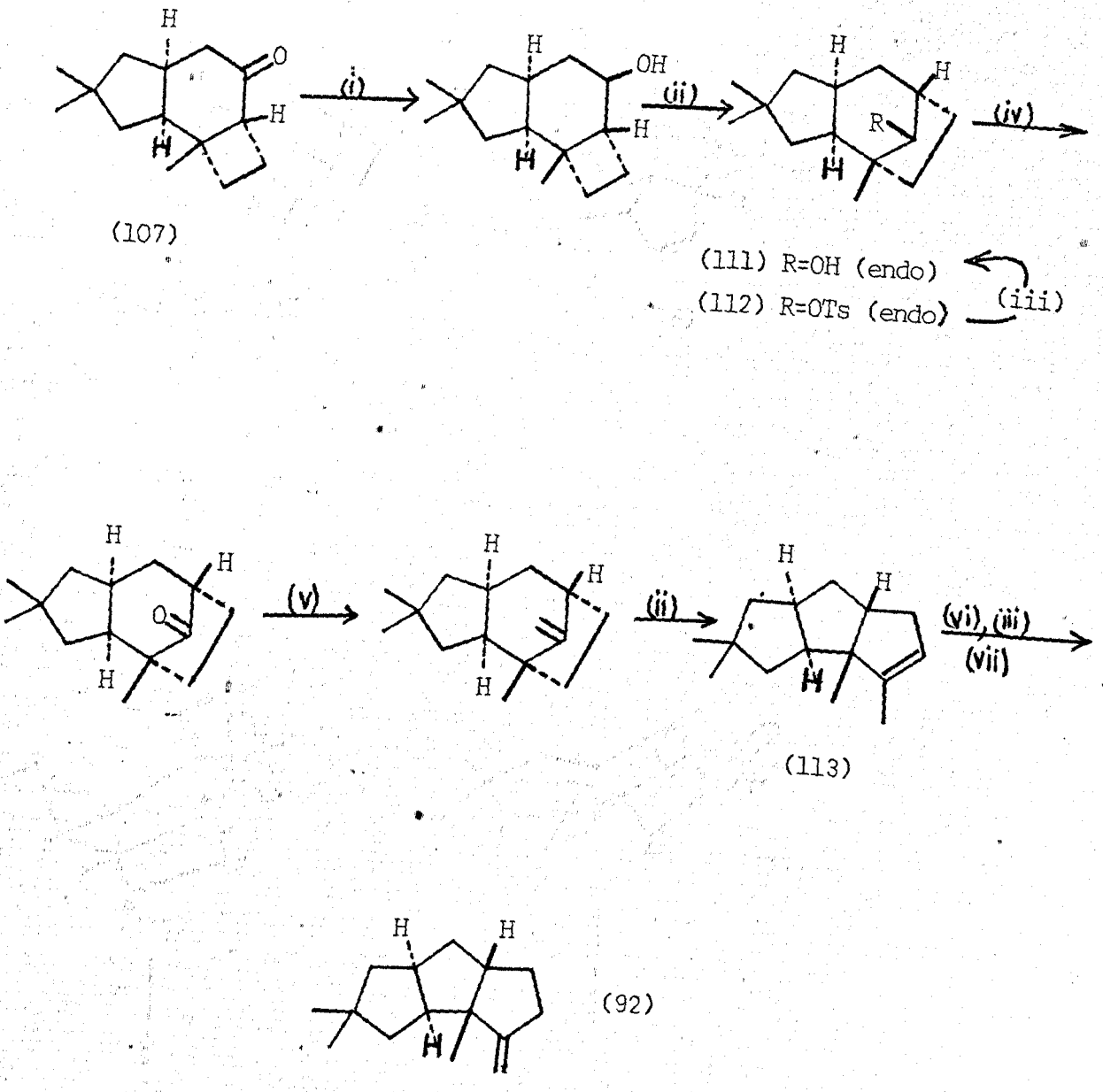
(iii) $Tl(ClO_4)_3$/^{t}-BuOH/H_2O;

(iv) MeMgI/Et_2O;

(v) $Hg(OAc)_2$/THF/H_2O;

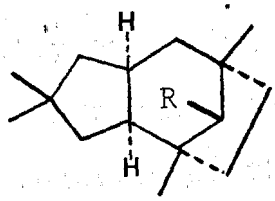
(vi) $NaBH_4$

Figure 20

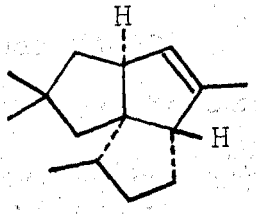


Reagents: (i) $NaBH_4/EtOH$; (ii) $p\text{-}TsOH/C_6H_6$;
(iii) $LiAlH_4/THF$; (iv) CrO_3/H^+;
(v) $Ph_3PCH_3Br/{}^tAmONa/C_6H_6$;
(vi) m-ClPBA/CH_2Cl_2;
(vii) MsCl/pyr.

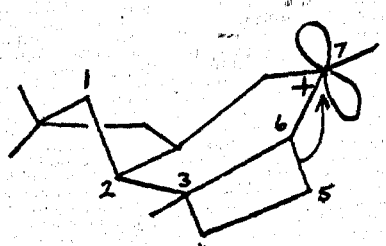
Figure 21



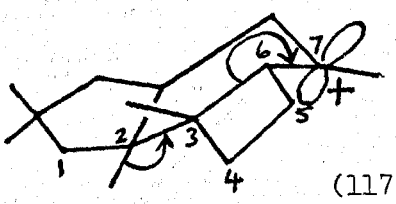
(114) R = OCHO



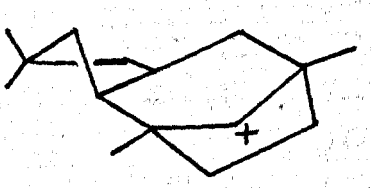
(115)



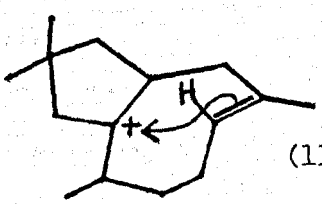
(116)



(117)



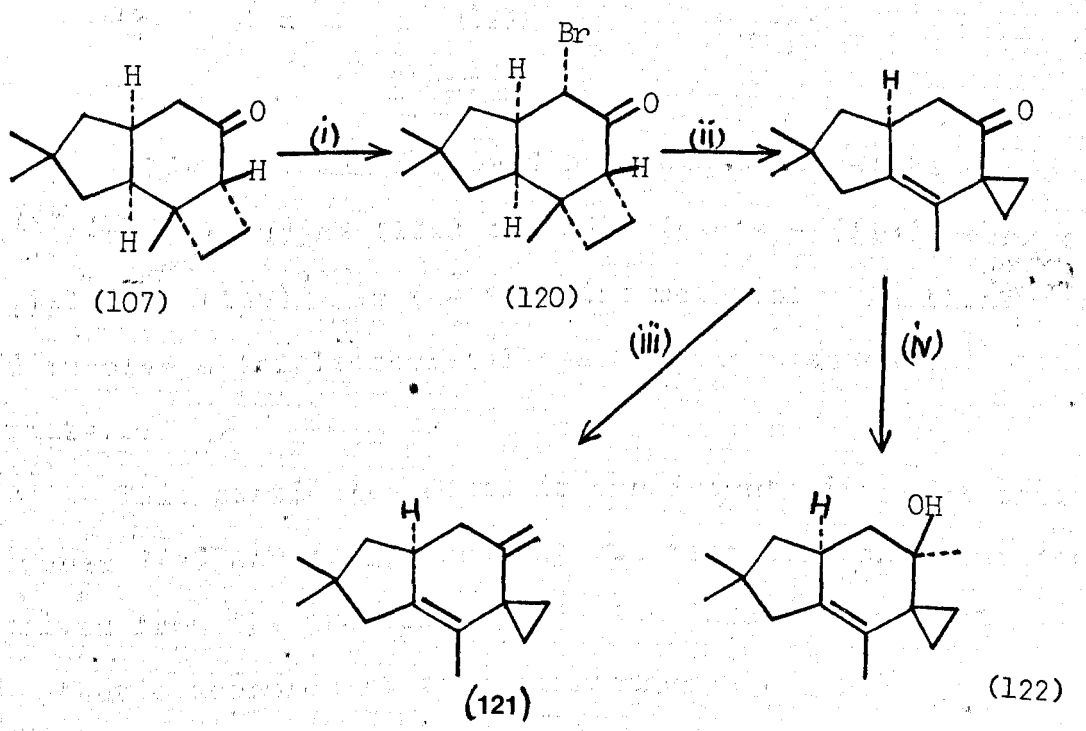
(118)



(119)

the C-3-C-6 bond, H-shift and then rearrangement to give (119) which leads to (115). Thus although no illudoids were produced, a naturally-occurring skeleton was found.

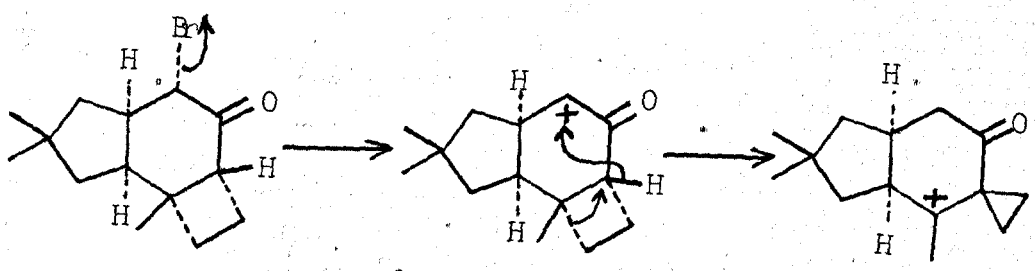
A further development[101] by the same group involved ring contraction of the cyclobutyl ketone (107) to give $\Delta^{2(3),7(13)}$ illudadiene (121) and $\Delta^{2(3)}$-7β-illudenol (122) both having the illudane skeleton. Figure 22 outlines the reaction sequence employed.



Reagents: (i) Br_2/CCl_4;
(ii) AgOAc/AcOH;
(iii) $Ph_3PCH_3Br/{}^tAmONa/C_6H_6$;
(iv) $MeMgI/Et_2O$.

Figure 22

The α-isomer (120) of the mixture of bromides underwent an unusual ring contraction via an α-keto carbenium ion[102] (123) as illustrated in Figure 23.



(120) (123)

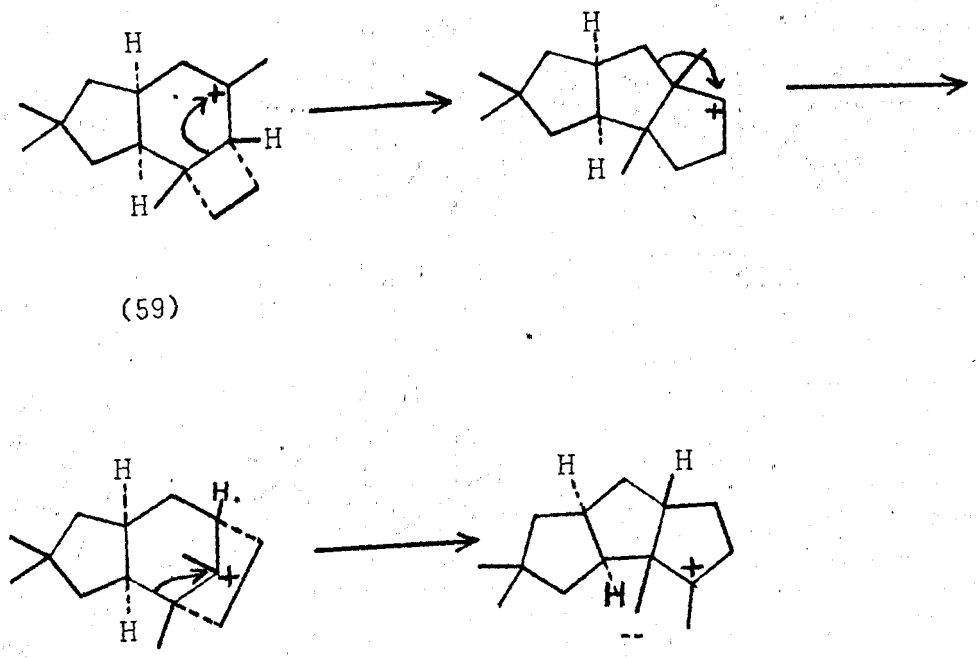
Figure 23

This work was followed by a biogenetic-like conversion[103] of $\Delta^{7(8)}$-protoilludene (124) to endo-hirsutene (113) using $\Delta^{7(13)}$-protoilludene (109)[99] as the starting material. In theory, this would involve a triple skeletal rearrangement as shown in Figure 24.

This particular route is consistent with that postulated in Figures 7(a) and 17(b), showing the hirsutene skeleton (58) to be derived from the protoilludyl cation (59). Figure 25 outlines the synthetic approach to this rearrangement.

A method has already been discussed for the conversion of endo-hirsutene (113) to hirsutene (92)[100] and so this is effectively a synthetic route to hirsutene (92) itself from a protoilludyl cation equivalent.

In parallel with this research, the same group were carrying out experiments on humulene (1) itself in an attempt to cyclise the hydrocarbon directly. In 1976, humulene (1) was cyclised using mercuric acetate[104] by an oxymercuration-demercuration

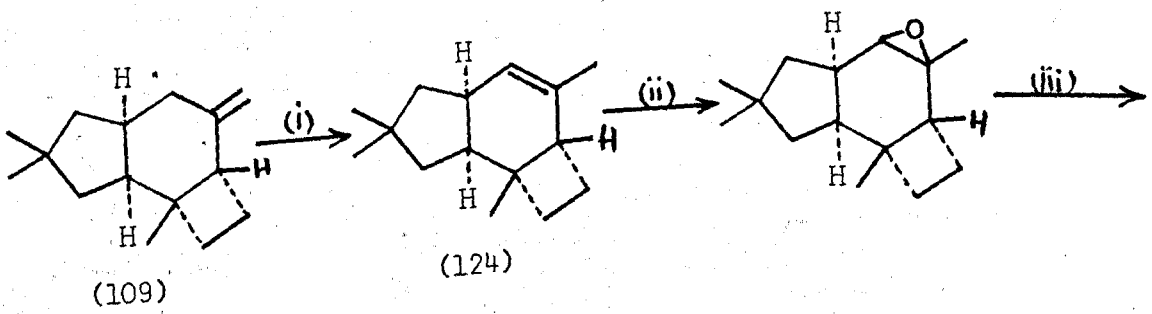


(59)

(58)

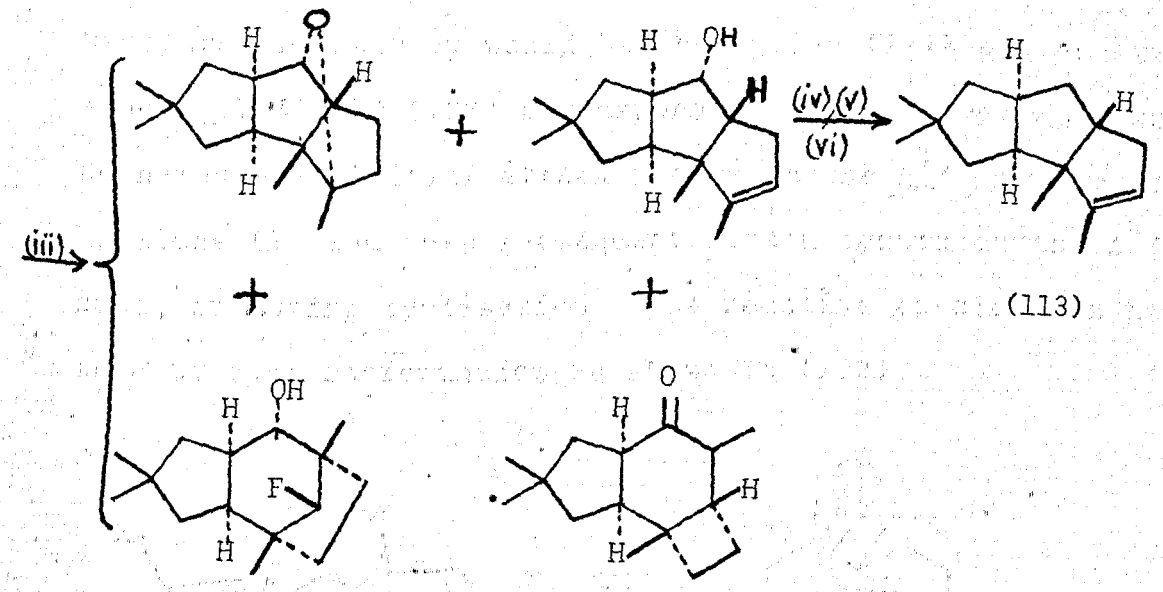
Figure 24

procedure to give two products, both identified as cyclic ethers having the structures (125) and (126).



(109)

(124)

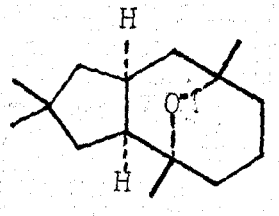


Reagents: (i) I_2/Toluene; (ii) m-ClPBA/CH_2Cl_2;

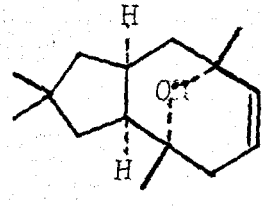
(iii) $BF_3.Et_2O$/hexane; (iv) CrO_3/H^+;

(v) $TosNHNH_2$; (vi) $NaBH_3CN$/p-TsOH/DMF/sulpholane.

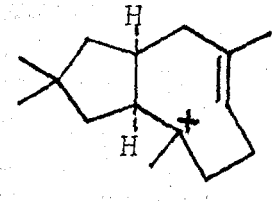
Figure 25



(125)

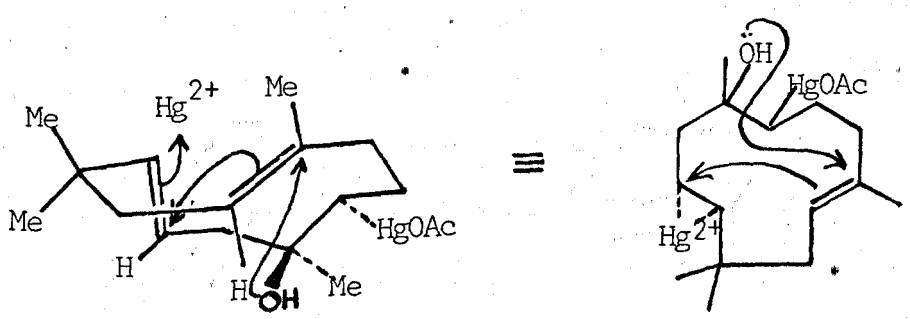


(126)



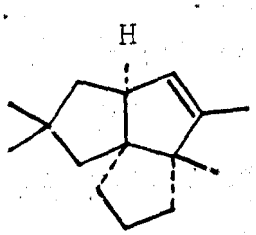
(127)

The hypothetical intermediate in the equivalent biosynthetic pathway would be the cation (127) and the two ethers (125) and (126) correspond to this. However it should be noted that initial attack occurs on the $\Delta^{1,2}$ double bond of humulene (1) and then subsequent attack occurs on the $\Delta^{4,5}$ double bond, promoting cyclisation. The reactive species can be drawn in a CT type conformation as shown in (128).

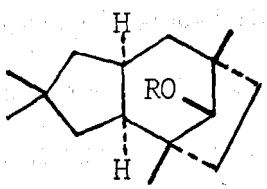


(128)

Matsumoto et al.[105] took the cyclic ether (125) and upon treating it with boron trifluoride etherate in acetic anhydride produced two compounds (129) and (130).

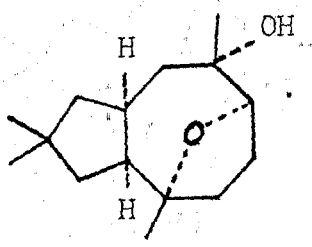


(129)

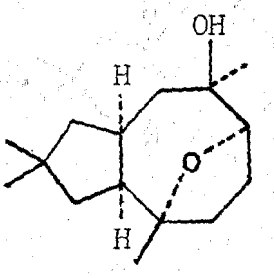


(130) R=Ac
(114) R=CHO

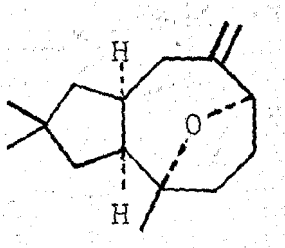
It was also found that if humulene (1) was cyclised with mercuric nitrate in aqueous acetic acid, new cyclic ethers (131), (132) and (133) were isolated. (133), upon treatment with lithium and



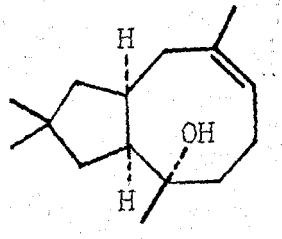
(131)



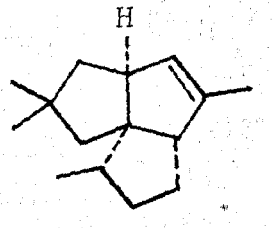
(132)



(133)

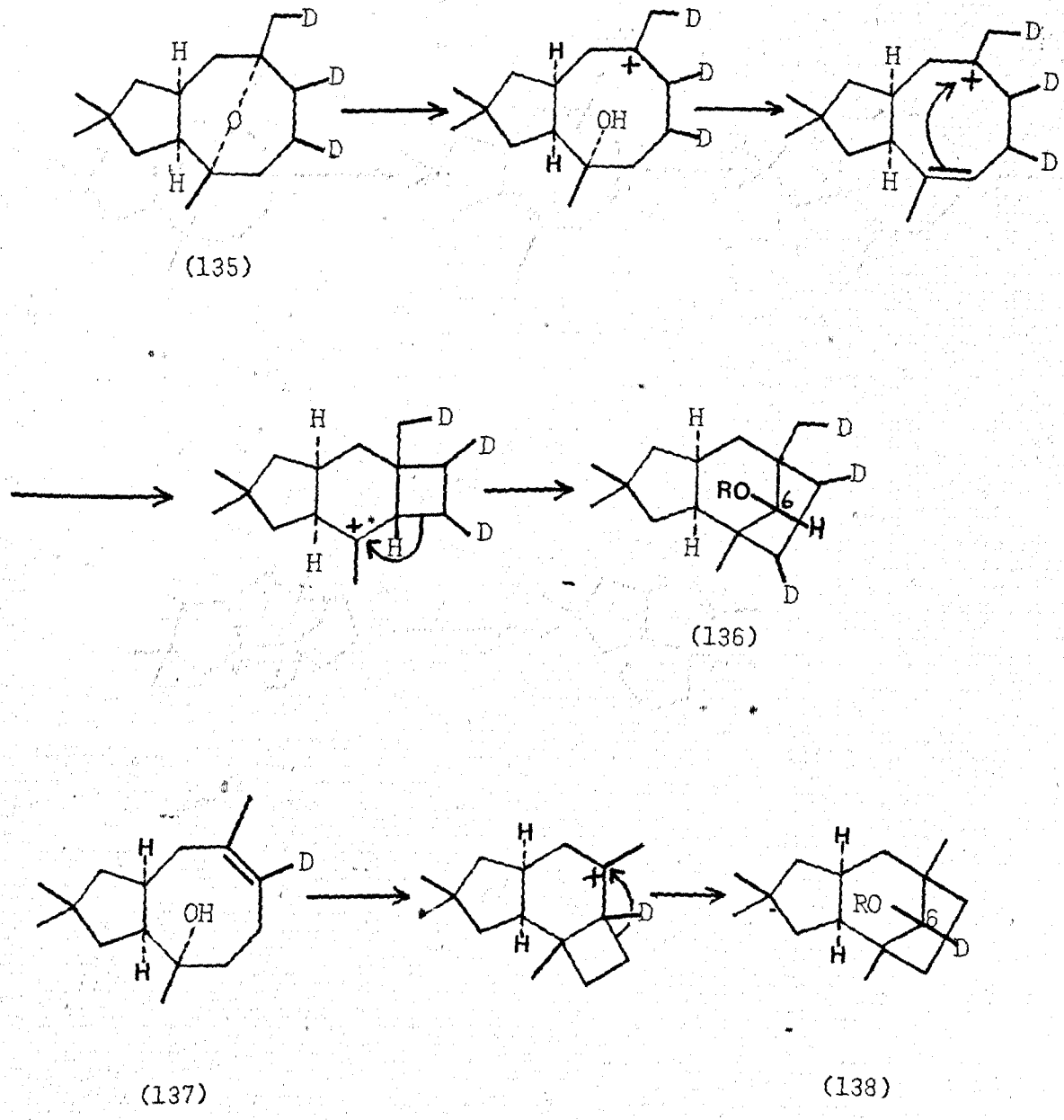


(134)



(115)

ethylamine, was converted to the alcohol (134) which on formolysis gave (114) and (115), both of which had already been prepared synthetically.[69] Deuterium labelling experiments[105] were carried out to ascertain if the protoilludyl cation (59) was intermediary in any of the sequences. An nmr examination of the labelled products indicated that in the rearrangement of (135) to (136), no protoilludyl intermediate could be envisaged whereas (137) rearranging to give (138) would appear to involve a protoilludyl cation intermediate. The labelling patterns arose by the rearrangements outlined in Figure 26.



(135)

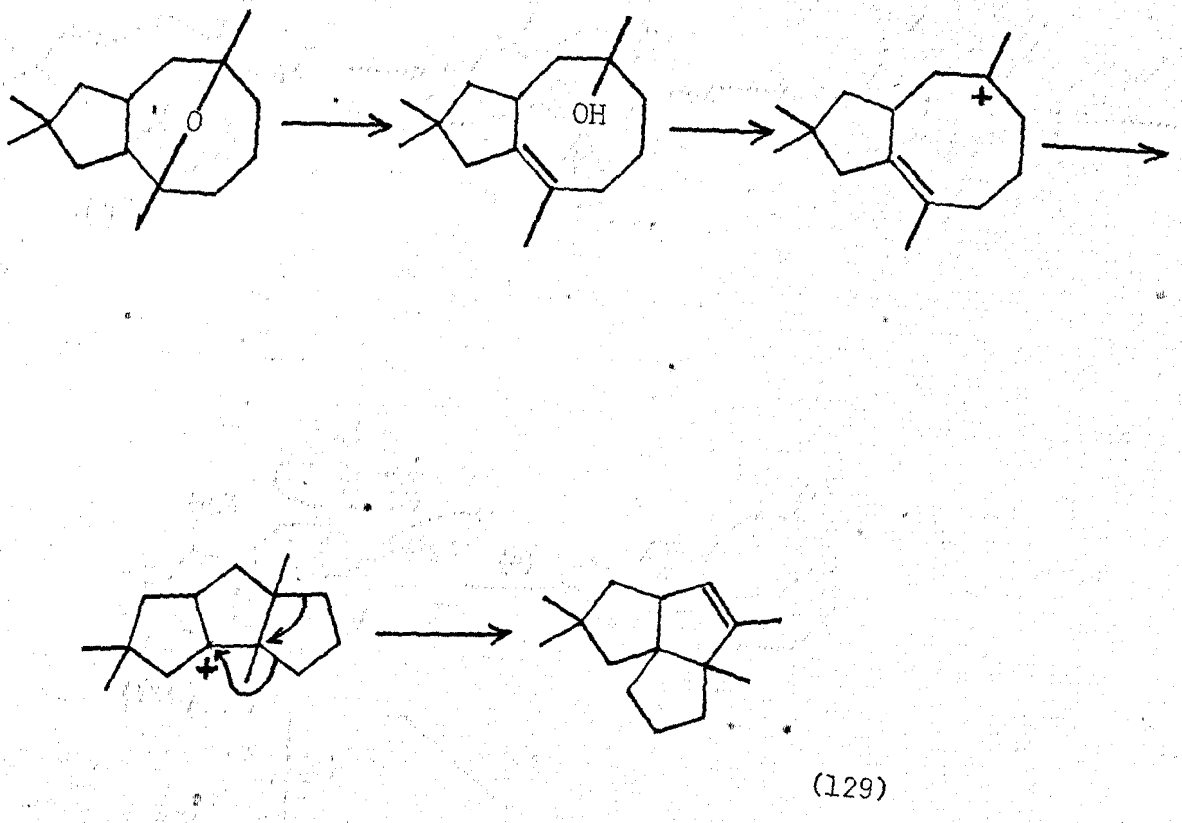
(136)

(137)

(138)

Figure 26

In (136) isolated from the reaction mixture, nmr showed the C-6 proton whereas in (138), no C-6 proton appeared, implying that a deuterium atom was present. However although the evidence points to the presence of a protoilludyl cation, no protoilludyl derivatives have been isolated from the reaction mixture. The tricyclic compound (129) arose by the rearrangement shown in Figure 27.



(129)

Figure 27

A recent investigation of the mechanism of cyclisation of humulene (1) with mercuric salts[106] has shown that although different products are obtained with mercuric acetate than with mercuric nitrate, the initial cyclisation is the same as shown in Figure 28.

The labelling patterns expected from deuteric borohydride reduction of the intermediate (139) did not match the observed results and mechanistically the results could be rationalised by examining the conformational differences between the pyranoid (140) and the furanoid (141) compounds. The

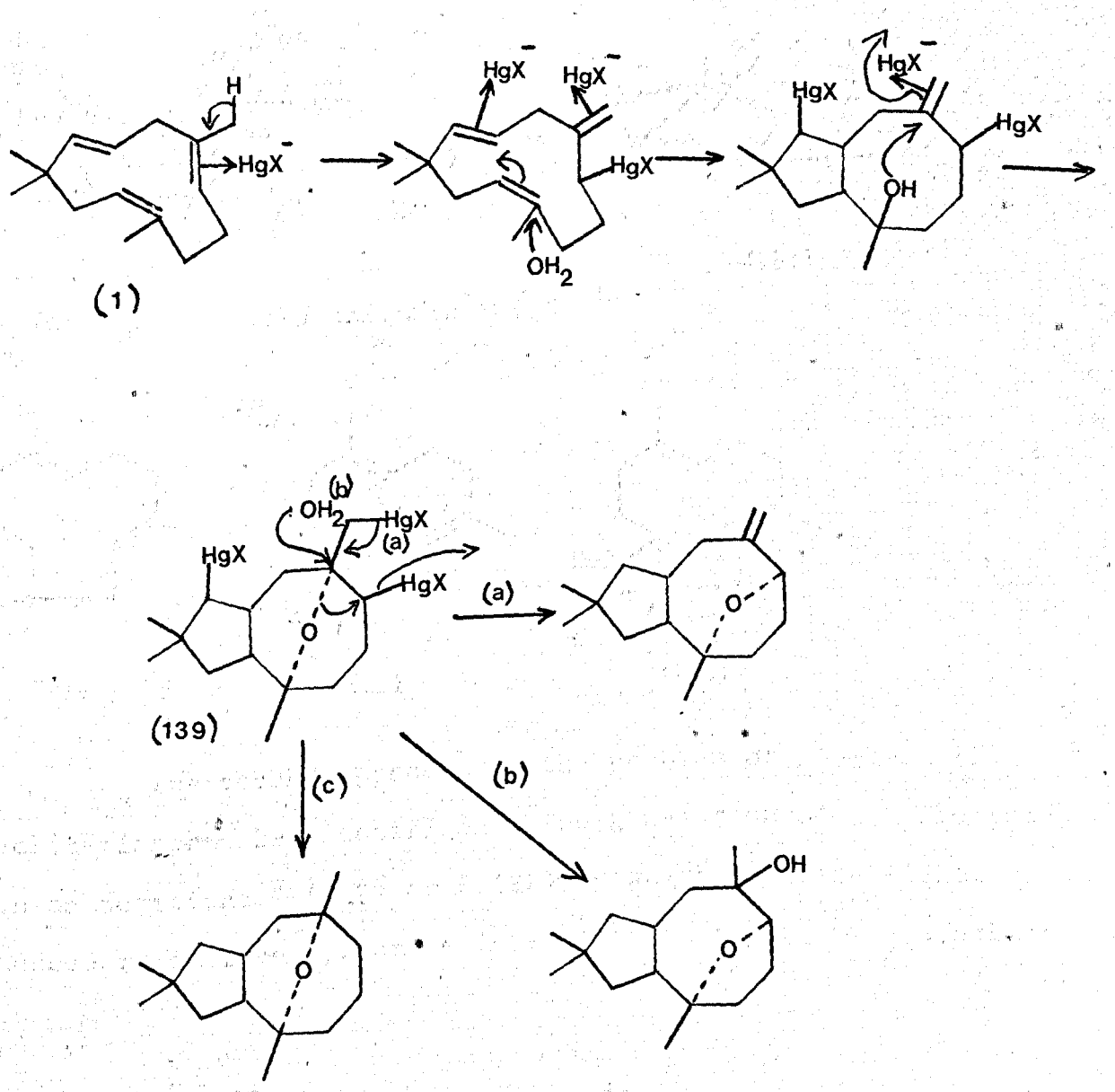
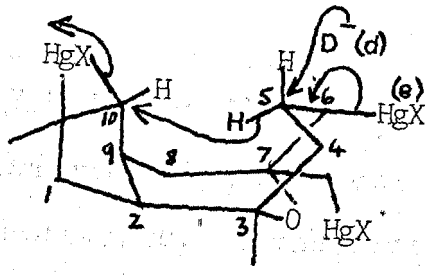


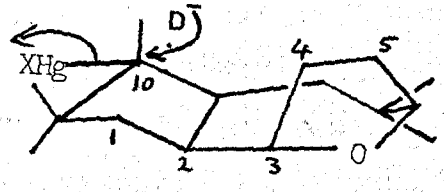
Figure 28

furanoid compounds followed the expected labelling pattern but there was a deuterium label missing from C-10 in the pyranoid compounds.

This could be explained in terms of a 1,6-hydride shift, allowed by the proximity of C-10 and C-5 in (140) but disallowed in (141) because of the distance between the two sites.

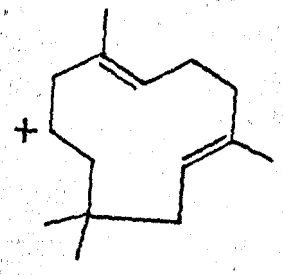


(140)

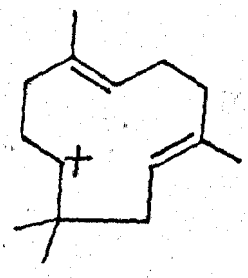


(141)

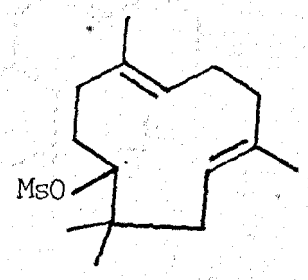
(d) S_N2 (e) *Elimination of HgX*



(142)

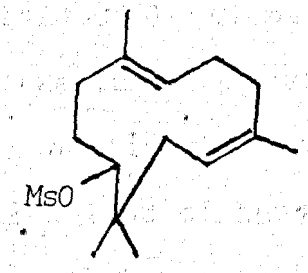


(21)

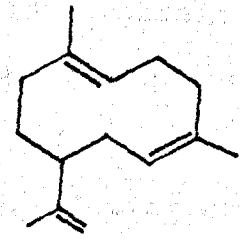


(143)

The second approach to the problem of humulene (1) cyclisation can be classified as the generation of a carbonium ion on positions 4 (142) or 5 (21) by which humulene (1) can be induced to cyclise. From an intermediate in the total synthesis



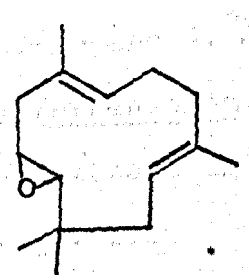
(144)



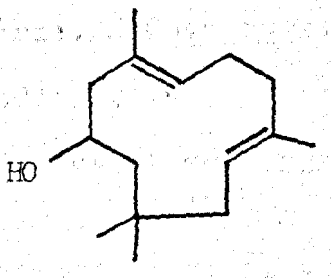
(145)

of humulene (1) previously discussed,[24] Nozaki *et al.* formed the mesylate (143)[107] which, on solvolysis in aqueous acetone and in dimethylaluminium phenoxide, gave humulene (1) in high yield. This was upheld by results obtained from solvolysis of the 5-tosylate by this research group.[108] However solvolysis of the

Z,E-isomer (144) in presence of various reagents yielded varying amounts of germacrene (145) and other 10-membered ring products. Noteworthy is the fact that no caryophyllene (22) was found as a



(10)



(146)

product in these particular experiments despite the proposal by Hendrickson[21] that caryophyllene (22) arose from the cis-$\Delta^{8,9}$ double bond isomer of humulene (1) or its equivalent precursor.

Preliminary research by this group into the possibility of functionalisation of the $\Delta^{4,5}$ double bond of humulene (1) had produced indirect methods whereby the 4,5-epoxide (10) and 4-alcohol (146) of humulene (1) could be obtained in good yield.[109] The objective of the research in this thesis was to utilise these compounds in the expectation of achieving satisfactory and novel cyclisations of humulene (1), allowing entry into any or all of the skeletal types outlined earlier and thus making a contribution to the growing understanding of the complexities of sesquiterpene biogenesis.

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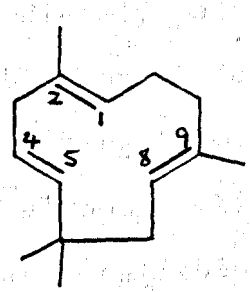
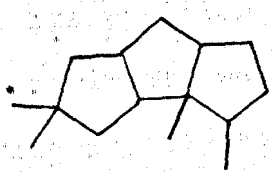
CHAPTER 1

DISCUSSION

As discussed in the Introduction, most previous attempts to cyclise humulene (1) itself and thus gain entry into the required skeletal types (2) - (7) have been unsuccessful. Initiation of cyclisation occurred at the $\Delta^{1,2}$ double bond and



(1)



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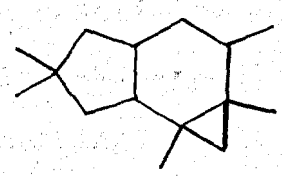

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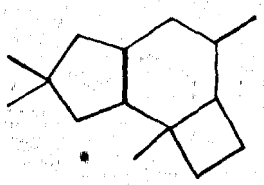

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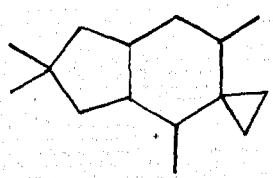

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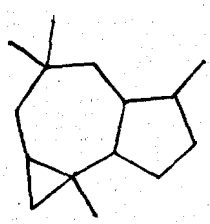

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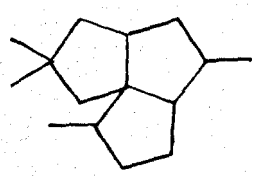
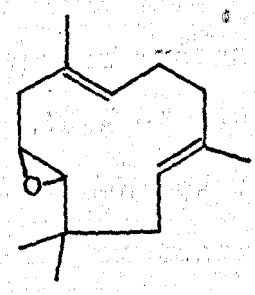


(7)

Produced, in general, compounds which did not exist in Nature, these results emphasising the known order of reactivity of the double bonds in humulene.[1] This order of reactivity has been explained in terms of relative strain about each of the reactive bonds viz. $\Delta^{1,2} > \Delta^{8,9} > \Delta^{4,5}$. In a sense the humulene problem was not unlike that which had beset Johnson and others[2,3] in their

early work on polyene cyclisation studies, viz. the difficulty of controlling the precise site of initial electrophilic attack. In the light of previous work it was felt that a prerequisite of any biomimetic cyclisation of humulene was the introduction of an appropriate group at C-4 and/or C-5 which could then be induced to react selectively to generate a cationic site. This however immediately raised problems in attempts to functionalise the $\Delta^{4,5}$ double bond because of its position in the reactivity sequence. A key target for our experiments was humulene-4,5-epoxide (8) but although this epoxide is naturally occurring, it is only a minor constituent as is the 8,9-epoxide (9), the 1,2-epoxide (10) being the predominant isomer. This is to be expected of course in



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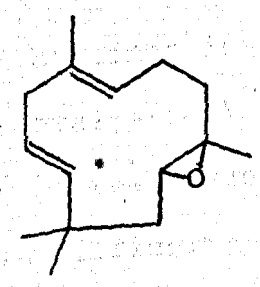

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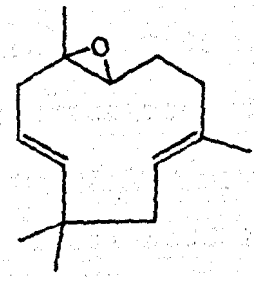
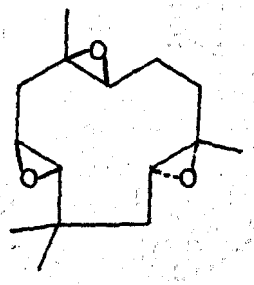


(10)

view of the proposed order of reactivity of the double bonds. Therefore indirect methods had to be devised to allow introduction of functionality specifically at the 4,5-positions. Such a method for the synthesis of humulene-4,5-epoxide (8) was established within this group[4] and involved peroxy acid epoxidation of humulene to give a mixture of trisepoxides from which the crystalline isomer[5] (11) was separated by repeated crystallisation.

Subsequent stereoselective deoxygenation of the trisepoxide (11)



(11)

using tungsten hexachloride and n-butyl lithium[6,7] gave the required 4,5-epoxide (8) in reasonable yield. This method proved to be the only one permitting stereoselective reduction of humulene trisepoxide (11) to the required monoepoxide.[4] The proposed mechanism is outlined in Figure 1. This mechanism consists of direct frontal attack of the tungsten complex and subsequent extraction of the oxygen atom of the epoxide.

Considerable difficulty was experienced for the first period of this project in trying to synthesise sufficient amounts of the starting 4,5-epoxide (8) by following the prescribed route.[4] The initial obstacle was the epoxidation reaction, carried out in chloroform as solvent with solid sodium bicarbonate added as a scavenger for the free m-chlorobenzoic acid generated in the reaction. The temperature had to be rigidly maintained below $4^{o}C$ for the initial addition of the peroxyacid and during the holding period. The reaction time was long ($\sim$ 36 hours) for complete epoxidation and the resultant crude product after work-up was extremely gummy and difficult to handle. Isolation of the required trisepoxide (11) involved a painstaking series of

$$WCl_6 + 2\,BuLi \longrightarrow Li_2WCl_6 + Bu\text{-}Bu$$

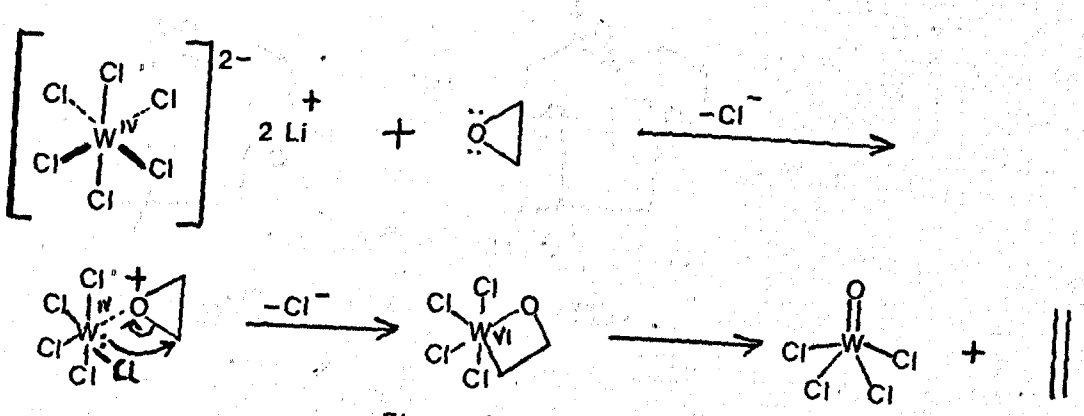
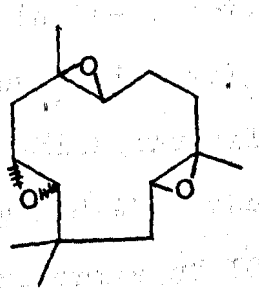
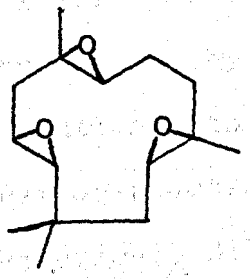


Figure 1

crystallisations, filtrations and evaporations and to overcome this, a known literature method involving an alkaline two-phase solvent system[8] was applied. This method was originally devised for acid-sensitive olefinic compounds but was found to be particularly suited to the inherent problems of humulene epoxidation. Dichloromethane was used as solvent and an 0.5M aqueous sodium bicarbonate solution acted as buffer. The benefits accrued from this method were numerous; the reaction was carried out at room temperature allowing fairly rapid addition of the peracid, the reaction time was much less (8-12 hours), the crude product was tacky but not gummy and nmr analysis of the crude product showed practically pure trisepoxide (11). This was an added bonus because the previous method produced two other trisepoxides (12) and (13) which had to be removed by crystallisation. In this modification, the crude product required only minimal purification before continuing to the deoxygenation stage. It has been noted in the past that there is a definite connection between choice of solvent and conformation of the substrate in



(12)

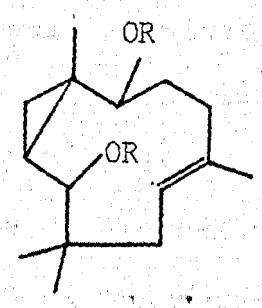


(13)

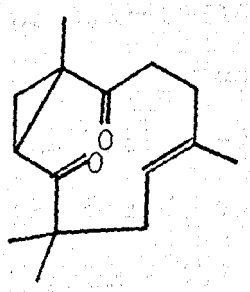
solution[3] in relation to oxidation of squalene and this has been confirmed by this group in solvent-related studies of mono-epoxidation of humulene (1).[9]

Having obtained the required trisepoxide (11), the next difficulty was encountered in the deoxygenation reaction. Initial attempts to follow the prescribed route[4] met with very little success, extremely low yields ($\sim$ 10%) of the 4,5-epoxide (8) being obtained. A fairly exhaustive study of the reaction conditions concluded that (i) it was vital that the tetrahydrofuran and the nitrogen gas used in the reaction be absolutely dry, (ii) the temperature during the addition of the n-butyllithium be kept below -65°C, (iii) the optimum ratio of humulene trisepoxide:tungsten hexachloride:n-butyl lithium was 1:2.3:6.9, (iv) heating of the mixture from room temperature to 45-50°C after addition of the trisepoxide (11) had to be as rapid as possible and (v) work-up required the use of aqueous sodium tartrate/sodium hydroxide solution[6] to ensure removal of any trace of tungsten salts. The optimum yield of material isolated by column chromatography was 58%, being considerably less than the quoted 80% yield by glc.[4]

Apart from the traces of humulene and diepoxides produced in the reaction, purification of the crude deoxygenation mixture by silica gel column chromatography yielded a further compound which crystallised on elution with ether. This however only happened when the deoxygenation reaction was carried out in the laboratories of ICI, Pharmaceuticals Division.[10] (The reason for this work being carried out at ICI will be revealed in Chapter 4.) Ir and polarity characteristics suggested the presence of two hydroxyls and ^{13}C nmr spectroscopy confirmed the presence of two carbons adjacent to oxygen. ^{1}H nmr spectroscopy suggested the presence of a cyclopropyl group (multiplet, 0.3-0.6 ppm), a vinylic methyl (3H,s,1.63 ppm) and what appeared to be carbinyl proton signals at unusually high field (2.78 and 2.95 ppm). The tentative structure (14) was assigned initially, the stereochemistry of the cyclopropyl ring fusion being unknown. The assignment of the structure was further enhanced by conversion



(14) R=H
(17) R=Ac



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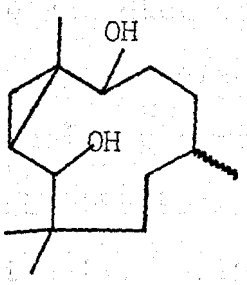
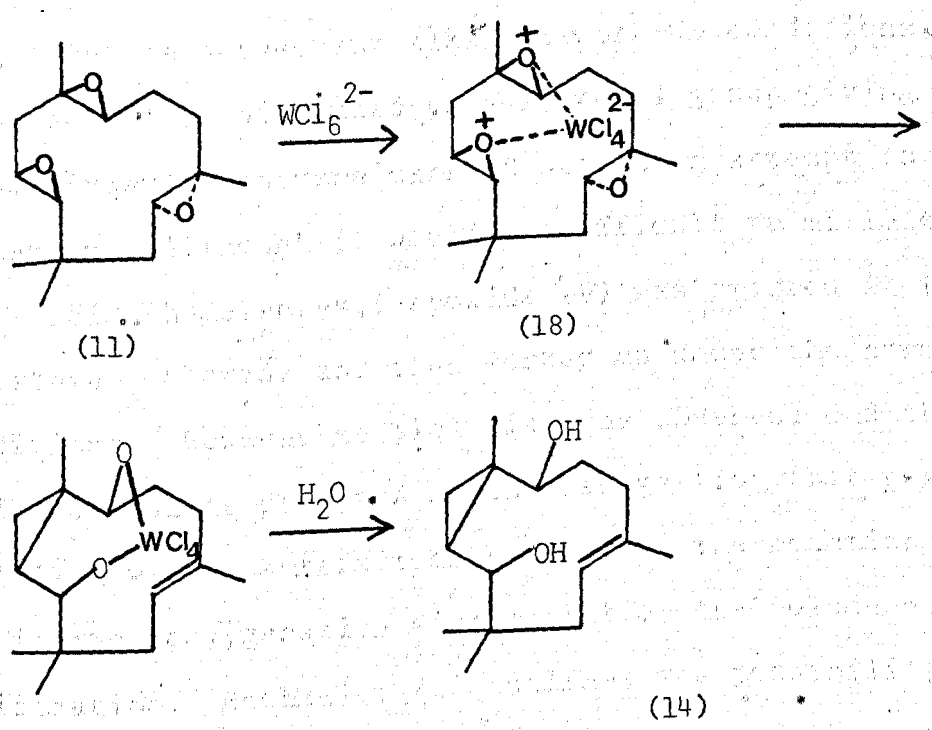


(16)

of the diol (14) to the diketone (15). The high field position of the carbinyl protons in the diol (14) could arise from shielding effects due to the carbinyl protons lying in the shielding zone of the cyclopropyl ring[11] and/or above or below

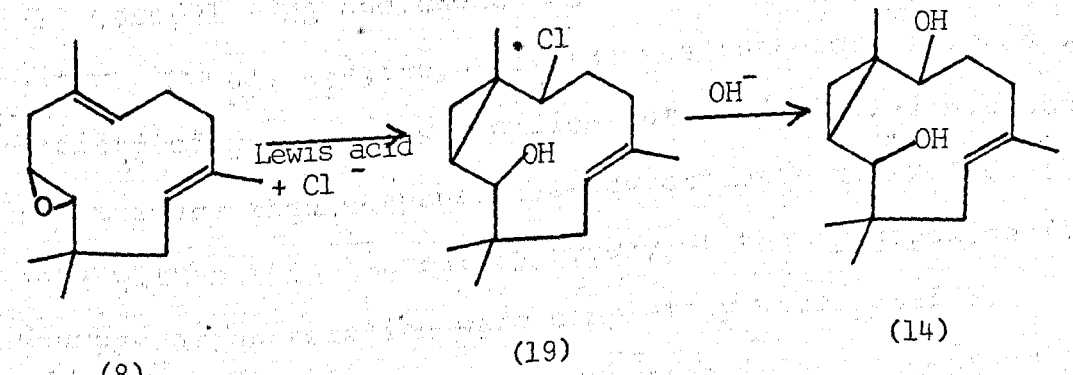
the plane of the double bond. Hydrogenation of the diol (14) gave the dihydrodiol (16) which still exhibited the high field signals for the carbinyl protons, thus implying that the high field position of these protons is due to the shielding effect of the cyclopropyl ring and that the two protons are cis- to each other. Decoupling experiments carried out at ICI and $Eu(fod)_3$ shift spectra carried out in this department verified the assignments but still did not unambiguously identify the nature of the cyclopropyl ring fusion. A crystalline acetate derivative (17) of the diol (14) has been prepared[10] and X-ray analysis would certainly clarify this situation. From models it is seen that both a cis- and a trans-ring fusion could be accommodated in a ten-membered ring system but which stereochemistry is present depends upon the mechanism of formation of the cyclopropyl ring. The original mechanism proposed by Dr. Ray Carman is outlined in Figure 2(a). The coordination of one tungsten with two epoxides of the starting material (either before or after the normal elimination of the 8,9-epoxide) gave an intermediate tungsten complex which eventually underwent cyclic electron flow and hydrolysis to give the diol (14). The driving force behind the deoxygenation reaction would appear to be the formation of the strong W=O bond. Therefore the mechanism (a) would seem to be unlikely because almost certainly the two single W-O bonds would not survive until hydrolysis was carried out. An alternative mechanism (b), suggested at Stirling, involved the action of a Lewis acid, possibly a tungsten species, upon the 4,5-epoxide (8), opening the epoxide ring, inducing cyclisation and producing an intermediate chlorohydrin (19). It is easy to visualise that lithium chloride is produced in the deoxygenation reaction and so chloride ion can act as an external nucleophile, quenching the

(a)



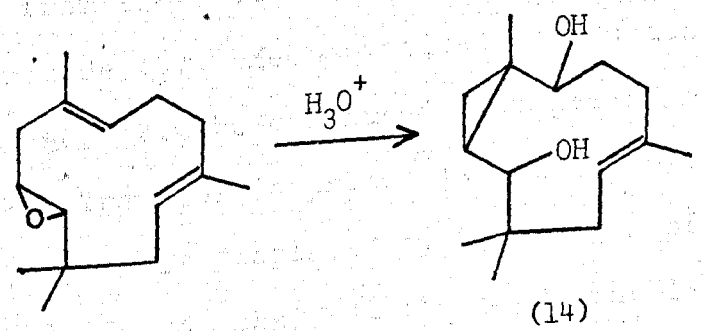
(11) (18) (14)

(b)



(8) (19) (14)

(c)

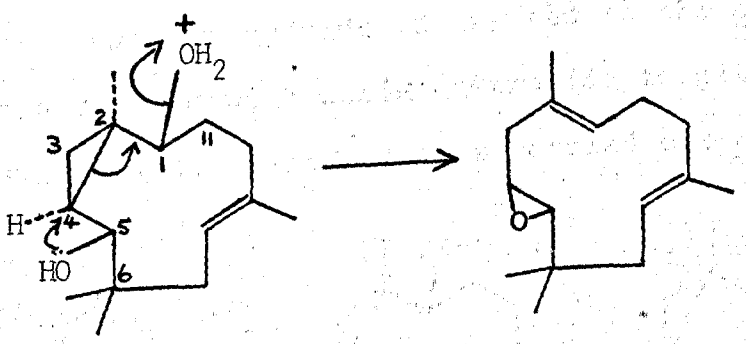


(8) (14)

Figure 2

carbonium ion formed at C-1 after formation of the cyclopropyl ring. In the subsequent alkaline work-up conditions, the chloride ion is displaced by a hydroxyl group giving the diol (14). Experiments were carried out in an attempt to prove this mechanism. Although it would be difficult to mimic the supposed Lewis acid, humulene-4,5-epoxide (8) was stirred in the presence of lithium chloride and then worked up under the normal alkaline conditions. However no diol (14) was observed and the lack of positive results prompted another suggestion that perhaps the diol (14) was an artefact of hydration, the reaction taking place not in the deoxygenation mixture but on the column during purification. Mechanism (c) outlines the possibility that wet acidic silica could induce protonation of the epoxide (8), followed by opening of the epoxide, cyclisation to give the cyclopropyl ring and quenching of the C-1 carbonium ion with water. Humulene-4,5-epoxide (8) was therefore stirred with a variety of grades of wet silica but no diol (14) was observed. The mystery then deepened because attempts to produce the diol (14) within this laboratory by way of the trisepoxide (11) deoxygenation reaction were completely unsuccessful.

Carman also reported[10] that in an attempt to form an acetonide from the diol (14), the product formed was surprisingly the 4,5-epoxide (8). Mechanistically this can be explained as shown in Figure 3, the cis-fused cyclopropyl ring being used to illustrate. The diol is protonated at the C-1 hydroxyl, then the cyclopropyl ring is displaced by S_N2 attack of the C-5 hydroxyl on C-4. This implies that all the bonds involved must be coplanar and particularly that there is an antiperiplanar relationship



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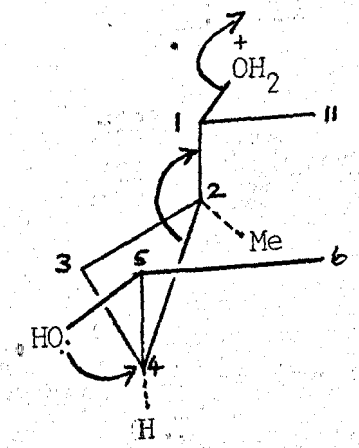
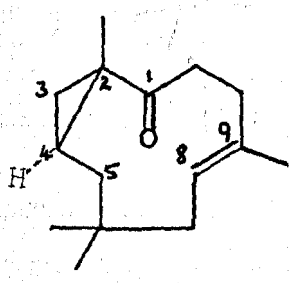


(20)

Figure 3

between C-2 - C-4 and C-1 - OH and also between C-5 - OH and C-2 - C-4 as seen in partial structure (20). Unfortunately this reversion does not necessarily prove the ring junction stereochemistry of the cyclopropyl ring since molecular models indicate that a conformation of the trans-fused compound might also be able to regenerate the 4,5-epoxide (8). However, it is interesting to note the isolation of the bicyclic ketone bicyclohumulenone (21) from the liverwort Plagiochila acanthophylla subsp. japonica,[12] the structure embodying a trans-fused cyclo-

propyl ring. The ketone was reduced to the alcohol (22) using lithium aluminium hydride, converted to the p-bromobenzoate (23) and then reacted with osmium tetroxide to give the triol derivative (24). X-ray studies carried out on this compound



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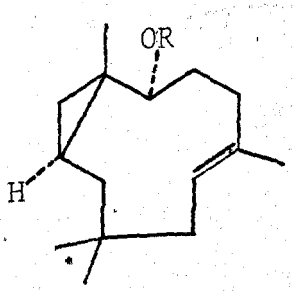
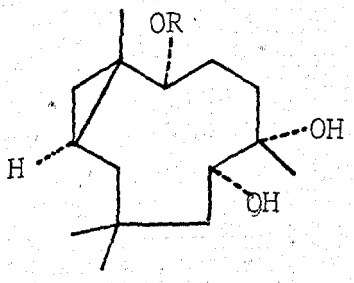


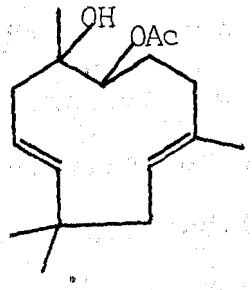
(22) R=H

(23) R=COC_6H_4Br-p



(24) R=COC_6H_4Br-p

gave the stereochemistry of the molecule and it would appear from the structure that the C-1 proton lies outwith the field of the cyclopropyl group. The nmr shift value for the C-1 proton would certainly be of interest but no nmr data was quoted for this compound. Very recently, Matsumoto et al.[13] have reported that treatment of humulene-4,5-epoxide (8) with boron trifluoride/acetic acid gives the compound (25) via the presumed intermediate (17). This report has prompted Bryson to carry out the reaction of the 4,5-epoxide (8) with boron trifluoride etherate in wet ether. This has led to the formation of the mysterious diol (14) and, although not conclusive, it does strongly suggest that the diol (14) obtained by Dr. Carman resulted from an acid-catalysed hydration of humulene-4,5-epoxide (8), most probably occurring on the silica column. Work is in progress in this laboratory in an effort to clarify some aspects of this chemistry.



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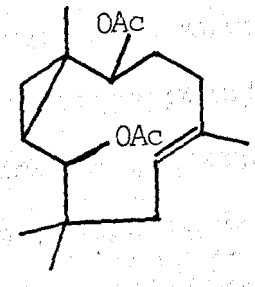


(17)

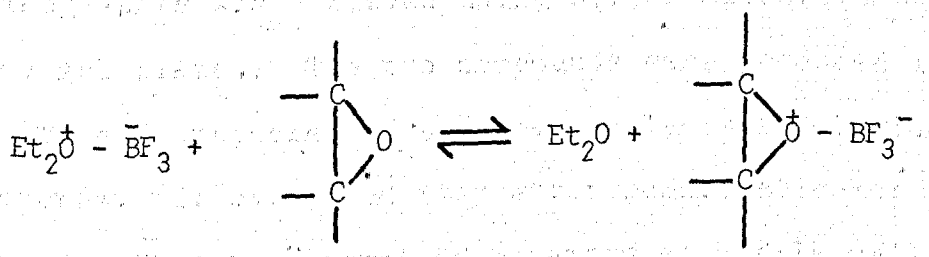
Studies carried out by van Tamelen[3,14] and Sutherland[15] showed that the epoxide function provided an excellent trigger for subsequent cyclisation of polyenes and both protic and aprotic catalyst types have been employed to initiate opening of the epoxide ring. The objective of this study was to find a suitable catalyst which would induce opening of the epoxide ring and subsequent cyclisation of the polyene to yield alcoholic products and to give a clean reaction with a small number of products.

Various protic agents such as 20% aqueous sulphuric, trifluoroacetic, glacial acetic, formic, p-toluenesulphonic acids and acid alumina, all stirred at room temperature, were tried without any notable success, either producing nothing or else a broad spectrum of compounds too complex to isolate and identify. Picric acid, in a 1:1 ratio with the epoxide (8) in nitromethane as solvent, although reacting very slowly at room temperature, produced an interesting result. After 11 days stirring there was some unreacted starting material but also several new compounds detected by analytical tlc, some of which were more polar than the 4,5-epoxide (8). Preparative tlc separated out

a polar spot which appeared by ^1H nmr spectroscopy to have only one olefinic proton at 5.30 ppm and a multiplet at 3.20 ppm, integrating for two protons. The compound also appeared to have lost a vinylic methyl and gained a quaternary methyl. This evidence indicated the loss of a double bond possibly by rearrangement and/or cyclisation. Also noted was the presence of a series of signals in the region 0.3-0.8 ppm but the significance of these was not fully appreciated at this stage. Ir spectroscopy showed the presence of a hydroxyl group.

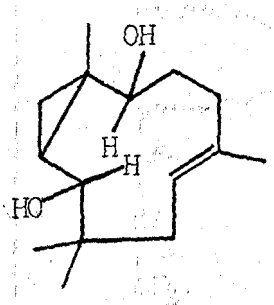
Attention was turned to Lewis acid catalysts, these having enjoyed considerable success in earlier polyene cyclisation experiments, in particular boron trifluoride etherate[3,14,16] and stannic chloride.[3,14,16] Boron trifluoride etherate in benzene at 0°C gave a very rapid reaction with the epoxide (8), the mixture very quickly turning purple then brown (2-5 minutes). After work-up, analytical tlc showed a large range of products and again some in the same polar region as that isolated from the picric acid reaction. Preparative tlc allowed the isolation of a polar spot with exactly the same R_f and nmr/ir characteristics as the product from the picric acid/nitromethane reaction. The evidence was puzzling because integration showed a ratio of one olefinic proton to two carbinyl protons and it was difficult to envisage a simple cyclisation which could result in such a situation. It was known from literature precedent[17] that different solvents could give different products and product ratios, ether and benzene with boron trifluoride etherate giving different reaction pathways, the former giving intermediate fluorohydrins.

The suggestion was that opposite modes of cleavage of the epoxide were induced and that the overall rate in ether was much slower than in benzene. The explanation given[18] was that upon consideration of the equilibrium the relative concentration



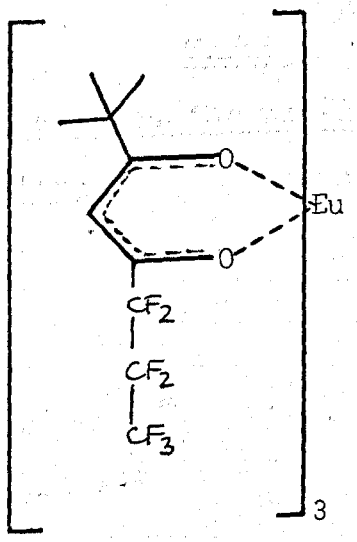
of the boron trifluoride-epoxide complex may be increased by the use of an inert solvent such as benzene rather than ether. The increased concentration of this complex would then tally with the increased rate of reaction observed. In other words when ether is used as solvent, the concentration of ether on the right hand side of the equilibrium is increased and so the equilibrium swings to the left. When the reaction of humulene-4,5-epoxide (8) with boron trifluoride etherate was carried out in dry ether at -70°C to further reduce the rate, it was found that the reaction did not go. However on warming the reaction mixture to room temperature and stirring for 8-12 hours, a much cleaner reaction was observed by analytical tlc, three major spots appearing. Apart from unreacted starting material, there was a non-polar spot and a polar spot running with the same R_f as the compound previously isolated from the picric acid/nitromethane and boron trifluoride etherate/benzene reactions. Isolation by preparative tlc gave a yellowish oil again with identical spectral characteristics

to those previously described. Again the nmr integration did not fit any immediately recognisable cyclisation pattern so analytical tlc experiments were carried out to see if the compound was pure. Initial variation of solvent polarity suggested that there was more than one spot and final separation into two distinct spots was achieved using silver nitrate-impregnated silica gel plates. The two compounds were produced in larger quantity and subjected to spectral analysis to elucidate their structures. It was noted that after purification of the two compounds, the minor compound remained as a pale yellow oil whilst the major compound solidified after a sample had been left at $0^{o}C$ for a number of weeks. The ^{1}H nmr spectra of both compounds again showed the multiplet at 0.4-0.8 ppm and it was then realised that this signal was significant and could in fact be attributed to the presence of a cyclopropyl ring. No olefinic protons were observed in the spectrum of the major compound but what appeared to be a vinylic methyl signal was observed as a broad singlet at 1.67 ppm suggesting that a tetrasubstituted double bond was present in the molecule. A broad doublet integrating for one proton was observed at 3.23 ppm with a coupling constant of about 9 Hz. This is quite high field for the carbinyl proton of a secondary alcohol, which is usually found in the 4-5 ppm region. However, if there is a cyclopropyl ring in the molecule, comparison can be made with the diol (14) in which both carbinyl protons appear at higher field due to the shielding effect of the cyclopropyl ring. Also observed were three quaternary methyl groups and a D_2O shake caused the collapse of a broad



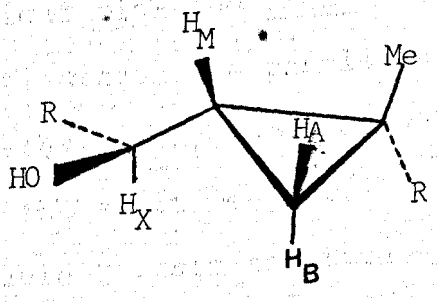
(14)

singlet at 1.55 ppm denoting the presence of a hydroxyl group. A study of the ir spectrum confirmed this, a distinct hydroxyl peak being observed at 3610 cm^{-1}. A weak peak at 3040 cm^{-1} confirmed the presence of the cyclopropyl ring, the typical gem-dimethyl doublet was observed at 1380 and 1370 cm^{-1} and C-O stretch signals appeared in the region 1000-1100 cm^{-1}. Initial spin decoupling and INDOR experiments showed that the carbinyl proton signal at 3.23 ppm was coupling with something in the cyclopropyl region but it was difficult to be specific about any assignments further than this because of the complexity of the nmr spectrum. It was decided to utilise a lanthanide shift reagent[19] to simplify the spectrum and so tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionato)europium (26), hereafter called $Eu(fod)_3$, was chosen as a suitable reagent. A series of measured additions of $Eu(fod)_3$/deuterochloroform solution were made and the observed induced shifts for the various signals were noted. Concurrently, spin decoupling and INDOR experiments were run after each addition to clarify the couplings and coupling constants of the related signals as the



(26)

spectrum was stretched out and simplified. A downfield shift of the three quaternary methyls together with a simplification of the cyclopropyl region was observed and decoupling experiments suggested an ABMX system, as shown in the partial structure (27). The coupling constants are shown in Table 1.



(27)

As the $Eu(fod)_3$ was added, H_X moved downfield fastest because of its proximity to the europium-complexed oxygen. The next proton to appear out of the methylene envelope was H_M which showed as a complex multiplet, potentially an eight-line signal. Irradiation on H_M caused H_X to collapse from a doublet to a broad

TABLE 1

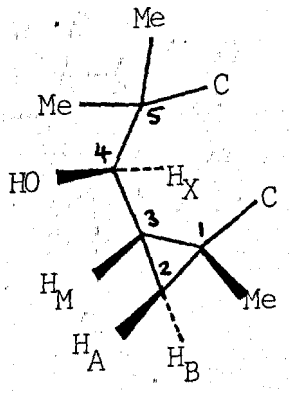
Coupling constants for partial structure (27)

Coupling	Coupling constant (Hz)
J_{AB}	-4.5
J_{AM}	8.5
J_{BM}	6.0
J_{MX}	9.5

singlet. Irradiation on H_X simplified H_M to a four line signal. Because of the similarity between the measured coupling constants J_{MX} and J_{AM} (9.5 and 8.5 Hz respectively), irradiation on H_B simplifies the H_M signal to a broad triplet. However since J_{MX} and J_{BM} are not similar (9.5 and 6.0 Hz respectively) irradiation on H_A collapses the H_M signal to a doublet of doublets. J_{AM} and J_{BM} are typical cis- and trans-cyclopropyl proton couplings.[11] From these findings, the partial structure (28) was proposed, the gem-dimethyl group and the cyclopropyl methyl also being affected by the shift reagent.

Table 2 lists the induced shifts observed in this experiment. The numbering system refers only to the partial structure (28) and is not intended to reflect the actual numbering of the complete molecule. The induced shifts of H_A, H_B and H_M were difficult to follow because for most of the experiment they were hidden under the methylene envelope.

On the basis of the spectral data coupled with off-resonance ^{13}C nmr spectra and mechanistic considerations, the structure (29) was assigned for the major compound.[20] Although



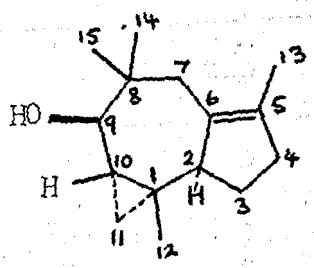
(28)

TABLE 2

$Eu(fod)_3$ shifts (ppm) for partial structure (28)

Proton	Amount of $Eu(fod)_3$ solution			
	0	20µℓ	40µℓ	60µℓ
C-1 Me	1.00	1.13	1.33	1.55
H_X	3.24	4.92	6.43	8.06
C-5 Me	0.91	1.66	2.26	2.93
C-5 Me'	0.90	1.45	1.95	2.51

the ^{13}C nmr data cannot be assigned completely unambiguously, Table 3 lists the signals with their proposed assignments.



(29)

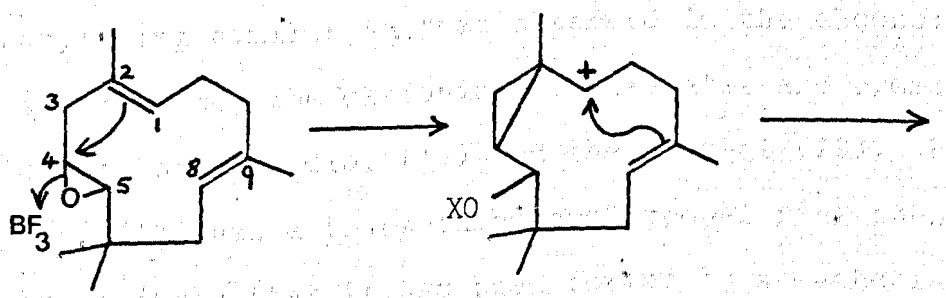
Mechanistically the formation of alcohol (29) can be rationalised by boron trifluoride-assisted opening of the epoxide ring followed by anti-Markovnikov cyclisation of the $\Delta^{1,2}$ double bond to form the cyclopropyl ring and then Markovnikov cyclisation of the $\Delta^{8,9}$ double bond on to the transient carbonium ion (30) to give the five-membered ring carbonium ion (31) which deprotonates to yield the tetrasubstituted olefinic alcohol (140). This sequence is shown in Figure 4. Note that humulene-4,5-epoxide (8), if drawn in a CT^{21} conformation (32) similar to that observed in the silver nitrate complex of humulene (1),[19] permits favourable alignment of the p-orbital of the developing carbonium ion on C-4 with the $\Delta^{1,2}$ π-orbital thus allowing backside S_N2-type attack of the C-1 π-orbital as the epoxide ring opens. As discussed by van Tamelen this reaction illustrates the concept of S_N2 epoxide opening with neighbouring π-bond participation. The cation (30) proposed in Figure 4 as an intermediate in the cyclisation of the starting epoxide (8) is of interest because it is identical to the cationic intermediate in the proposed mechanism for the formation of the diol (14) from humulene-4,5-

TABLE 3

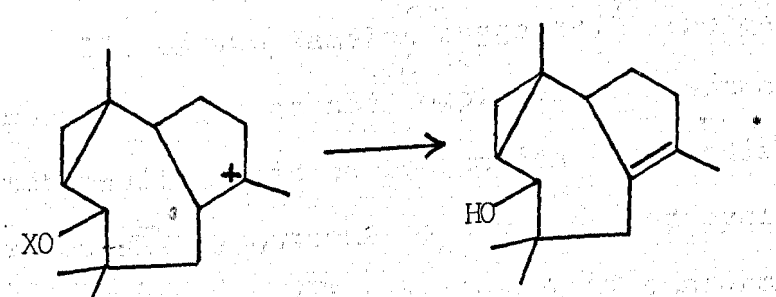
Off-resonance ^{13}C nmr data for (29)

Chemical shift (ppm)	Multiplicity	Assignment*
13.74	q	C-12
20.21	s	C-1
20.21	q	C-14
20.28	q	C-15
21.94	t	C-11
26.38	t	C-7
27.74	q	C-13
29.58	d	C-10
37.72	t	C-3
39.29	s	C-8
42.18	t	C-4
54.33	d	C-2
80.38	d	C-9
133.11	s	C-5
135.24	s	C-6

* Some difficulty was experienced in assigning certain of the peaks particularly in the high field region (∿ 20 ppm) because of the overlap of the singlet and quartet due to C-1 and C-14. However on the expanded scale spectrum it was seen that the 20.21 singlet lay slightly to the right of the quartet mid-point and that the quartet peaks were slightly broadened compared to the downfield quartet (20.28). Therefore the assignment was made as shown above.

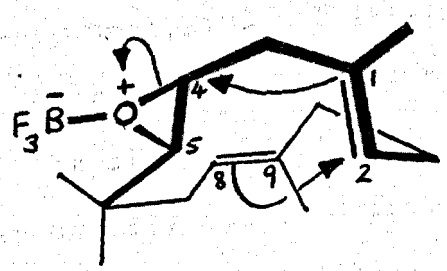


(8) (30)



(31) (29)

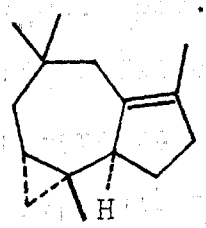
Figure 4



(32)

epoxide (8) in the deoxygenation of humulene trisepoxide (11).[93] This similarity only applies if the diol (14) has a cis-fused cyclopropyl ring similar to that observed in the alcohol (29) and in such a case, the cationic intermediates are common to the formation of both the diol (14) and the alcohol (29). However if the diol (14) has a trans-fused cyclopropyl ring then it is extremely unlikely that it has been formed by a mechanism based on Lewis-acid acid-catalysed opening of the epoxide ring of humulene-4,5-epoxide (8) followed by cyclisation involving the $\Delta^{1,2}$ double bond.

If, as van Tamelen suggests,[22] the mechanism of polyene cyclisation is not completely concerted but that conformationally rigid carbonium ion intermediates are involved, the stereochemical outcome of the proposed cyclisation of humulene-4,5-epoxide (8) held in the CT conformation (32) would be such that the C-1 methyl and C-10 hydrogen atom would bear a cis-relationship to each other based on the numbering system of alcohol (29). Also there should be a trans-relationship between the C-10 and C-9 hydrogens and between the C-2 hydrogen and C-1 methyl. The structure assigned for the alcohol (29) embodies these proposed stereochemical relationships and confirmation was obtained by X-ray analysis[20] of the p-bromobenzoate of the alcohol (29). Further evidence was obtained by comparison of the spectra of the alcohol (29) with those of an authentic sample of the hydrocarbon (33) obtained from the Belgian workers who originally isolated africanol (34) and its dehydration product, the hydrocarbon (33).[23]



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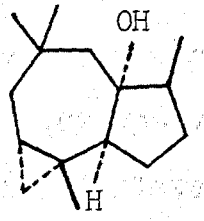
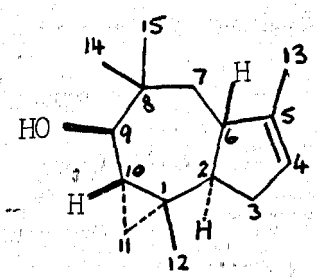


(34)

Attention was turned to the minor compound from the boron trifluoride-catalysed rearrangement of humulene-4,5-oxide (8). It should be noted that although these two compounds are referred to as the major and minor products, this was only observed in the early reactions, subsequent syntheses producing these compounds in approximately equal amounts. However, for ease of recognition at this stage they have been referred to as major and minor. The minor compound showed very similar spectral characteristics to those of the alcohol (29). The nmr spectrum revealed one olefinic proton at 5.33 ppm, a broad doublet at 3.23 ppm, a vinylic methyl at 1.64 ppm, a nine-proton broad singlet at 1.02 ppm, and a multiplet in the range 0.3-0.9 ppm. The ir spectrum showed hydroxyl, cyclopropyl and possibly tri-substituted olefin signals (1650 and 825 cm^{-1}). Lanthanide shift studies using $Eu(fod)_3$ were carried out on a sample of the minor alcohol and, coupled with INDOR and spin decoupling nmr techniques, similar results were obtained as those for the major alcohol (29).

Although INDOR was not as sensitive in this case, quite definite coupling was observed between the doublet at 3.23 ppm and the cyclopropyl multiplet at higher field. The doublet at 3.23 ppm moved downfield fastest under the influence of the shift reagent and the nine-proton singlet at 1.02 ppm very rapidly resolved into three separate three-proton singlets, two of which moved downfield much more rapidly than the third. Based on the spectral characteristics and the similarity between it and the major alcohol (29), the structure (35) was proposed. Table 4 shows the



(35)

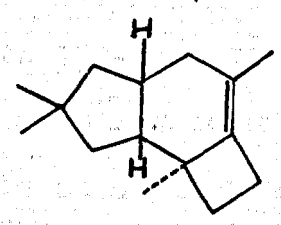
induced shifts observed in the lanthanide shift experiments.

TABLE 4

$Eu(fod)_3$ shifts (ppm) for alcohol (35)

Proton	Amount of $Eu(fod)_3/CDCl_3$ solution added			
	0	20µℓ	45µℓ	55µℓ
C-9-H	3.23	5.24	7.83	8.94
C-8-Me	1.02	1.81	2.89	3.31
C-8-Me'	1.02	1.68	2.52	2.89
C-1-Me	1.02	1.27	1.61	1.75

This structure can be rationalised as arising from cation (31) by an alternative deprotonation. Considerable effort was expended in trying to interconvert the two alcohols (29) and (35) by isomerisation of the double bond in an attempt to establish the relationship between the two compounds. The reagents used were trifluoroacetic acid in dichloromethane, p-toluenesulphonic acid in benzene, Amberlyst-15(H^+) resin, and boron trifluoride etherate in ether for the conversion of the trisubstituted to the tetrasubstituted olefin. These were also tried for the reverse conversion and also tried were boron trifluoride etherate with silver tetrafluoroborate in ether, the reason being that because the trisubstituted olefin alcohol (35) runs slower than the tetrasubstituted isomer (29) on silver nitrate tlc, (35) forms a more stable silver complex and inclusion of silver ions could perhaps swing the equilibrium in favour of the trisubstituted isomer (35). Hydrogenation i.e. 10% palladium/charcoal, methanol and hydrogen under atmospheric conditions, was tried because a literature example is known where Δ^6-protoilludene (36), on subjection to these hydrogenation conditions, gave the isomer (37).[24] Despite all attempts, however, no conversion was



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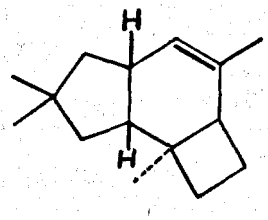
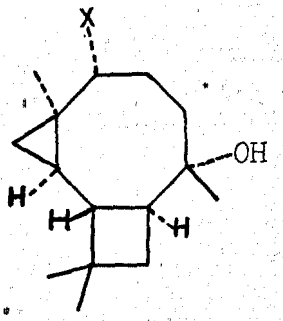


(37)

achieved in either direction.

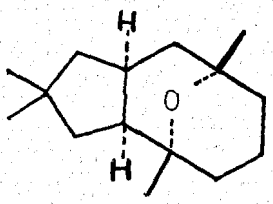
Several points arise from the structures of these two alcohols. Although alcohol (35) has been assigned a trans-ring fusion between the 7- and 5-membered rings, the nmr experiments did not conclusively establish this. However, many of the reactions discussed in the introduction to this thesis involve the participation of an internal trans-double bond in polyene cyclisations and it is obvious that where this occurs, trans-ring fusion is observed unless of course there is deprotonation and reprotonation of intermediates. These observations are in keeping with the proposals of the Stork-Eschenmoser hypothesis[25,26] and a study of molecular models clearly show the probability of a trans-ring junction.

Another consideration is the net overall cis-addition to the $\Delta^{1,2}$ double bond which is uncommon in olefin reactions unless the synchronous attack of a reagent occurs at both carbons on the same face of the double bond as in the formation of a cis-diol using permanganate. Trans-addition normally occurs as in bromination, etc. but it must be pointed out that ample literature precedent exists for this net cis-addition. Sutherland,[27] in reporting the formation of the tricyclobromohydrin (38), McKervey[28] and Namikawa[29] in the formation of tricyclohumuladiol (39) all propose that the $\Delta^{4,5}$ double bond of humulene or its 1,2-epoxide (10) undergoes net cis-addition. Furthermore Matsumoto and co-workers[30] proposed a net cis-addition to the $\Delta^{8,9}$ double bond of humulene in the oxymercuration-demercuration reaction with mercuric acetate to produce the cyclic ether (40). A suggestion as to how this cis-addition can arise is that when



(38) X = Br

(39) X = OH

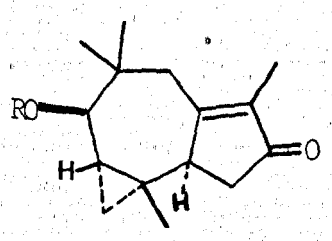


(40)

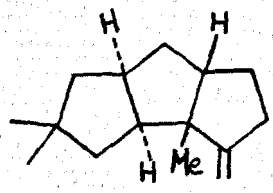
the transient carbonium ion at C-1 (30) is produced, some form of external nucleophile, perhaps involving complexation with the ether molecules or attack of a fluoride ion, participates to give a "pseudo" trans-addition, quickly followed by an S_N2-type reaction involving displacement of the nucleophile by the π-electrons of the $\Delta^{8,9}$ double bond.

Recent isolation of a new sesquiterpenoid keto-angelate (41) by Bohlmann and Zdero[31] provided another compound containing the africane-type skeleton. This particular compound was of even more interest than africanol (34) because its biogenesis could be postulated as arising from humulene-4,5-epoxide (8) whereas africanol (34) appeared to be derived from humulene itself. Of all the sesquiterpenoids thought to arise from humulene cyclisation, none to date had shown the oxygen functionality in a position which could be postulated as having originated from the oxygen of humulene-4,5-epoxide (8). In fact non-oxygenated hydrocarbons, thought to arise by cyclisation of a humulene precursor, have been isolated from fungal metabolites in which the highly-oxygenated

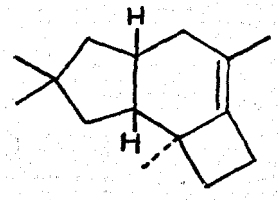
analogues co-exist, thus suggesting that oxygenation of these metabolites occurs at a late stage in the biosynthesis by incorporation of molecular oxygen. Such examples are hirsutene[32] (42) and protoilludene[24] (43).



(41) R = Ang



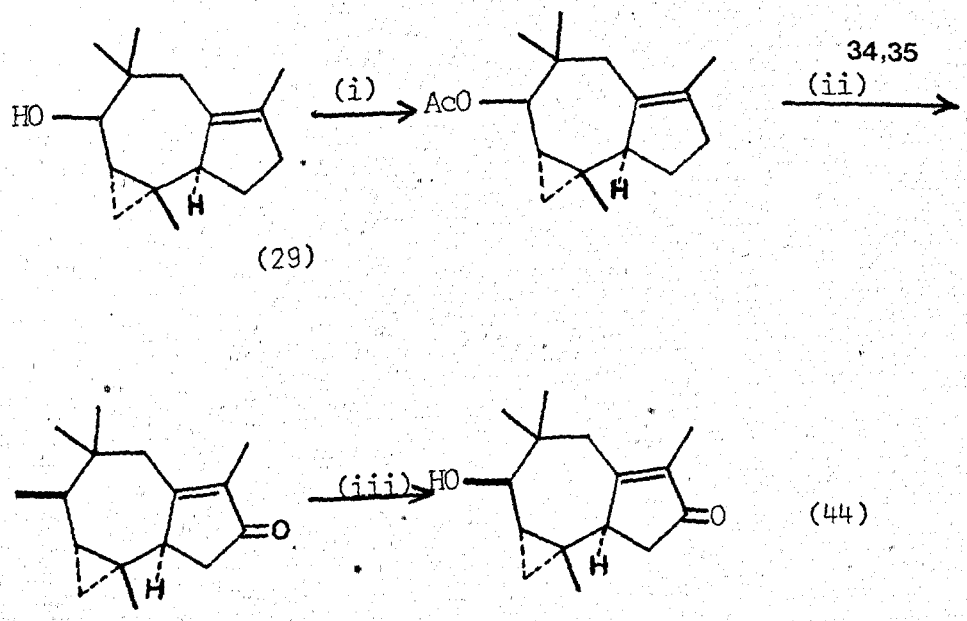
(42)



(43)

Because of the obvious similarity between the alcohol (29) and the keto-angelate (41), a synthetic conversion[33] of alcohol (29) to the keto-alcohol (44) was carried out as illustrated in Figure 5. The spectral characteristics of keto-alcohol (44) compared favourably with the published values for an authentic sample and further confirmation was obtained when a sample of (44) was sent to Professor Bohlmann for comparison with the authentic sample.

The reactions of various other Lewis acid catalysts with humulene-4,5-epoxide (8) were tried without much success except for stannic chloride in benzene at 0°C. A rapid reaction occurred and after twenty minutes, work-up and isolation by column chromatography produced some of the alcohols (29) and (35) and a new compound which was a clear oil and which was more polar on analytical tlc than the starting epoxide (8) but less polar



Reagents: (i) Ac_2O/pyr.; (ii) CrO_3/pyr.; (iii) MeOH/NaOH.

Figure 5

than the alcohols (29) and (35). Nmr spectroscopy showed signals in the region 3.5-4.0 ppm which looked like a doublet of doublets with a small coupling and a doublet with a large coupling. Otherwise the nmr spectrum was very similar to that of the starting epoxide (8). Mass spectroscopy gave a molecular ion of m/e 256 plus another peak at m/e 258. Analysis of the spectrum suggested the presence of chloro and hydroxyl groups in the compound and this could arise mechanistically by the acid-catalysed opening of the epoxide (8) followed by quenching of the cations (45) or (46) by chloride ion to give the chlorohydrin (47) or (48) as shown in Figure 6. The two structures (47) and (48) could not

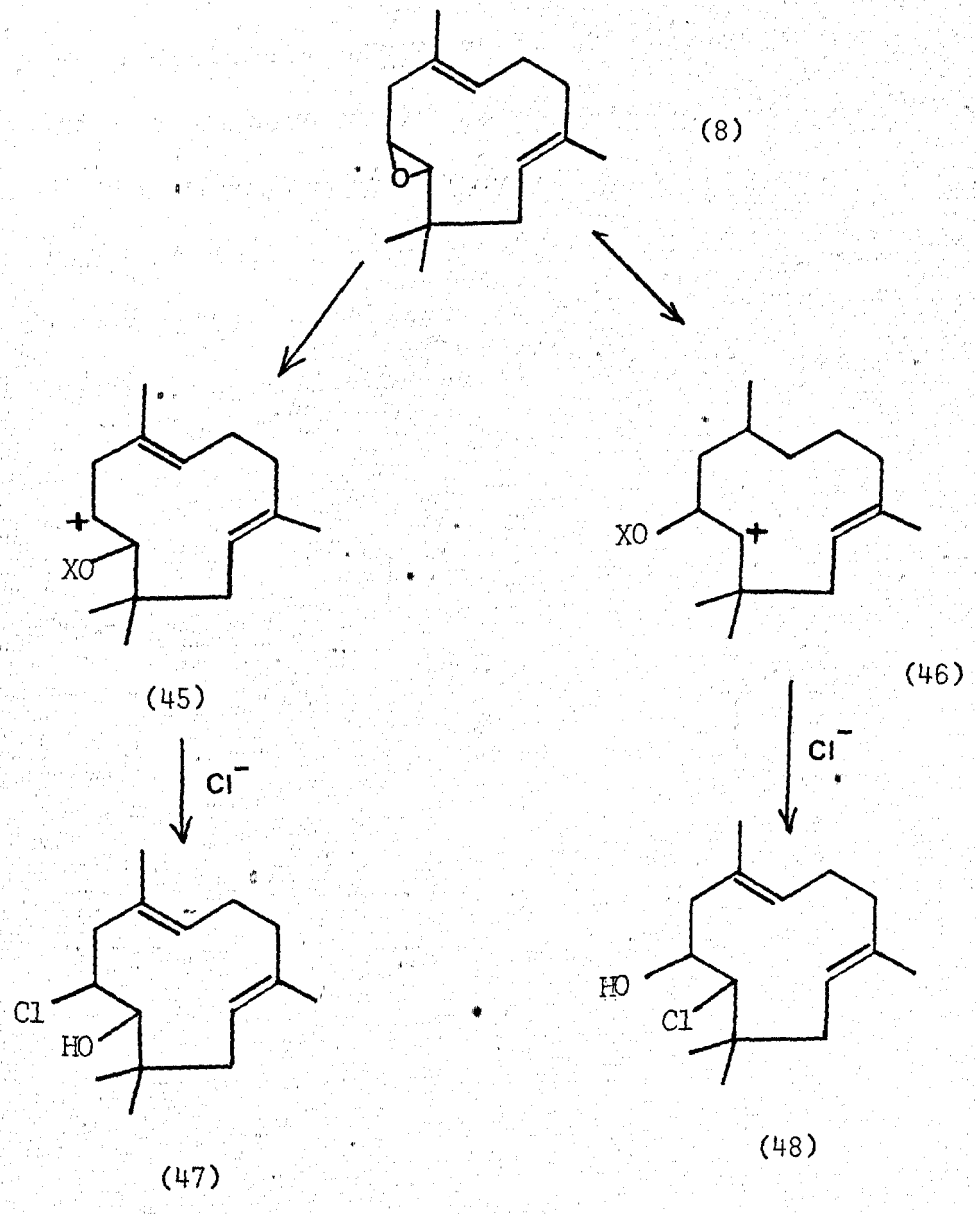
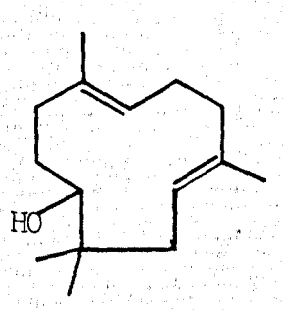


Figure 6

be distinguished either by mass spectroscopy or by nmr spectroscopy because both the carbinyl proton and the proton α- to the chlorine atom will give signals in approximately the same region of the spectrum. Of the two possible structures, (47) would seem to be more likely based on the fact that lithium aluminium hydride reduction of humulene-4,5-epoxide (8) gives humulen-5-ol (49) with no trace of the 4-alcohol (50). Also, if the epoxide opened

to give a carbonium ion on C-5, this constitutes a neopentyl carbonium ion which would be expected to undergo rearrangement, at least inducing ring contraction to give the ten-membered ring structure of type (51).[36] This rearrangement does not take place as no isopentyl or related group is observed by spectral analysis. The structure could also be elucidated by chemical means.



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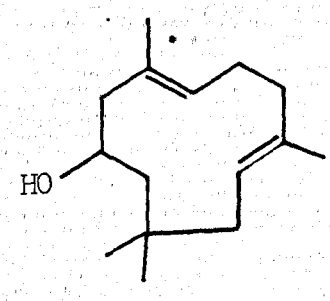

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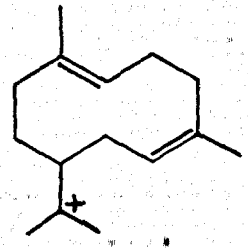


(51)

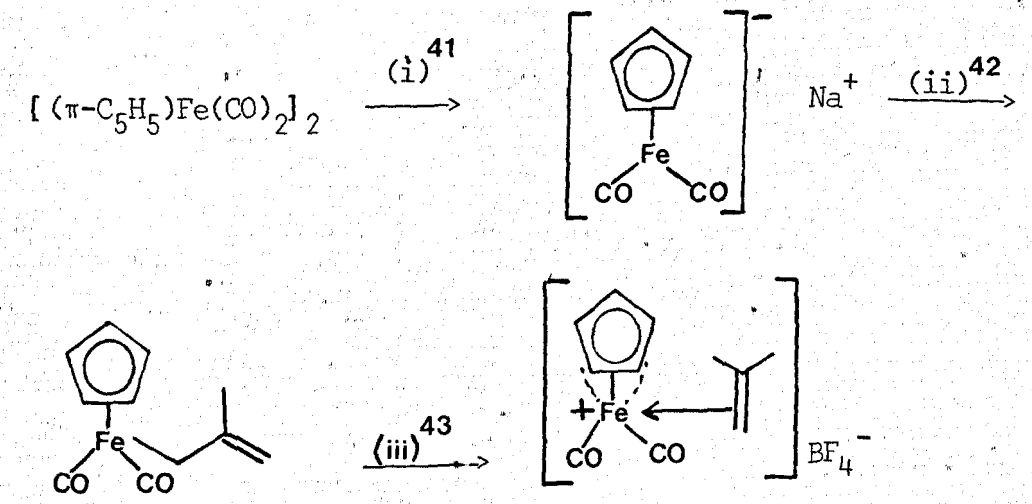
CHAPTER 2

From the cyclisation of humulene-4,5-epoxide (8) to give the alcohols (29) and (35) and from a study of molecular models of humulene-4,5-epoxide (8), it became obvious that the 1,2-double bond could align itself close to the backside of the epoxide group particularly at C-4 thus permitting S_N2-type attack of the π-orbitals of the $\Delta^{1,2}$ double bond on the epoxide as it opened, assisted by Lewis acid catalysis. van Tamelen[22,37] has shown that the correct alignment of the epoxide C-O bond and the π-orbital of the neighbouring double bond is absolutely critical, very little margin for deviation being allowed. Therefore it could be seen that there would be preferential involvement of the $\Delta^{1,2}$ double bond over the $\Delta^{8,9}$ double bond in any cyclisation reaction unless a different conformation of the starting epoxide (8) could be induced which would allow the $\Delta^{8,9}$ double bond to draw closer to the epoxide ring or the nucleophilicity of the $\Delta^{1,2}$ double bond could be either reduced or temporarily removed. A variety of potential methods were available for doing this and several were tried in the hope that an alternative cyclisation of the epoxide (8) could be induced thus allowing access into some or all of the other carbon skeletons which are proposed as being derived from humulene.

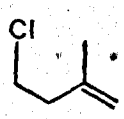
The first approach considered was to complex selectively the $\Delta^{1,2}$ double bond[38] to a metal as a π-olefin complex[39] and then to promote cyclisation of the 4,5-epoxide (8) by participation of the $\Delta^{8,9}$ double bond. On the basis that humulene (1) forms a 1:2 complex with two silver ions[40], at the $\Delta^{1,2}$ and $\Delta^{4,5}$ positions humulene-4,5-epoxide (8) was treated with a 50% aqueous silver nitrate solution in ether but no visible reaction occurred. In

an attempt to induce precipitation of any complex formed the experiment was tried using silver tetrafluoroborate in ether, thus introducing a large counter ion. A white precipitate was observed but on isolation, the yield was found to be very low and the compound appeared to be very unstable. Similar experiments using benzene and nitromethane as solvent were unsuccessful. It was decided that if a silver complex of the 4,5-epoxide(8) had been formed in solution then the alternative approach would be to attempt <u>in situ</u> cyclisation without isolation of the complex. The experiment was repeated with silver tetrafluoroborate in ether and, upon formation of the white precipitate, a one molar equivalent of boron trifluoride etherate was added to the reaction mixture, the reaction progress being monitored by analytical tlc. It was observed that a polar spot with R_f identical to that of the alcohol (29) and possibly (35) were slowly produced. Using nitromethane as solvent and following the same procedure gave a much faster reaction, but the products appeared to be the same. It would seem that the complex, if formed at all, equilibrates at different rates in different solvents, ether allowing a slow equilibration between the complexed 4,5-epoxide and the free 4,5-epoxide (8) and so giving rise to a slow production of the rearranged alcohols (29) and (35) on reaction with the Lewis acid. On the other hand, nitromethane must induce fast equilibration between the two species hence the fast rate of reaction. An alternative reason could be that already discussed in terms of the effect of solvent upon the complex-formation equilibrium between the epoxide and the boron trifluoride.[18]

π-olefin complexes with iron are known and could be prepared as shown in Figure 7. The σ-methallyl complex (52)



(52) (53)

Reagents: (i) 1% Na-amalgam/THF; (ii) 

;

(iii) 40% HBF_4/Ac_2O (1:1).

Figure 7

is converted to the π-isobutene complex (53) and it is known[44] that an olefin exchange reaction can be induced as shown in Figure 8. Work already carried out within this department had achieved the formation of such an iron-π-complex of humulene[45]. However the reaction occurred in very low yield, the complex formed was unstable and there was uncertainty as to which double bond of humulene had been complexed. Certainly it would appear that it was on one of the two trisubstituted double

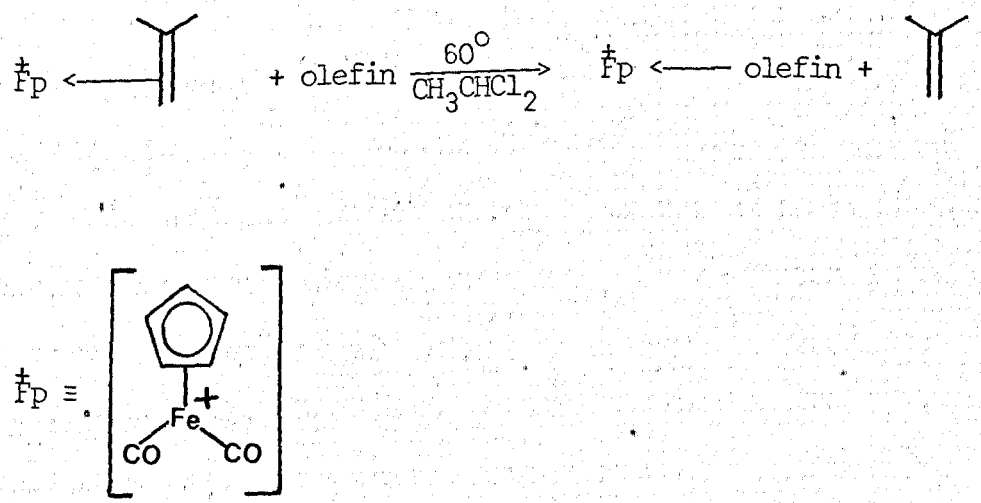


Figure 8

bonds based on the nmr data which showed definite downfield shifts of the vinylic methyls by 0.17 ppm. This could be confirmed using the theoretical consideration that the two tri-substituted double bonds in humulene were more likely to undergo complexation compared with the disubstituted double bond because of their greater reactivity in terms of torsional strain.[1]

Humulene-4,5-epoxide (8) was treated in the same manner as in Figures 7 and 8, the product, a solid, being extremely unstable and isolable in only very small yield, the largest amount obtained being 10 mg, which rapidly decomposed.

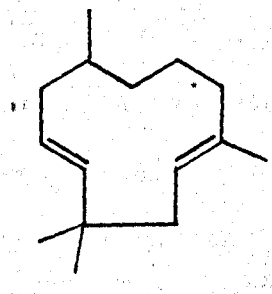
Sharp and Sharpe[46] noted the formation of what they proposed as complexes from the interaction of the cuprous salts of fluoro-acids and aromatic hydrocarbons. Copper (I) tetrafluoroborate was obtained by the displacement of silver from its tetrafluoroborate by copper. The resultant copper salt, suspended in toluene and n-pentane, was reacted with humulene in an effort to form the humulene-1,2-π complex. A white solid appeared but on filtration it dissolved, probably in atmospheric moisture. The reaction was then repeated but using humulene-4,5-epoxide (8) as

substrate. On addition of the copper salt in toluene to the epoxide (8) dissolved in pentane, a very fine pale green solution appeared. To prevent any handling difficulties with attempted isolation, it was decided to continue the reaction in situ by adding boron trifluoride etherate to the stirred mixture. An immediate reaction, recognised by the rapid change in colour from green to yellow to purple, took place and after work-up gave mostly non-polar material by analytical tlc, similar by nmr to the non-polar fraction from the reaction of humulene-4,5-epoxide (8) with boron trifluoride etherate in benzene at $0^{o}C$.

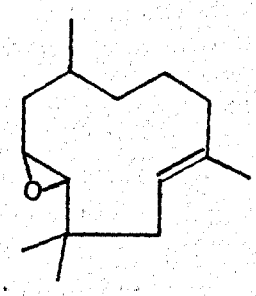
From these experiments, it would appear that the $\Delta^{8,9}$ double bond in humulene-4,5-epoxide (8) could not be induced to cyclise on to C-4 or C-5 when the nucleophilicity of the 1,2-double bond had been reduced by metal complexation. This can be explained by examining molecular models which suggest that the $\Delta^{1,2}$ double bond, when present, causes a fair degree of rigidity in the epoxide (8) such that insufficient flexibility exists to allow the proper alignment of the $\Delta^{8,9}$ double bond behind the epoxide ring.

Several approaches dealing with the problem of ring flexibility were attempted which basically involved conversion of the bond between carbons 1 and 2 from having sp^2 to sp^3 character. Models of 1,2-dihydrohumulene (54) and 1,2-dihydrohumulene-4,5-epoxide (55) showed enhanced flexibility of the ring system allowing greater mobility of the $\Delta^{8,9}$ double bond.

Initially, as a model, it was decided to take humulene and attempt the selective uptake of one molar equivalent of hydrogen by the 1,2-double bond based on the order of reactivity of the double bonds in humulene. The isolated dihydrohumulene (54)



(54)



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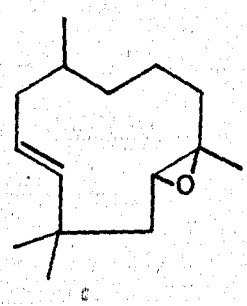

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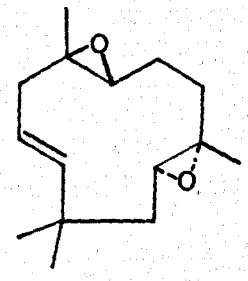
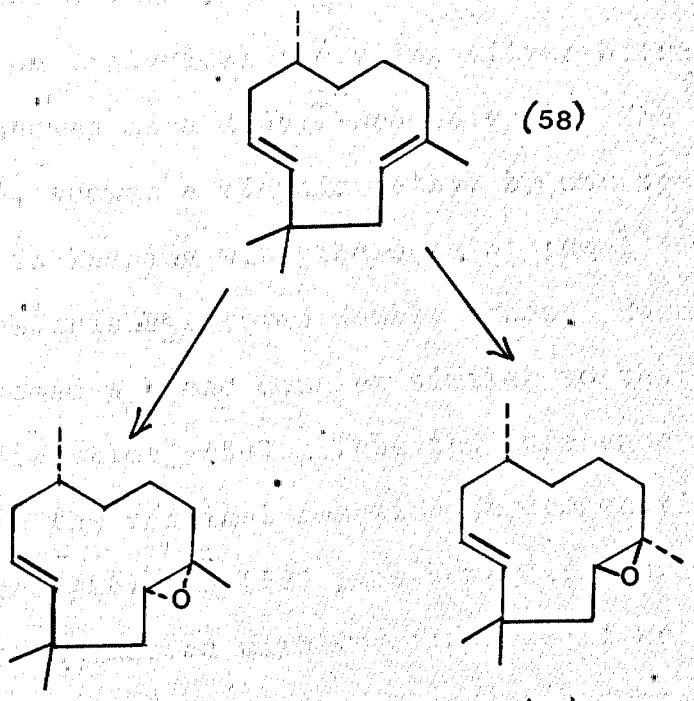


(57)

would then be monoepoxidised to give possibly a mixture of the 1,2-dihydro-4,5 and 1,2-dihydro-8,9-epoxides (55) and (56) respectively. Careful monitoring of the hydrogenation of humulene using Adam's catalyst in ethanol gave a product mixture which was separated by column chromatography using silver nitrate impregnated silica. A fraction isolated from the column displayed a sharp peak at 977 cm^{-1} in the ir spectrum which was attributed to the trans-disubstituted $\Delta^{4,5}$ double bond as seen in humulene[47]. The nmr spectrum showed an olefinic proton region very similar to that of humulene-1,2-epoxide[74] (10) and the disappearance of the doubly allylic protons on C-3 at approximately 2.5 ppm confirmed that the major product from the hydrogenation was 1,2-dihydro-

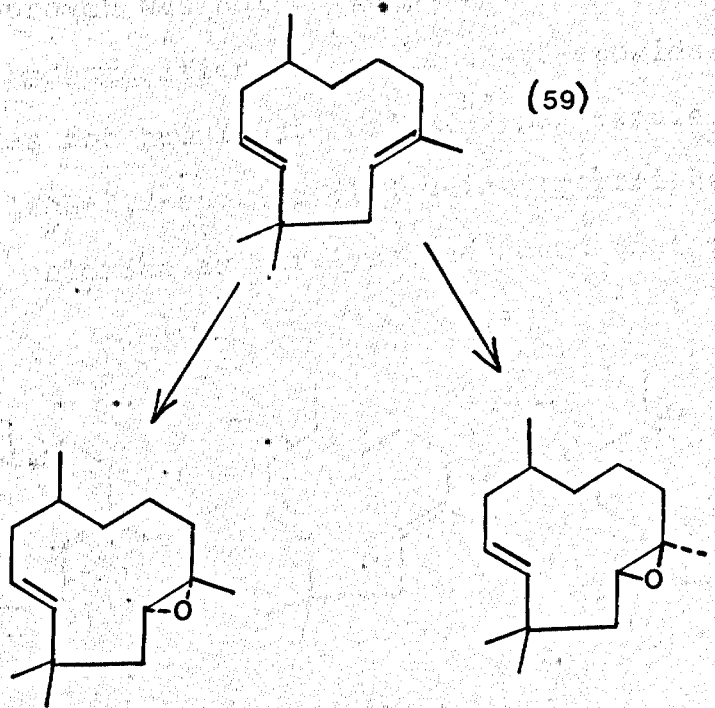
humulene (54). Monoepoxidation of 1,2-dihydrohumulene with m-chloroperoxybenzoic acid did not give any of the desired 1,2-dihydro-4,5-epoxy compound (55), several other compounds being formed. Analytical tlc showed that no starting material remained and several more polar products were observed. Silica gel column separation of the reaction products gave two compounds R_f 0.41 and 0.38 which, on examination of the nmr spectra, did not show a change in the olefinic proton region characteristic of the loss of the $\Delta^{4,5}$ double bond. Instead the faster running spot showed an olefinic region consistent with loss of the $\Delta^{8,9}$ double bond leaving only the 4,5-double bond system. This was diagnosed by comparison with the authentic nmr. spectrum of humulene-1,2-8,9-diepoxide[4] (57) which shows a complex ABMX system in the olefinic region. However in the region 2.5-2.9 ppm the signal for the C-8 oxirane proton is much more complex than expected, giving what would appear to be two sets of superimposed signals not unlike a twofold doublet of doublets having practically identical coupling constants. This can be rationalised by considering that hydrogenation of the $\Delta^{1,2}$ double bond can take place from either face of the double bond due to ring flip allowing rotation of the bond. Thus hydrogen can be introduced α- or β- on C-2 with respect to the C-2 methyl thus producing two enantiomers (58) and (59). With the introduction of the asymmetric centre, mono-epoxidation produces two racemic pairs of epoxides as seen in (60) - (63). (60) and (63) constitute a racemic pair as do (61) and (62). Therefore initially, upon hydrogenation, the enantiomers (58) and (59) produced cannot be separated unless by resolution. Upon monoepoxidation, however, the two racemic pairs of epoxides should be separable and this



(58)

(60)

(61)

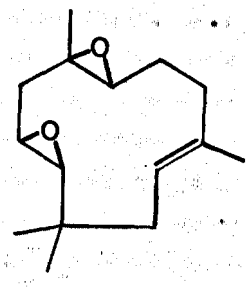


(59)

(62)

(63)

was suggested when it was observed that the compound R_f 0.41, when run on analytical tlc using silver nitrate impregnated silica, showed as a double-headed spot. The slower moving spot (R_f 0.38), showed a vinylic methyl by nmr spectroscopy which could be in keeping with the loss of the $\Delta^{4,5}$ double bond and one of the trisubstituted double bonds. However the olefinic region showed a broad doublet similar to that seen in humulene-1,2-4,5-diepoxide[4] (64). Thus the inference from the nmr data is that in the original reaction, hydrogenation produced the enantiomers (58) and (59) plus some 4,5-dihydrohumulene (65), this being in accord with the findings of Wright.[48] Because there is no asymmetric centre introduced by hydrogenation of the $\Delta^{4,5}$ double bond, there is only one compound (65) which, on epoxidation, gives the humulene-4,5-dihydro-1,2-epoxide (66). This approach was not followed further but attention was turned to the hydrogenation of humulene-4,5-epoxide (8) using the same system as for humulene. Wright[48] had carried out similar reactions on humulene-1,2-epoxide (10) using tristriphenylphosphine rhodium chloride as catalyst and found that the major product (84%)



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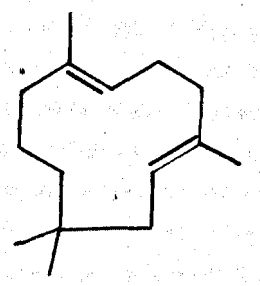

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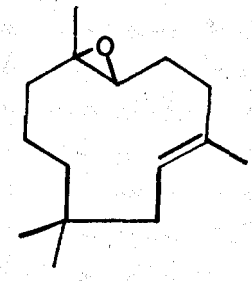
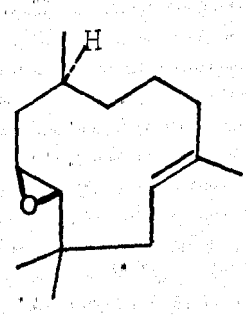


(66)

was 4,5-dihydro-1,2-humulene epoxide (66), the reported nmr data comparing very favourably with the hydrogenation-mono-epoxidation product from humulene reported above. A broad one proton doublet appeared in the olefinic proton region at 5.10 ppm consistent with the signal observed for the olefinic proton in humulene-1,2-4,5-diepoxide[4] (64). Also observed were the gem-dimethyl protons at 0.89 and 0.96 ppm, the C-2 methyl adjacent to the oxygen at 1.12 ppm, a vinylic methyl at 1.50 ppm and the C-1 oxirane proton at 2.59-2.77 ppm showing as a doublet of doublets. Based on the findings of Wright, it follows that careful hydrogenation of humulene-4,5-epoxide (8) should give the 1,2-dihydrohumulene-4,5-epoxide (55). However, with the experience gained from the reaction of humulene, hydrogenation of the 1,2-double bond should give the isomeric epoxides (67) and (68) and their racemates. When the reaction was carried out, and the reaction mixture run on analytical tlc using silver nitrate impregnated



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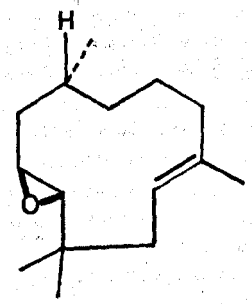


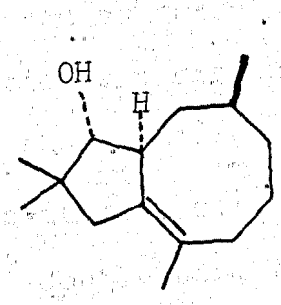
(68)

silica plates, a material R_f 0.52 and less polar than the starting epoxide (8) was observed, consisting of a double-headed spot. A separation of the two components was obtained by silver nitrate/silica column chromatography and nmr data showed that the

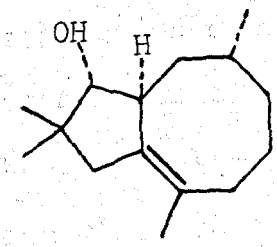
two components were practically identical, as would be expected from what should be two racemic pairs of epoxides. What appeared to be secondary methyls at 0.81 ppm in one compound and 0.89 ppm in the other were masked, probably by the overlapping signals of the racemic partners. The gem-dimethyl signals appeared as singlets at 1.06 and 1.09 ppm, the vinylic methyls as broad singlets at 1.58 and 1.59 ppm and the olefinic proton on C-8 as a doublet of doublets at 5.17 ppm and a broad doublet at 5.18 ppm respectively. The region 2.50 - 3.00 ppm and 2.40 to 2.80 ppm respectively showed a very complex multiplet derived from the C-4 and C-5 oxirane protons. These observations are consistent with the epoxides (67) and (68) and their racemates. However, further attempts to separate and purify the two compounds proved to be extremely difficult, in fact impossible because using either multiple elution of silver nitrate preparative plates or column chromatography using silver nitrate/silica and non-polar solvents only succeeded in producing new compounds at the expense of the dihydroepoxides (67) and (68) and their racemates. These new compounds appeared by analytical tlc to be much more polar than even the starting 4,5-epoxide (8) and it was decided to try some experiments on the dihydroepoxide mixture without separation. The mixture was reacted with boron trifluoride etherate (1:1) in ether for 30 minutes at -70°C initially then at room temperature for 1 hour. Analytical tlc of the oily product obtained from work-up showed that there was practically no starting material and that the major spot was very polar. This region was isolated using a 20 x 20 cm analytical plate but subsequent analytical tlc using silver nitrate/silica showed at least two compounds. Nmr investigation proved to be fairly inconclusive except that there

were olefinic proton signals and what appeared to be two distinct doublets in the region 3-4 ppm, where a carbinyl proton signal would be expected to appear. Further silver nitrate/silica column chromatography of the dihydroepoxide mixture induced disappearance of the starting material and subsequent stripping of the column with ether gave several new polar compounds. The nmr spectrum of the crude mixture did not allow assignment of any peaks except that a D_2O shake of the mixture caused the disappearance of a large broad singlet at 3.55 ppm implies the presence of a hydroxyl group. Further separation proved to be extremely difficult, all subsequent fractions appearing to be impure. However, it was obvious that in each of the fractions isolated there was a complex multiplet in the 3-4 ppm range. One very polar spot was isolated which was purified using preparative tlc and subsequently appeared as one spot by analytical silver nitrate/silica tlc. The ir spectrum showed a sharp-OH peak at 3610 cm^{-1}, a gem-dimethyl doublet at 1370 and 1380 cm^{-1} and C-O stretch bands between 1000 and 1100 cm^{-1}. The nmr spectrum indicated possibly a secondary methyl at 0.90 ppm, the gem-dimethyl signals as two three-proton singlets at 1.02 and 1.18 ppm, a vinylic methyl as a broad singlet at 1.56 ppm and a broad doublet at 3.10 ppm. On shaking the nmr sample with D_2O, nothing conclusive was observed although the broad signal at 1.56 ppm may have been marginally blunted. The major observation was that there were no olefinic protons visible and attempts using lanthanide shift experiments with $Eu(fod)_3$ in association with decoupling and INDOR experiments introduced some complications. It was noted that the doublet at 3.10 ppm rapidly moved downfield as the $Eu(fod)_3$ solution was progressively added but that soon the broad doublet altered its

appearance initially to a triplet then to two doublets as if two separate signals were superimposed. What at first looked like a quintet also appeared from the methylene envelope and moved downfield at practically the same pace as the carbinyl doublet. The methyl region simplified, the gradual appearance of two quite distinct secondary methyl doublets becoming obvious. The previously simple gem-dimethyl signals now also began to show as two pairs of singlets and a pair of methyl singlets also began to show at lower field, possibly due to the vinylic methyl group but the absolute assignment became very difficult. It would certainly appear that more than one compound was present and quite probably a similar situation has arisen as discussed previously in the case of the 1,2-dihydrohumulene-8,9-epoxides (60) - (63). In this case hydrogenation has given two racemic pairs of dihydro-epoxides which on opening of the epoxide followed by cyclisation gave the two isomers (69) and (70) plus their mirror images. The stereochemistry of the hydroxyl group relative to the α-methine



(69)



(70)

proton is quite clear cut because of the nature of the opening of the epoxide ring but the compounds (69) and (70) are epimeric

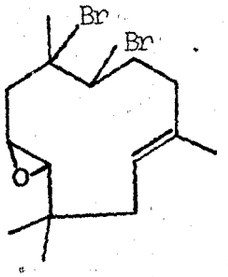
about the methyl group. The 'quintet' mentioned earlier is probably a complex multiplet composed of the superimposed signals derived from the methine proton of the two racemic pairs. This fits with the fact that the protons causing these signals must be very close to the europium-bound oxygen. A low resolution mass spectrum did not prove to be any more enlightening.

The less polar alcohol fraction obtained from the column separation was oxidised with pyridinium chlorochromate and the ir spectrum of the crude product showed a broad twin peak signal in the region 1700 to 1800 cm^{-1}, denoting the presence of carbonyl groups. Further purification by column chromatography gave an oil which showed a carbonyl peak at 1730 cm^{-1} in the ir spectrum and loss of the carbinyl proton multiplet between 3.00 and 4.00 ppm in the nmr spectrum. Otherwise the nmr spectrum was fairly inconclusive and it was decided to derivatise the total polar fraction obtained from the rearrangement of the 1,2-dihydrohumulene-4,5-epoxide mixture as the p-bromobenzoates in an effort to facilitate separation of the compounds. This approach did not simplify the problem and so the study of the 1,2-dihydrohumulene-4,5-epoxide cyclisation was discontinued. A conclusion drawn from this study is that the complete removal of the $\Delta^{1,2}$ double bond allows much more flexibility of the ring and hence permits closer approach of the $\Delta^{8,9}$ double bond to the backside of the epoxide group on the 4,5-positions of the ring. It would appear that the silver ions are acting as Lewis acids to catalyse the opening of the epoxide ring and hence allowing very rapid cyclisation of the molecule by participation of the neighbouring $\Delta^{8,9}$ double bond. Certainly the flexibility of the ring is manifest by the rapid reaction rate of

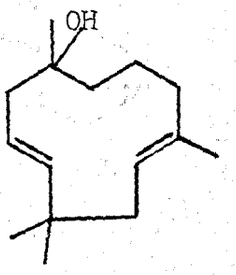
the cyclisation of the 1,2-dihydrohumulene-4,5-epoxides in presence of the silver ions compared with the inertness of humulene-4,5-epoxide (8) under similar conditions. Thus possibly the best approach would be a half-way house between the rigidity of the $\Delta^{1,2}$ double bond and the floppiness of the 1,2-dihydro compound.

Even if the cyclisation of the 1,2-dihydrohumulene-4,5-epoxides had been effected, it would only have displayed a part-cyclisation alternative to that seen in the cyclopropyl alcohol (29) formation. No functionality would have remained on C-1 or C-2 for further cyclisation. Thus the answer might lie in the introduction of a functional group at C-1 and/or C-2 in the form of a latent double bond. This would then permit the initial cyclisation to take place and subsequently the double bond could be reinstated for participation in the final ring closure. Initial attempts concentrated on forming the 1,2-dibromide (71) of humulene-4,5-epoxide in the hope that after Lewis acid-induced opening of the epoxide ring with subsequent cyclisation by participation of the $\Delta^{8,9}$ double bond, the $\Delta^{1,2}$ double bond could be reintroduced by elimination of the dibromide functionality.[49] Bromination was attempted using bromine in carbon tetrachloride, pyridinium hydrobromide perbromide in dichloromethane, and pyridine perbromide in dichloromethane, all at room temperature but each of these reactions produced a multitude of spots on analytical tlc.

Naya and Hirose reported the acid-catalysed reaction of humulene to give, amongst other products, the alcohol humulol (72).[50] The possibility of using humulol (72) (i) to functionalise specifically the $\Delta^{4,5}$ double bond, (ii) to induce cyclisation on to C-4 or C-5 with $\Delta^{8,9}$ participation and (iii) to induce a further



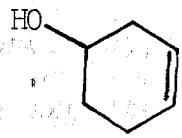
(71)



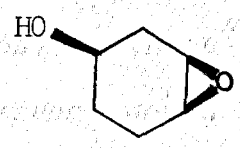
(72)

cyclisation of the rearranged molecule by way of the C-2 hydroxyl, could lead to a whole new range of cyclised products. It is known that allylic and homoallylic alcohols can be utilised to induce epoxidation in a highly stereo- and regioselective manner using vanadium[51-54] or molybdenum[51,52] complexes with t-butyl hydroperoxide. It would appear that the hydroxyl group anchors the metal complex by coordination of the oxygen to the metal and then the hydroperoxide complexes to the metal. The bound hydroperoxide can then epoxidise syn to the alcohol and it is observed that the reaction rate is much faster than for a peroxyacid epoxidation. An example quoted in the literature[51] is the epoxidation of 3-cyclohexen-1-ol (73) using vanadyl acetyl acetonate and t-butyl hydroperoxide to give the epoxide (74). It was noted that the epoxide was formed syn to the hydroxyl group with high stereospecificity and that the reaction rate was much faster relative to peroxyacid epoxidation. The general mechanism is illustrated in Figure 9.

A model of humulol (72) shows that the hydroxyl on C-2 can position itself close in space to the $\Delta^{4,5}$ double bond if the



(73)



(74)

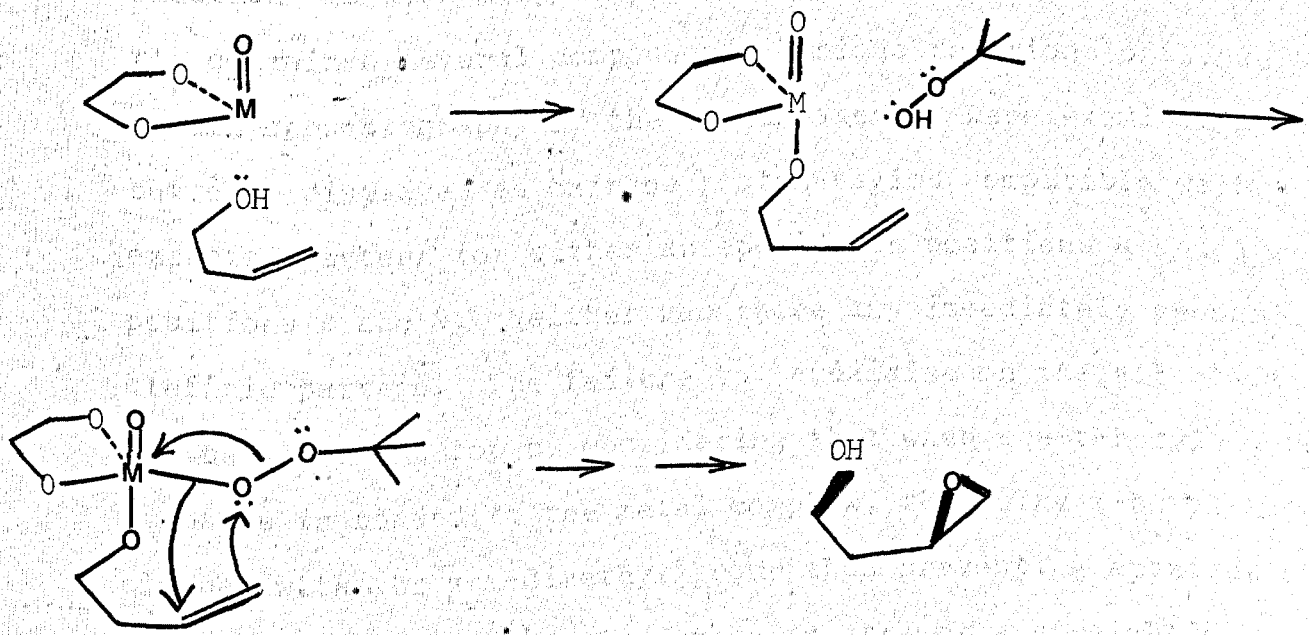
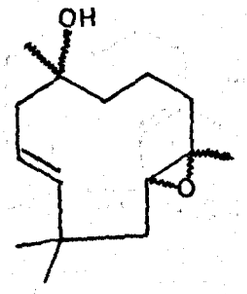


Figure 9

CT conformation of humulene is adopted. However the hydroxyl group cannot approach as close to the $\Delta^{8,9}$ double bond and so it would be expected that preference would be given to epoxidation of the $\Delta^{4,5}$ double bond. Furthermore epoxidation on the $\Delta^{4,5}$ double bond would give a 6-membered cyclic transition state whereas epoxidation on the $\Delta^{8,9}$ double bond would progress via an 8-membered cyclic transition state. In general, the 6-membered transition state is preferred. When the reaction was tried, the disappearance of humulol (72) and the appearance of a new polar spot was noted but the reaction proceeded extremely slowly. After nine days, traces of even more polar spots began to appear so the reaction was worked up to produce a yellow oil which by analytical tlc contained several compounds. Various fractions obtained by column chromatography of the crude material were examined by nmr but none displayed an oxirane proton region compatible with the expected pattern for either an epoxide on positions 4 and 5 or positions 8 and 9. Neither was there any immediately recognisable olefinic pattern. The failure to epoxidise on the 4,5 positions may be accounted for by considering that when the t-butyl hydroperoxide is bonded to the metal complex, the bulky t-butyl group clashes with the gem-dimethyl group thus preventing epoxidation taking place. It was decided then to attempt a straightforward peroxyacid oxidation of humulol (72) using m-chloroperoxybenzoic acid but the major product is the crystalline 8,9-epoxyhumulol (75) of undefined stereochemistry, based on ir and nmr spectra. The same arguments as previously discussed will hold here, the probable products being two racemic pairs of epoxy humulols. The ir spectrum shows a strong peak at 977 cm^{-1} which is characteristic of the $\Delta^{4,5}$ double bond C-H bend.[55] The presence of the



(75)

$\Delta^{4,5}$ double bond is recognisable on inspection of the olefinic proton region of the nmr spectrum. Also obvious is the doublet of doublets at 2.60 ppm which arises from the C-8 oxirane proton.

An alternative approach considered was first to protect the hydroxyl group of humulol (72), then attempt to induce cyclisation by selective attack upon the $\Delta^{4,5}$ double bond. A proposed reaction pathway is shown in Figure 10. Possible entry into the caryophyllane or potentially some of the tricyclic skeletons could be achieved by this route. Matsumoto *et al.*[30] had made use of mercuric salts in the cyclisation of humulene to give the cyclic ethers (40) and (76). Initial removal of the $\Delta^{1,2}$ double bond by reaction with one mole of the mercuric salt was followed by electrophilic attack of another mole of mercuric salt on the $\Delta^{4,5}$ double bond initiating cyclisation with participation of the $\Delta^{8,9}$ double bond closing over on to C-4. If the alcohol function of humulol (72) could be protected, this perhaps would prevent subsequent cyclisation to produce a cyclic ether and allow manipulation of any intermediate compounds to promote a second cyclisation as shown in Figure 10. Japanese workers had reported

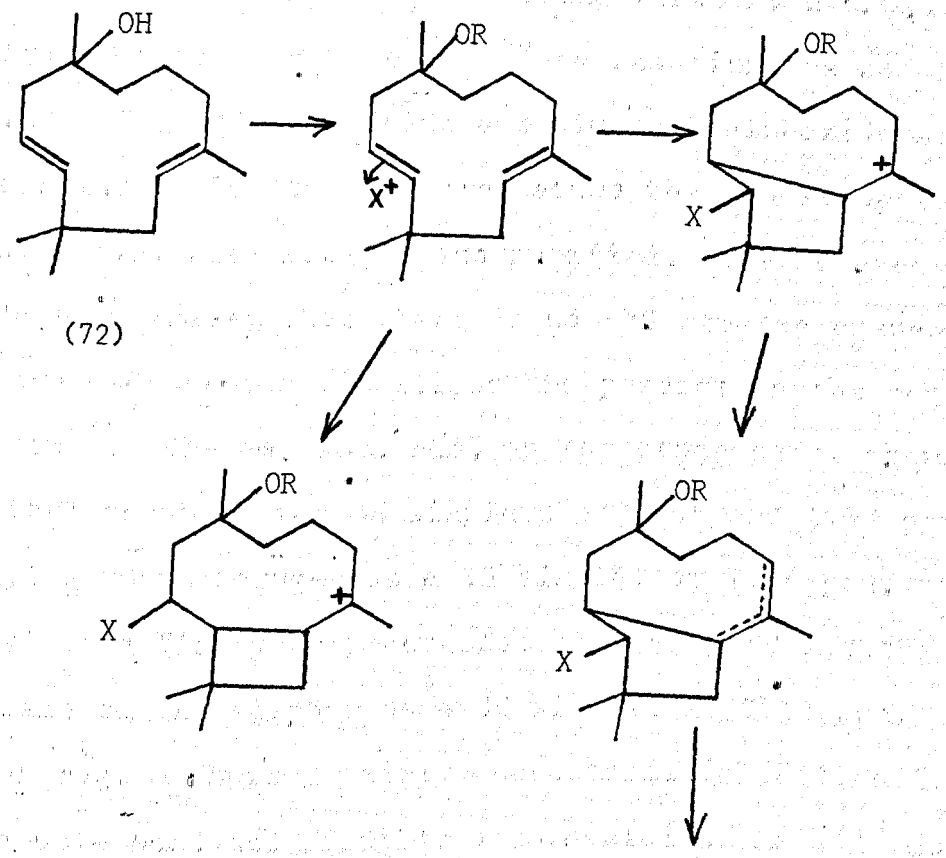
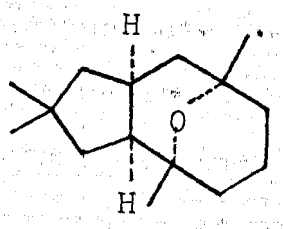


Figure 10



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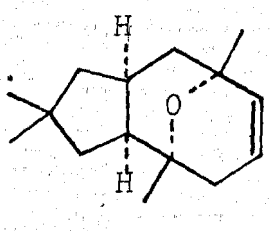
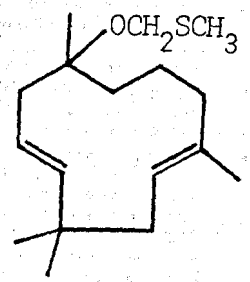


(76)



(77)

a satisfactory method of protecting tertiary hydroxyl groups as methylthiomethyl (MTM) ethers[56] by reacting the alcohol with a mixture of acetic anhydride and dimethylsulphoxide at room temperature. Following their method the MTM ether (77) of humulol (72) was prepared and purified. The ir spectrum of the product indicates that there is no -OH stretch present and that the C-S and C-O stretch vibrations are present in the region 1000 - 1100 cm^{-1}. The nmr spectrum, on the other hand, shows the gem-dimethyl group as two 3H singlets at 1.05 and 1.07 ppm, the C-2 methyl α to the oxygen as a 3H singlet at 1.16 ppm, the vinylic methyl as a finely split doublet at 1.59 ppm, and the methyl adjacent to the sulphur as a 3H singlet at 2.18 ppm. The olefinic proton region shows a fairly characteristic pattern for the 4,5-double bond and possibly also contains the $\Delta^{8,9}$ double bond proton signal masked by the dimethylsulphoxide peak. The nmr spectral evidence is consistent with the formation of the desired humulol MTM ether (77) and the resultant product was treated with mercuric trifluoroacetate in dry ether at -70°C in an attempt to induce cyclisation. After work-up, a clear oil was obtained which by nmr appeared to be humulol (72). Cleavage of MTM ethers to regenerate the tertiary alcohols is generally achieved using a mercuric salt in acetonitrile/water at 50°C. It had been reported[57] that examination of the cleavage of MTM ethers of tertiary alcohols at room temperature had mainly resulted in recovery of the MTM ethers after a fairly long stirring period. To preclude any cleavage of the humulol MTM ether (77), it was thought that low temperature and non-aqueous conditions might suffice. However it would appear that the MTM ether (77) cleaved rapidly,

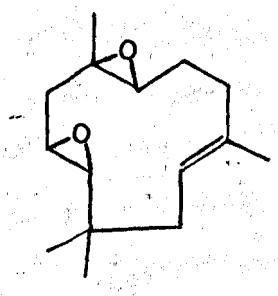
regenerating humulol (72) and showing no traces of any other products.

Little success therefore was achieved by completely taking out the sp^2 character of the 1,2-bond in humulene and its derivatives. Perhaps an intermediate state between the rigidity of sp^2 and the total flexibility of the sp^3 type derivatives tried would reduce the flexibility of the molecule sufficiently to reduce the rate of reaction observed with the dihydro compounds. Models showed that if some form of ring were introduced on to the 1,2-bond, then some rigidity remained whilst still allowing a certain amount of flexibility. The most obvious derivatives to try were those in which the 1,2-bond bore the epoxide functionality. A series of experiments run on humulene-1,2-epoxide (10) using mercuric acetate in methanol[58] and 50% tetrahydrofuran/water[59] and mercuric trifluoroacetate in methanol gave a large range of products with the methanol reactions and no reaction with aqueous tetrahydrofuran. In those reactions which gave products, it was decided that because of the large number of compounds involved, further time would not be spent in attempting to isolate any.

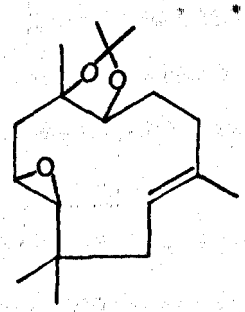
Remaining with the 1,2-epoxide function, humulene-<u>cis</u>-1,2-4,5-diepoxide (64) was treated with boron trifluoride etherate in both ether and benzene respectively at room temperature but the crude product mixture showed a multitude of polar spots by analytical tlc.

One final attempt was made to introduce a protecting group on the 1,2-double bond. Models showed that a five-membered ring appended to the 1,2-bond permitted close approach of the

8,9-double bond to the 4,5-epoxide group in humulene-4,5-epoxide (8). Flexibility, whilst present, was restricted due to a reasonable amount of rigidity being enforced by the ring and also to steric interactions as a result of the ring. A method was reported whereby an epoxide could be converted directly to an acetonide by reaction with copper sulphate and acetone.[60] It was hoped then that the epoxy acetonide (78) obtained from the diepoxide (64) could undergo epoxide ring opening followed by cyclisation. The acetonide could then be removed to regenerate



(64)



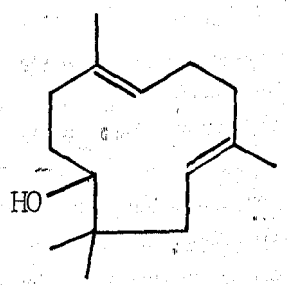
(78)

the olefin via the diol.[61] However, no reaction occurred on attempted preparation of the acetonide (78) and with a similar reaction on humulene-1,2-epoxide (10) itself.

Although there was very little success achieved in approaches made to modify the nucleophilicity of the 1,2-double bond in humulene and humulene-4,5-epoxide (8), it would certainly seem that further work in this area could prove to be fruitful, the difficulty being in finding a suitable protecting group for the $\Delta^{1,2}$ double bond.

CHAPTER 3

As seen from the previous chapters, use of the epoxide function to generate alternative skeleton types from cyclisation reactions was not very successful. As the main objective at the outset of this research work was to generate carbonium ions on C-4 or C-5 of humulene or its derivatives, attention was turned to find a satisfactory means of accomplishing this goal. The alcohol (49) has been synthesised[4,36] and its mesylate[36] and tosylate[62] solvolysed to produce humulene. It



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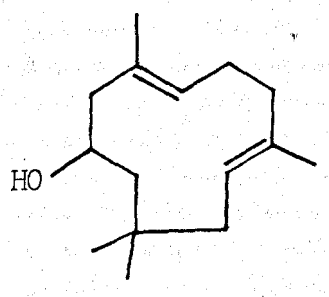
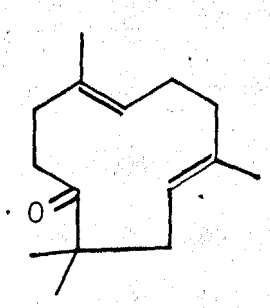


(50)

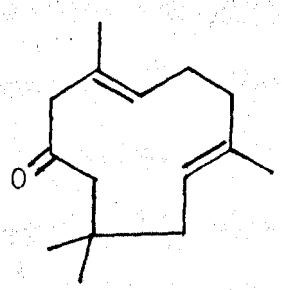
was thought that if the humulen-4-ol (50) was prepared then solvolysis of this alcohol or its derivative might promote cyclisation. The route used[4] to produce the alcohol (50) is fairly long and expensive, necessitating protection of the $\Delta^{1,2}$ and $\Delta^{8,9}$ double bonds of humulene as the corresponding epoxides followed by selective hydroboration of the $\Delta^{4,5}$ double bond to give the diepoxy-4-ol. Protection of the hydroxyl group as the corresponding acetate, subsequent deoxygenation of the epoxide groups, and finally removal of the acetate protecting group produced the 4-ol (50). Alternative methods were therefore

sought in an effort to shorten and/or simplify the synthesis of the alcohol (50).

One method tried was the 1,2-transposition of the ketone function to give the ketone (80) from the ketone (79), easily obtained by pyridinium chlorochromate oxidation[63,64] of the alcohol[4] (49). The nmr spectrum of the resultant ketone (79), isolated in quantitative yield, shows the disappearance of the carbinyl proton as a broad triplet at 3.50 ppm and a distinct downfield shift of the gem-dimethyl singlets at 0.83 and 1.07 ppm. to combine as a 6-proton singlet at 1.19 ppm, which can be explained by the proximity of the carbonyl group to the gem-dimethyl group. Ir spectroscopy shows the appearance of a carbonyl peak at 1698 cm^{-1} consistent with the value expected for a large ring ketone.[65]

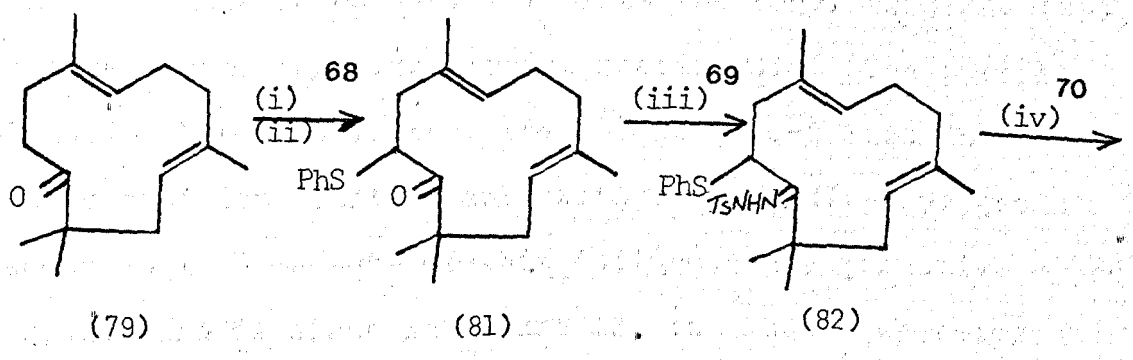


(79)

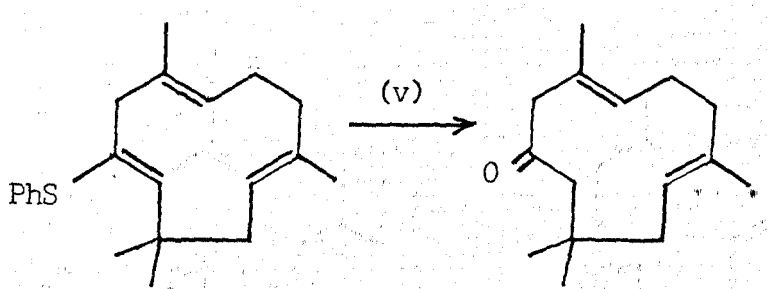


(80)

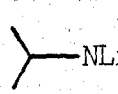
Several literature methods are known for the 1,2-transposition of ketones and two such methods[66,67] were tried in an effort to transpose the ketone function from C-5 to C-4 to give the humulen-4-one (80). The first method[66] is outlined in Figure 11.



(79) (81) (82)



(80)

Reagents: (i)  NLi/THF/-78°C; (ii) PhSSPh;

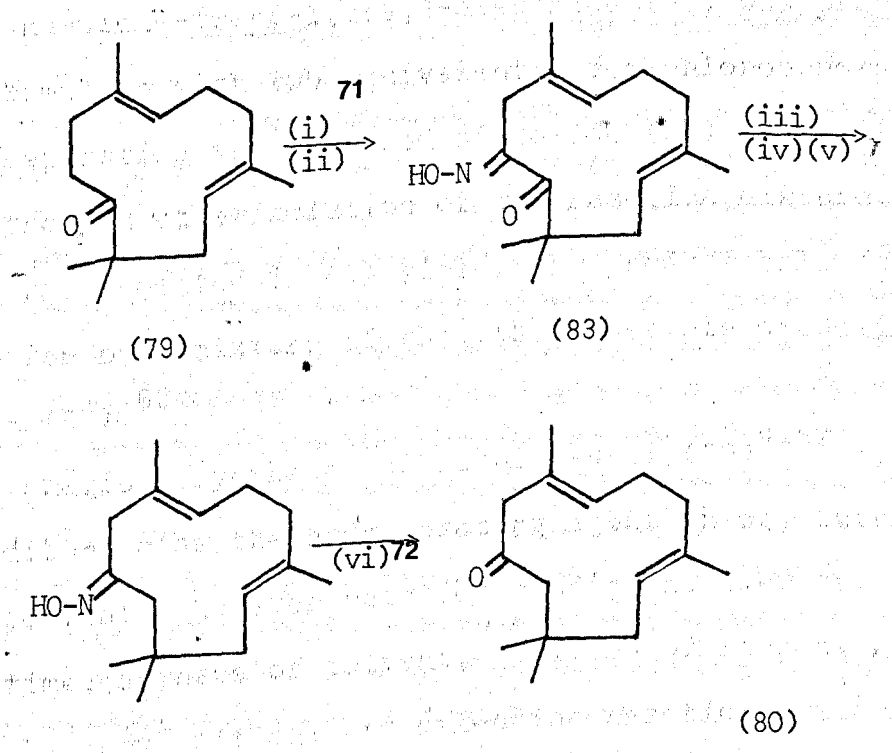
(iii) $TsNNH_2/EtOH$; (iv) $MeLi/Et_2O$;

(v) $HgCl_2/CH_3CN/H_2O$.

Figure 11

The first step was achieved satisfactorily, the phenylthioether (81) being produced in good yield as a crystalline solid. Nmr spectroscopy showed the appearance of a one-proton doublet of doublets at 3.80 ppm and five aromatic protons at 7.16 ppm as a broad singlet. The product, purified by column chromatography, was then carried on to the next stage where an unsuccessful

attempt was made to convert (81) into the tosylhydrazone (82), even after refluxing with glacial acetic acid. The possible explanation for this is that the ketone on C-5 lies in a sterically hindered position, particularly with the gem-dimethyl group adjacent to it. Because of this failure, an alternative approach was sought and is shown in Figure 12, the last step being a modification to the published route.[67]



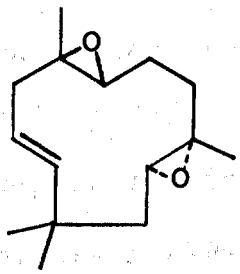
Reagents: (i) t-BuOK/t-BuOH; (ii) i-AmONO;
(iii) $NH_2NH_2.H_2O$/KOH/OH OH;
(iv) HCl; (v) MeOH;
(vi) pyr.chlorochromate/CH_2Cl_2.

Figure 12

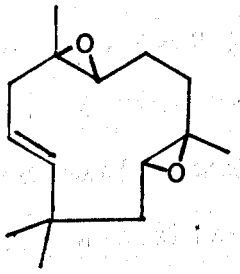
Although the α-keto derivative (81) was obtained successfully in the previous sequence, the formation of the α-keto-oxime (83) could not be achieved. After stirring for two days only starting ketone (79) was observed by analytical tlc and no reaction could be encouraged even by changing the base to lithium-N-isopropyl-amide. In view of these results, it was decided to abandon this approach and concentrate on the original method[4] with a view to refining the process as some difficulties had been encountered during the hydroboration step.

Humulene-trans-1,2:8,9-diepoxide (57) was prepared by treating humulene with two equivalents of m-chloroperoxybenzoic acid and separating the required diepoxide (57) by crystallisation from a petroleum ether solution of the reaction mixture.[62] The diepoxide was isolated in 60% yield, the product balance being accounted for by a mixture of the other possible diepoxides (64) and (84) - (87).[62]

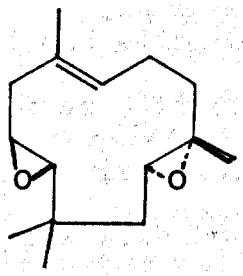
Previous syntheses of humulen-4-ol (50)[4,62] had utilised diborane as the hydroborating agent in dry tetrahydro-furan as solvent. However reaction conditions i.e. temperature, reaction time, dryness of solvent, addition of reagent, etc., had proved to be very critical and often the reaction could not be repeated successfully, a multitude of compounds being produced. 9-Borabicyclo[3.3.1]nonane[73] (88) had also been tried unsuccessfully, presumably because of the steric hindrance in the $\Delta^{4,5}$ double bond region.[62] The major difficulty, when working with these borane reagents, seems to be their air-sensitivity, creating handling problems. Initial attempts to improve the reaction conditions using the borane-tetrahydrofuran complex were unsuccessful but it



(57)



(84)



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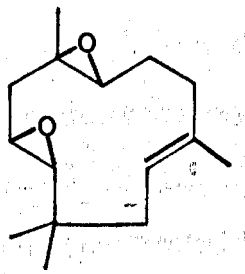
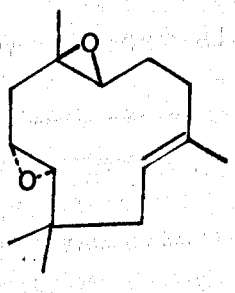


(64)



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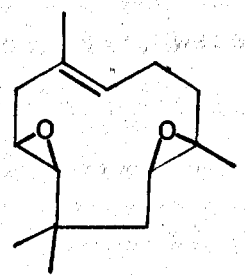
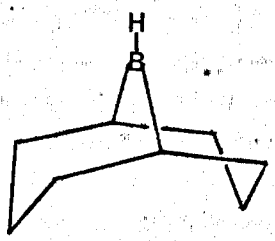
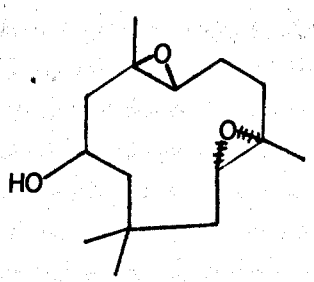


(87)



(88)



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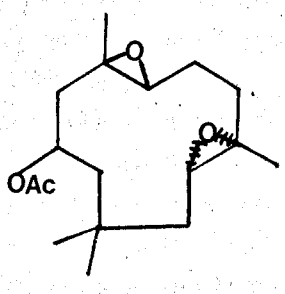
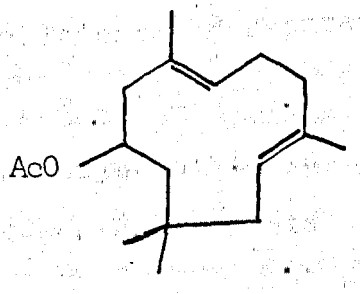
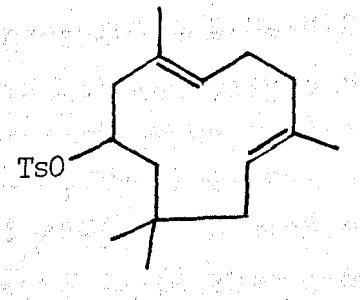


(90)

was subsequently found that the borane-dimethyl sulphide complex gave a clean reaction with good yield and eliminated much of the handling difficulties. Borane behaves as a strong electron acceptor forming coordination complexes with Lewis bases and so dimethyl sulphide forms a stable complex with borane.[74] The liquid complex has a molar concentration ten times that of borane-tetrahydrofuran, it can be stored at room temperature without loss of activity, is soluble in and unreactive towards many aprotic solvents and can be used under mild conditions. As a result, good yields of the diepoxyacetate (90), obtained by acetylation of the diepoxy alcohol (89), were isolated. Subsequent deoxygenation using tungsten hexachloride and n-butyl lithium followed by reduction of the acetate (91) with lithium aluminium hydride gave humulen-4-ol (50) which was then used in an attempt to prepare the tosylate (92) using the standard method.



(91)

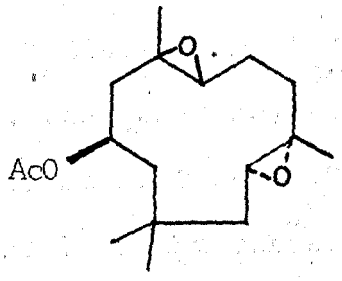


(92)

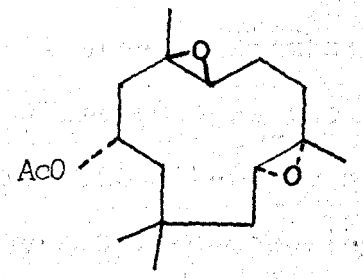
When the reaction was carefully worked up, analytical tlc showed mostly non-polar material and the nmr spectrum showed quite clearly that humulene had been formed. The tosylate must have been formed but only very briefly existing as a transient inter-

mediate before rapidly eliminating to give humulene. An alternative reason could be that the tosylate was unstable under the work-up conditions. The reaction was repeated, great care being taken during work-up but again the tosylate eliminated to give humulene.

A side issue which arose from the preparation of humulen-4-ol (50) was that during isolation of the desired diepoxy acetate (90) by precipitation from 3:1 petroleum ether/ethyl acetate, a white solid was obtained as a second crop of crystals. ^{1}H Nmr spectroscopy indicates the presence of an acetate group, a three-proton sharp singlet being observed at 2.00 ppm. Also observed is the same type of multiplet at 5.00 ppm as that associated with the C-4 proton in the known diepoxy acetate (90) and the 4-acetate (91). On comparison of the oxirane proton region 2.4 to 3.0 ppm of the known diepoxyacetate (90) and the unknown product, definite differences can be seen. The methyl region is distinctly different, the known compound (90) having two distinct and separate three-proton singlets at 0.97 and 1.07 ppm attributable to the gem-dimethyl group. The methyl groups adjacent to the epoxides on C-2 and C-9 are seen as two three-proton singlets at 1.25 and 1.29 ppm respectively. However the unknown compound shows the gem-dimethyl group as a closely associated pair of three-proton singlets at 1.02 and 1.05 ppm whilst the oxirane methyls are more widely spaced at 1.35 and 1.46 ppm. The inference from the spectral data is that the compounds are very similar and probably the difference between them is that they are epimeric at C-4. Thus, diepoxy acetates (93) and (94) and their mirror images can be envisaged,



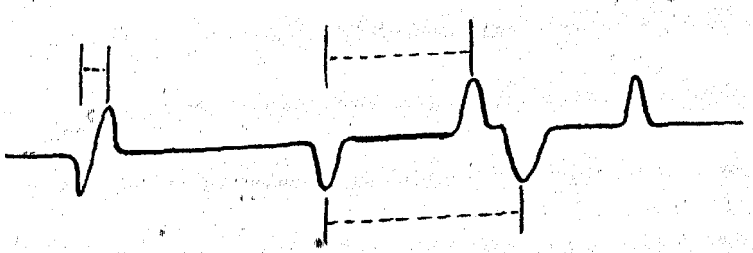
(93)



(94)

formed by hydroboration of the opposite faces of the $\Delta^{4,5}$ double bond, exposed by rotation of this double bond. Based on this assumption the mixture of diepoxyacetates was carried forward to the deoxygenation step to maximise the yield of 4-acetate (91) obtained. However analytical tlc run on the crude product mixture showed that the reaction was not as clean as any previous ones and a new spot R_f 0.35 was observed, more polar than the expected acetate (91) R_f 0.43, which still appeared as the major product. Column chromatography of the crude product mixture gave the expected 4-acetate (91) in addition to a white crystalline solid which exhibits rather strange nmr signals. The typical C-4 proton multiplet consistent with that expected for the acetate is quite obvious at 5.02 ppm and the three-proton methyl signal at 1.99 ppm is attributable to the acetate methyl. However, there do not appear to be any olefinic protons present unless they are masked by the acetate proton signal. Even stranger is the presence of a broad doublet at 3.65 ppm which is typical of a methine proton adjacent to a deshielding functional group. However, D_2O shaking did not cause any noticeable change in the nmr spectrum

and the ir spectrum shows no hydroxyl group to be present. There are four distinct methyl groups other than that of the acetate methyl, the gem-dimethyl being observed as two three-proton singlets at 0.99 and 1.05 ppm. The other two methyls appear at 1.21 and 1.50 ppm respectively. Spin decoupling experiments coupled with INDOR unravelled a small part of the molecule. Irradiation of the left hand side of the left hand line of the doublet at 3.65 ppm gave six lines in the region 1.15-1.85 ppm as shown in Figure 13. This can be assigned as an AMX system



Two couplings

Figure 13

with couplings J_{AX} = 1.5 Hz, J_{MX} = 10.5-11 Hz and J_{AM} = 13.5-14 Hz which can be illustrated as in Figure 14. This is a first-order

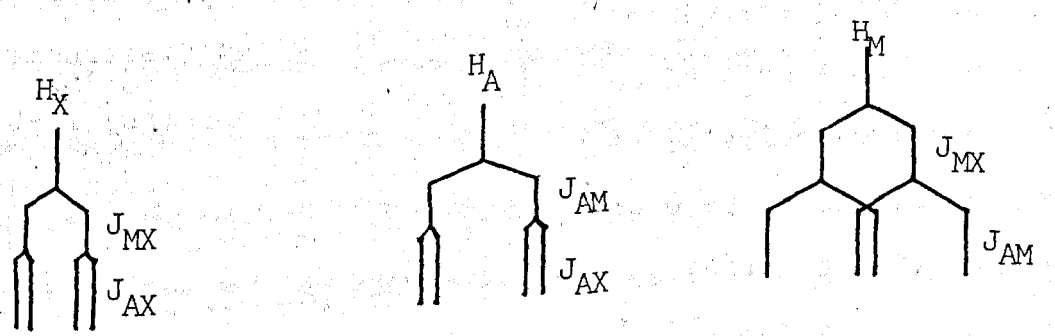
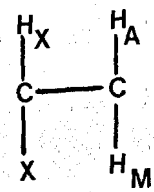


Figure 14

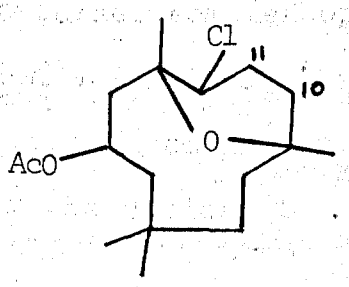
spectrum and is consistent with the partial structure (95) where X is a deshielding group and the coupling constants indicate dihedral angles of approximately 90° and 160° for the protons AX and MX respectively.[11] Closer examination of the ir spectrum shows the presence of distinctive C-O stretch



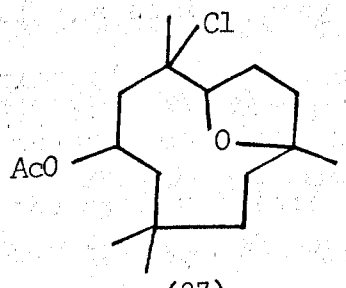
(95)

absorptions in the range 1000-1100 cm^{-1}, denoting the presence of an ether. A low resolution mass spectrum shows a base peak at m/e 43 which is assigned as the acetyl grouping but also noted are the two molecular ions at m/e 316 and m/e 318. When it was realised that they were in the ratio of 3:1, the presence of a chloro group was proposed. On the basis of all these observations, four structures could be proposed to fit the accurate mass of 316.1806, structures (96) - (99). Structures (96) and (97) can be immediately ruled out because the methylene protons on C-10 and C-11 (using humulene numbering) would give a much more complex spectrum than that observed in relation to the couplings of the proton α to the chloro group. As yet, no final assignment has been made as to which of structures (98) and (99) is correct. X-ray analysis would certainly solve the problem and certainly

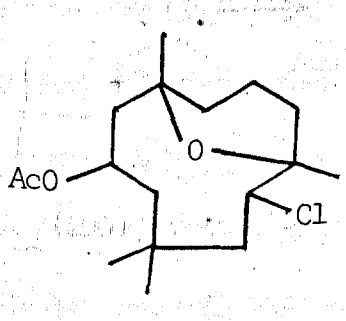
^{13}C nmr spectroscopy would probably differentiate between the carbon bearing the chloro group and the carbon bearing the oxygen group. With regard to mechanistic considerations for the formation of this new product, it is difficult to be precise because the unknown compound arose from a mixture of diepoxy acetate epimers (93) and (94) and their mirror images, carried through the deoxygenation step together. On hydrolysis of the tungsten hexa chloride used in the deoxygenation, it is possible that the hydrogen chloride formed may protonate a partially deoxygenated species such as the epoxy acetate (100) as shown in Figure 15. Initially the epoxide ring in (100) is opened, assisted by



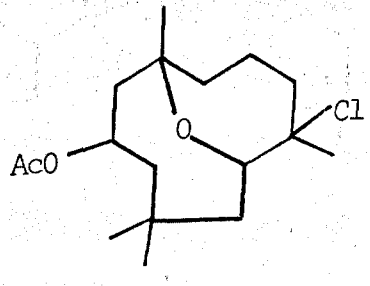
(96)



(97)



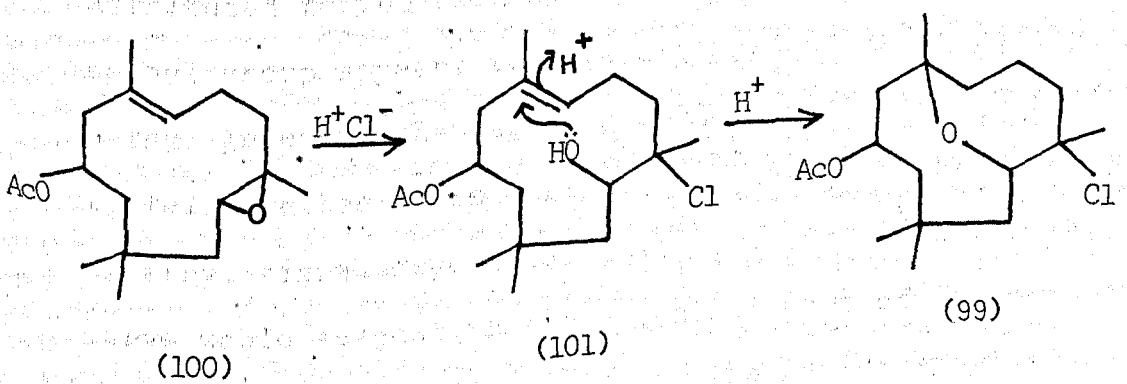
(98)



(99)

protonation of the oxygen and nucleophilic attack of the chloride ion to give the intermediate chlorohydrin (101). Subsequent protonation of the $\Delta^{1,2}$ double bond followed by cyclisation to give the cyclic ether (99) could quite simply explain the formation of the unknown compound. A similar mechanism could be postulated by anti-Markovnikov opening of the epoxide ring in (100) followed by protonation and cyclisation to give the alternative cyclic ether (98). The epimeric diepoxy acetate was in fact observed by Sattar[62] but no structural assignment was made.

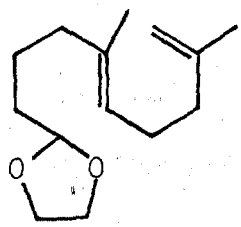
Johnson, in his attempts to induce polyene cyclisation, made use of the cyclic acetal functional group as an initiator.[2] Reaction of the acetal (102) with stannic chloride in nitromethane promoted opening of the acetal ring to give a carbonium ion which induced cyclisation of the diene system to give only trans-fused products, the major components being (103) and (104). It was decided to prepare the cyclic ketals (105) and (106) to



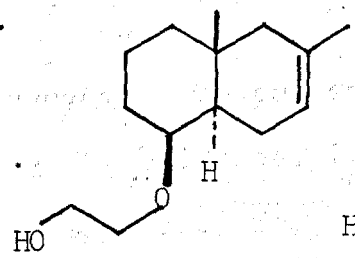
(100) (101) (99)

Figure 15

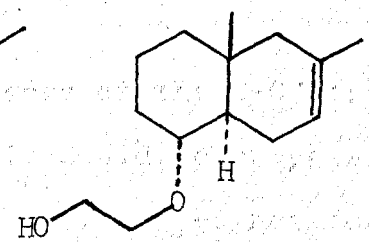
see if cyclisation of either or both of these compounds could be brought about. The 5-ketone (79) had already been prepared so it would appear to be a fairly straightforward process to form



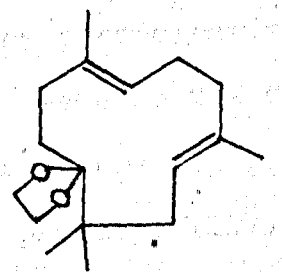
(102)



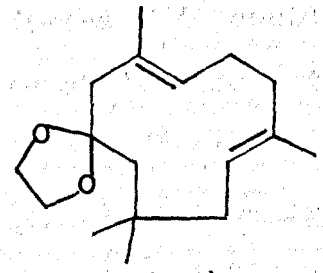
(103)



(104)



(105)

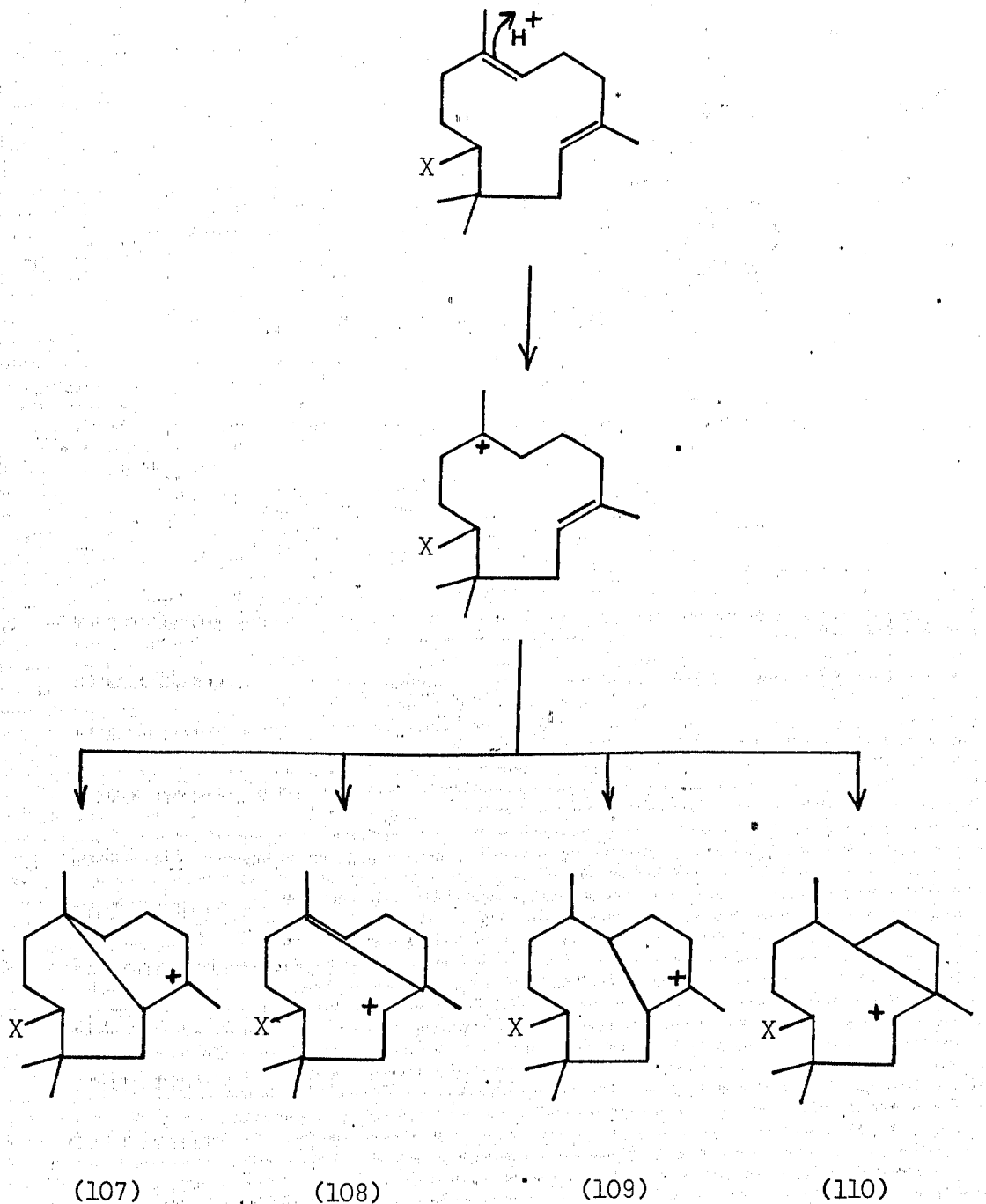


(106)

the corresponding ketal (105) unless steric hindrance causes any problems. Using a standard method for ketal preparation,[75] the 5-one (79) was refluxed in benzene and ethylene glycol with a catalytic amount of p-toluenesulphonic acid using a Dean and Stark apparatus. After an extended reflux period, the reaction was worked up and the nmr spectrum of the yellow oil produced showed that the major product did not appear to have any olefinic protons. A broad singlet appears at 3.82 ppm and can be attributed to the two methylene protons of the ketal. A vinylic methyl group appears to have disappeared and possibly there is a methyl group in the region 0.80-0.94 ppm although it is difficult to tell whether it is tertiary or quaternary because of the gem-dimethyl signal superimposed at 0.94 ppm. However the integration curve would suggest that the central part of the methyl signal is offset to the high field side of the gem-dimethyl signal and taken in consideration with a small peak on the high-field side of the gem-dimethyl signal, the spectrum could indicate a tertiary methyl doublet. The ir spectrum shows that neither hydroxyl nor carbonyl groups are present but there are several bands in the region

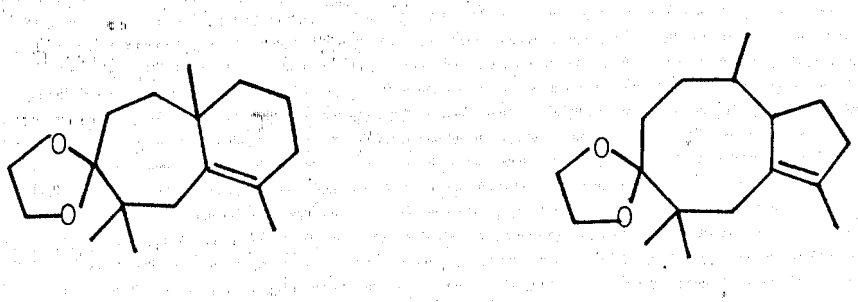
1000-1200 cm^{-1} implying, the presence of the C-O stretch. After deliberating the evidence, it could be proposed that one of two things has happened. The ketal on C-5 has formed then the acid catalyst has protonated the $\Delta^{1,2}$ double bond promoting cyclisation by participation of the $\Delta^{8,9}$ double bond or vice versa. Whatever the order of reaction, the outcome can be summarised in Figure 16 where X represents the ketal function. The cationic products (107) - (110), can be reduced to a possible choice between (107) and (109) because (108) and (110), on quenching, would result in olefinic proton signals in the nmr spectrum which of course are not observed. On quenching therefore the only possible compounds would be (111) and (112) since no product can be considered unless it has a tetrasubstituted double bond. The crude material was then treated with aqueous acid to remove the ketal and inspection of the nmr spectrum shows that no ketal is now present and that the gem-dimethyl signal has changed from a six-proton singlet at 0.94 ppm to two three-proton singlets at 1.08 and 1.14 ppm. This downfield shift is in accord with the protecting ketal being removed from the carbonyl thus allowing the ketone to exert a deshielding effect upon the gem-dimethyl group. The ir spectrum shows a strong carbonyl absorption at 1702 cm^{-1}, typical of a 7-membered or larger ring ketone.[65] Therefore the proposed structures for the ketone are (113) or (114). This problem was not investigated further.

Humulen-4-ol (50) was oxidised quantitatively with pyridinium chlorochromate[63] to produce the corresponding 4-one (80). The nmr spectrum shows the absence of the C-4 carbinyl proton signal and the apparent downfield shift of two protons to approximately 2.70 ppm. These can be assigned as the C-3

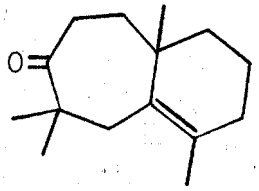


(107) (108) (109) (110)

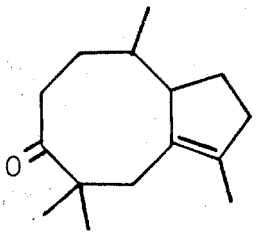
Figure 16



(111) (112)

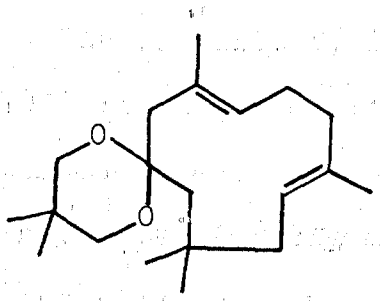


(113)



(114)

methylene protons which in humulene are doubly allylic and are observed at 2.45 ppm but which are now allylic and α to the carbonyl in humulen-4-one (80) and so would be expected to move downfield. The ir spectrum shows the presence of a carbonyl peak at 1702 cm^{-1}, consistent with that of a large ring ketone[65] and the mass spectrum/accurate mass confirms the structure (80). Preparation of the corresponding ketal (106) was tried using the same method as that for the 5-ketal (105) but it was found that the reaction was very slow and the product was extremely difficult to separate from the starting material using chromatographic methods. This method was abandoned and the preparation of the corresponding six-membered ketal (115), obtainable from 2,2-dimethyl-propan-1,3-diol, was attempted in the hope that separation of the desired product (115) might be facilitated. From preliminary investigations, it would appear that a ketal was formed but that cyclisation also occurred similar to that observed with the 5-ketal (105), no olefinic protons being observed in the nmr spectrum of the isolated product and so this work was abandoned.



(115)

Anderson et al[76] and Cooper and Harding[77] discussed the cationic cyclisation of α,β-unsaturated aldehydes and ketones using stannic chloride and perchloric acid respectively. Although humulen-4-one (80) is not an α,β-unsaturated ketone, it was decided to try the reaction of the ketone (80) with stannic chloride as a 'long shot'. Stirring for one hour at room temperature, the reaction mixture, on work-up, showed no starting ketone and a series of more polar spots on analytical tlc. However the nmr spectrum shows no carbinyl proton signal and the ir spectrum shows the presence of a carbonyl peak at 1700 cm^{-1}. These results are very puzzling and as yet no solution to this problem has been obtained.

Apparent from the work described in this chapter is the fact that (i) humulene is so stable that any attempts to solvolyse the 4- or 5-ol derivatives just cause elimination and/or ring contraction and (ii) the sensitivity of the reactive olefinic 1,2-bond is causing problems when even a trace of acid catalyst is used in attempted preparations of the 4- or 5-ketals. A method for ketal formation in a non-acidic medium is known using 2-chloro-

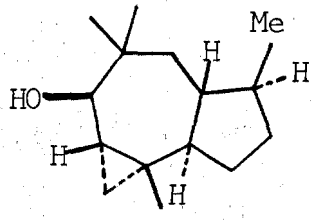
ethanol with lithium carbonate[78] and this could be a possible method to try. The possibility in the case of humulen-4-ol (50) and 4-ketal (106) is that even if cyclisation were induced, the most probable product would be one where the $\Delta^{1,2}$ double bond participates initially, forming a cyclopropyl ring as in the alcohols (29) and (35).

CHAPTER 4

Because of the background of biological activity associated with many of the fungal metabolites discussed in the introduction and because of the involvement of ICI, Pharmaceuticals Division, with this project, a sample of the alcohol (29) was sent to the ICI Research Laboratories for routine screening using the Yeast Lipogenesis Screen devised within the company. For reasons of company secrecy, the screen will not be described herein but basically has been designed such that the effect of compounds on certain aspects of cell growth could be initially monitored, the test being of a dual nature, testing for inhibition and toxicity. It was found that alcohol (29) inhibited lipogenesis having an inhibition value of $\frac{5}{0}$. This means that inhibition is still apparent after a series of five dilutions and the zero implies that no toxic effects upon the culture were observed at all. In effect, the alcohol (29) would appear to inhibit the production of free fatty acids within the culture but only temporarily because the culture is still capable of growing. This was of interest to ICI because a competitor in the field was preparing to market a similar compound (-)-hydroxycitrate, which also gave inhibition of lipogenesis but at much higher dosage levels. Therefore, to explore this further, more of the alcohol (29) was synthesised within the ICI Research Laboratories[10] and submitted for testing on rats with hydroxy citrate being run in parallel.

In the interim period, while preparations were being made to carry out the tests, submission of the trisubstituted olefinic alcohol (35) for YL screen showed an activity of $\frac{3}{0}$. A study[10] was launched to discover the structure/reactivity relationship involved in the two alcohols. From the preliminary screen results, it would appear that the olefin played some part

in the reactivity but hydrogenation of the alcohol (35) gave the dihydroalcohol (116) which showed an activity of $\frac{5}{0}$.



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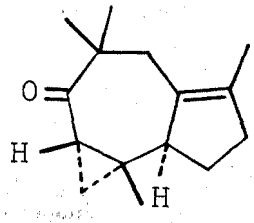

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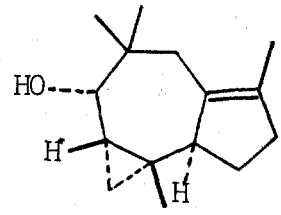
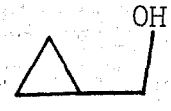


(118)

Oxidation of alcohol (29) gave ketone (117) which was inactive and stereoselective borohydride reduction of ketone (117) gave the epimeric alcohol (118) which displayed an activity of $\frac{4}{0}$. It would seem therefore that the double bond was unnecessary for YL activity and that the activity lay in the cyclopropyl carbinol region of the structure. A search of the ICI collection for compounds having the partial structure (119) provided a variety of compounds which were subjected to the YL screen. The results produced a broad spectrum of activity from active through



(119)

inactive to toxic and there appeared to be no rationale attached. A suggested reason to explain the activity was nucleophilic attack of an enzyme involved in lipogenesis upon the cyclopropyl ring, displacing the hydroxyl as shown in Figure 17.

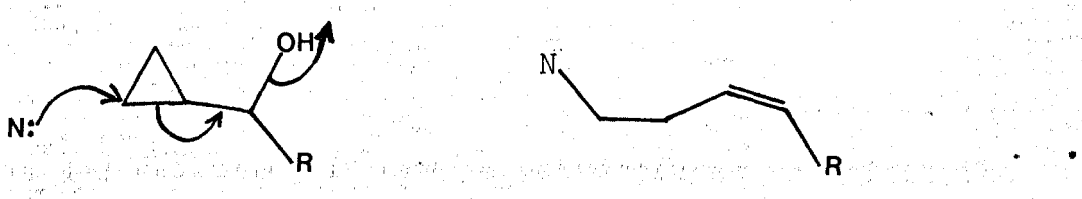
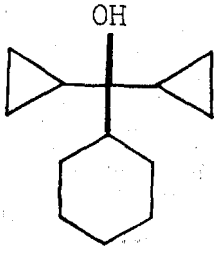


Figure 17

This would explain the observed enhanced activity of tertiary and secondary over primary alcohols. However, the activity is not fully understood.

On the basis of the activities observed, it was decided that a contribution could be made to understanding the structure/reactivity relationships involved by synthesising two compounds which would perhaps exhibit activity. During the screening of compounds from the ICI collection, the alcohol (120) was found to be as active as the alcohol (29). Because alcohol (120) is not a substituted neo-pentyl alcohol as alcohol (29) is, it was decided to synthesise alcohol (121) to see if the presence of the neo-pentyl system enhanced the activity in the YL screen. The reaction was carried out by forming the Grignard reagent from t-butyl bromide, adding dicyclopropyl



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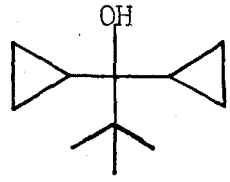
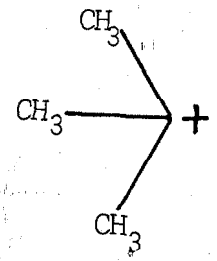


(121)

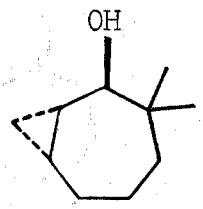
ketone and refluxing in dry tetrahydrofuran to effect the formation of the alcohol (121) after hydrolysis of the reaction mixture. The reflux was carried out in tetrahydrofuran because it was felt that as the ketone was extremely hindered, the reaction might not proceed readily and normal ether reflux might not provide a sufficiently high temperature to promote reaction. A sample of the desired alcohol (121) was obtained pure by column chromatography, preparative tlc, and distillation. The nmr spectrum shows a nine-proton singlet at 1.01 ppm and a ten-proton multiplet between 0.3-1.3 ppm, typical of the multiplets observed for cyclopropyl systems in the other compounds previously discussed. The ir spectrum shows peaks at 3600 and 3080 cm^{-1} attributable to the hydroxyl and cyclopropyl groups respectively. Low resolution m.s. did not give the expected molecular ion of m/e 168, an extremely complex fragmentation pattern occurring with very high m/e ratio fragments apparent. However, it was noted the maximum deflection peak was m/e 111 which could be due to $M^+ - 57$ or the loss of the fragment (122). Elemental



(122)

analysis confirmed the structure of the alcohol as being (121) and a sample was sent for YL screening. (Result = $\frac{0}{0}$).

The second proposed synthesis was to produce a model of the alcohol (29) such that the basic unit of suspected reactivity was present without the double bond and inhibition on the YL screen would verify that the reactivity is due to the cyclopropyl neopentyl alcohol part structure. The target alcohol (123) could be synthesised in various ways. One possible approach is shown in Figure 18. However, from work carried out[10] on the Simmons-Smith cyclopropanation[79] of cyclohex-2-en-1-ol (124), it was observed that great difficulty was experienced in achieving consistent results. Also it was felt that the gem-dialkylation would probably be difficult because of the possibility of opening the cyclopropyl ring under basic conditions. Therefore an alternative route, as shown in Figure 19, was proposed. On paper, the route looks fairly straightforward for the most part with difficulties perhaps being envisaged in the Birch cleavage of the phenylthio group with the associated alkylation of the resultant anion and also in the cyclopropanation step. The conversion of cycloheptanone (125) to the phenylthioether (126) was quite straightforward but difficulty was experienced in achieving a clean separation of the starting ketone (125) and



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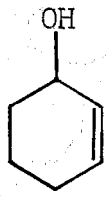

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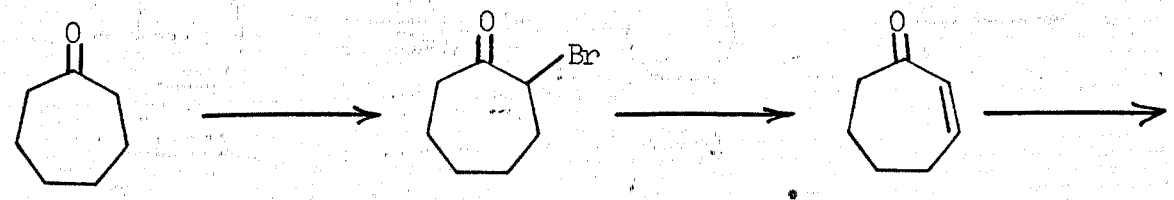
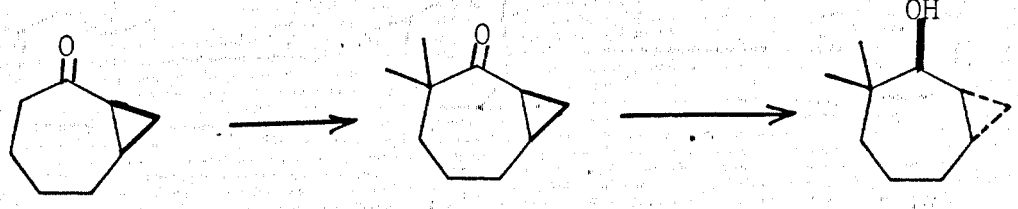
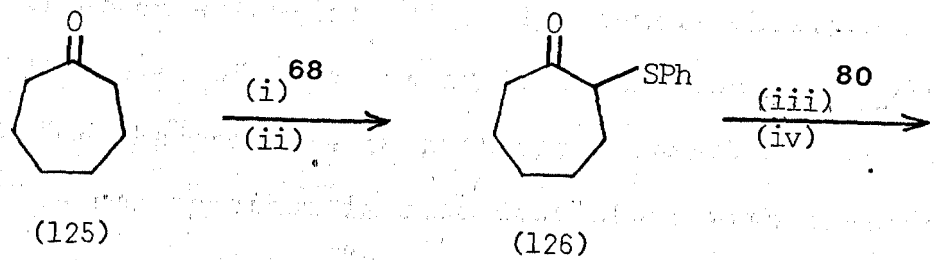


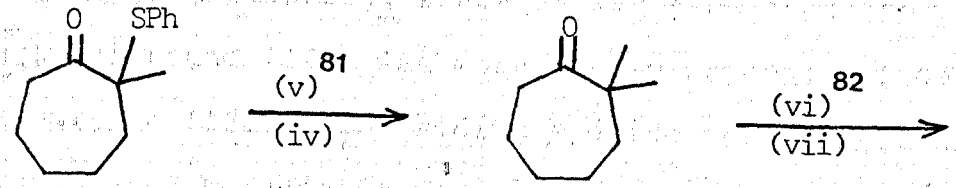


(123)

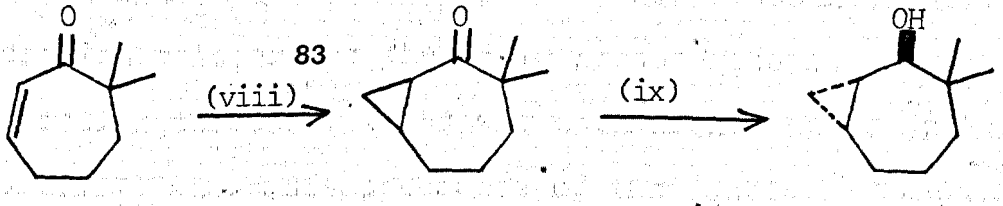
Figure 18



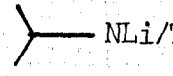
(125) (126)



(127) (128)



(129) (130) (123)

Reagents: (i)  NLi/THF/-78°C; (ii) PhSSPh;

(iii) NaH/THF; (iv) MeI;

(v) $Li/NH_3/Et_2O$; (vi) Br_2/CCl_4;

(vii) MgO/DMF/140°C;

(viii) $NaH/Me_3{}^{+}\overset{O}{\overset{\|}{S}}I^{-}$/DMSO; (ix) stereoselective redn. ($NaBH_4$)

Figure 19

the product (126) because of the fact that they ran very close to each other on analytical tlc. The sample obtained shows a low field doublet of doublets at 3.75 ppm consistent with that expected for the proton α to both the carbonyl and the phenylthio group. The nmr spectrum is also consistent with that of an authentic sample of (126)[84]. It was decided to carry this mixture of starting ketone (125) and product (126) through to the next stage, after initial column chromatographic separation of the diphenyl disulphide, since it was seen that the monomethylated compound (127) was more easily separated from the starting ketone (125). The sodium hydride used in the next stage was chosen because it was not too strong a base and therefore only the most acidic proton i.e. the proton α to both the phenylthio and carbonyl groups, would be abstracted. A stronger base might abstract a proton from the other α position and so give competitive alkylation. This methylation reaction to give the monomethyl thioether (127) went fairly smoothly but it was difficult to assess the yield because the quantity of actual starting thioether (126) was not known. Study of the nmr spectrum of (127) shows disappearance of the doublet of doublets at 3.75 ppm and appearance of a methyl group at 1.21 ppm. Low resolution m.s. and accurate mass results are consistent with the structure (127) and this was confirmed by elemental analysis. The following step involved cleavage of the phenylthio group from (127) and quenching of the resultant anion with methyl iodide to yield the gem-dimethyl ketone (128). The isolated yellow oil, purified by chromatographic methods, had a strong camphor-like smell and the yield was poor

(∿ 50%) even although great care was taken with experimental procedures. The nmr spectrum is consistent with literature values for the compound (128)[85] and shows a sharp six-proton singlet at 1.08 ppm, a broad six-proton singlet at 1.58 ppm and a two-proton multiplet at 2.48 ppm. The ir spectrum shows a carbonyl peak at 1702 cm^{-1} and a gem-dimethyl doublet at 1372 and 1382 cm^{-1}. These results, in conjunction with accurate mass measurement, are in accord with the structure (128). The need now was to introduce unsaturation, forming an α,β-unsaturated ketone and thus enabling a cyclopropane ring to be attached. Various methods are known such as introduction of the phenylthio group α to the carbonyl, oxidising it to the sulphoxide[86,87] then eliminating to give the enone.[86] However, when preliminary investigations were carried out on the phenylthio ketone (126), no successful oxidation of the thio group was achieved. An alternative method was found which involved bromination of the gem-dimethyl ketone (128) and subsequent elimination of hydrogen bromide using magnesium oxide and dimethyl formamide at high temperature.[82] No separation of the intermediate bromide compound was carried out after an initial small scale attempt resulted in decomposition of the bromide and dark brown oil being produced. However the nmr spectrum of the crude bromide indicated the appearance of a doublet of doublets at approximately 4 ppm consistent with that expected for the proton α to the bromo and carbonyl groups. Elimination of hydrogen bromide was carried out to give the enone (129) in very low yield, possibly due to either decomposition or volatility of the enone. The assignment of the nmr spectral signals showed an AB quartet with further splitting of

the peaks at 6.00 ppm and a gem-dimethyl six-proton singlet at 1.00 ppm. The ir spectrum indicates a shift in the carbonyl stretch to 1658 cm^{-1}, consistent with the introduction of unsaturation α,β- to the ketone. Low resolution m.s.. gives some peculiar fragmentations but this could possibly be due to polymer formation. A significant peak (25%) is observed at the m/e expected for the molecular ion and accurate mass confirms the structure (129). Introduction of the cyclopropyl group by means of the dimethylsulphoxonium ylide[83] was considered to be the best method because the literature examples quoted report high yields. Furthermore, enones specifically produce cyclopropyl groups whereas the straight ketone produces epoxides. The mechanism for cyclopropane ring formation is shown in Figure 20. The enone (129) was

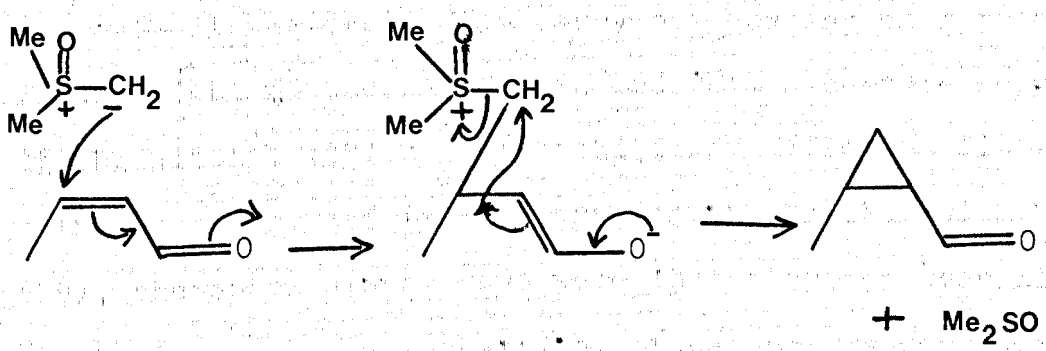


Figure 20

treated with the dimethylsulphoxonium ylide and the crude product obtained from the reaction was examined by nmr spectroscopy. The AB system, apparent in the enone (129), had definitely disappeared and there were discrete signals between 0.3 and 0.9 ppm, signifying the presence of cyclopropyl protons. However, attempts to isolate and purify this material resulted in total loss and it was thought that the product must be extremely volatile. The nmr evidence

would seem to indicate that the cyclopropyl ketone (130) had been formed but no further confirmation was obtained, mainly for two reasons. Firstly, the period spent at ICI came to an end and secondly, more searching biological testing of the alcohol (29) did not match the initial activity observed in the YL screen.

The results obtained in the YL screen led to feeding of the alcohol (29) to rats followed by examination of the liver and adipose tissue after alanine ^{14}C introduction. The radioactivity uptake into the fatty acids would suggest that the alcohol (29) is enhancing synthesis of free fatty acids. However, it was concluded that the alcohol (29) was inhibiting regeneration of the free fatty acid from the pool and thus promoting synthesis of new free fatty acid from the radioactive source. It was decided to carry out testing on rats checking the gross weight of the animals in order to ascertain unambiguously if the alcohol (29) had any inhibitory effect on lipogenesis. Using a control and (-)-hydroxycitrate together with the alcohol (29), the conclusions drawn were that although overall a very slight inhibition was observed, it was not as convincing as (-)-hydroxycitrate and certainly did not merit further investigation.

The second aspect of the work carried out during the ICI visit period involved the use of fungal cultures in attempts to induce microbial oxidation of humulene. Because of the then potential interest in the alcohol (29), an alternative, cheaper and more efficient route to humulene-4;5-epoxide (8) would be of great benefit in the event of a large scale synthesis of the alcohol (29) being required. A search was made of the ICI

Culture Collection based on literature reports of cultures known to epoxidise and hydroxylate[88] olefinic bonds. Microbially many of these cultures had previously been used in hydroxylation of C-9 and C-14 in certain steroids,[89] others specifically epoxidised olefinic bonds.[90,91] A list of forty one cultures were grown in preparation for the proposed microbial oxidation experiments and these are listed in Table 5.

The initial problem to be solved was that of finding a solvent suitable to dissolve humulene, to maintain a reasonable amount of homogeneity on addition of the humulene solution to the aqueous solution of the culture and medium and to exhibit a non-toxic effect upon the cultures. After trying several solvents, acetone was found to be the most suitable. Having found a suitable solvent, small scale experiments were carried out in 100 ml shake flasks using two cultures NTP1 and 2, details of which are included in the experimental section. In order to ascertain whether or not humulene could be detected at a low concentration after extraction of the grown culture with ethyl acetate and whether or not humulene was broken down during autoclaving, a series of flasks were charged with an aliquot of medium. Control experiments were run involving medium + acetone, medium + acetone + humulene using different concentrations of substrate and humulene on its own to see if it breaks down during autoclaving. It was noted that humulene was not degraded upon autoclaving and that it was detectable by analytical tlc right down to 50 µg per ml. of medium.

Attention was then turned to the behaviour of humulene in the presence of the cultures chosen. Initially a shake flask

was charged with medium, innoculated with culture, grown for several days then dosed with either acetone or acetone and substrate. The system was that for each culture there was a control containing culture + medium + acetone and a flask containing culture + medium + acetone + humulene. This was done for both media and there was a set of flasks for sampling each of four days. Also included was a set of flasks containing humulene + medium to ensure that there was no aerial oxidation of the substrate. Each day a set of flasks was removed, shaken with ethyl acetate, a sample removed from the solvent layer, evaporated to dryness under vacuum, the residue dissolved in a small amount of ethyl acetate and then spotted on analytical tlc plates. This procedure was repeated for every culture listed in Table 5. It was noted that a wide variety of growth types were observed ranging from minimal growth to prolific fluffy growth and also the growth of varying sized balls. The tlc results obtained from the ethyl acetate extracts varied, most of the culture extracts giving a large spot of unreacted humulene and spots of intermediate and low R_f values. It was immediately noted that practically all of the cultures studied gave a vast array of spots in the polar region where any trace of hydroxylated humulene would be expected to be observed. Therefore because of the time required to develop a visual detection method for any humulene alcohols and the short time available to carry out this project, it was decided to concentrate solely on the detection of any epoxides formed with particular emphasis on humulene-4,5-epoxide (8). Some cultures did give spots with an R_f value roughly comparable to humulene-1,2-epoxide (10) and some cultures also

TABLE 5

Organisms selected for microbial oxidation experiments

Organism	ACC No.	Organism	ACC No.
Cunninghamella blakesleeana	1346	*Aspergillus niger*	6742
Cunninghamella elegans	2548	Candida aquatica	7520
Aspergillus niger	5065	Aspergillus flavus var. columnaris	8527
Mucor griseocyanus	1870	Aspergillus flavus var. rufus	8528
Parasitella simplex	1868	Penicillium notatum mut. fulvescens	10915
Cochliobolus lunatus	1752	Penicillium notatum	26
Beauveria Bassiana	3675	Penicillium chrysogenum	423
Aspergillus niger	32	Penicillium chrysogenum	1426
Penicillium notatum	172	Beauveria bassiana	1655
Rhizopas stolonifer	1581	Cochliobolus lunatus	3035
Penicillium chrysogenum	1708	Aspergillus niger	3438
Mucor griseocyanus (-)	1869	Candida sp.	5604
Candida zeylanoides	2661	Penicillium notatum	5733
Candida guillermondii	2662	Aspergillus flavus	6442
Candida krasei	2663	Penicillium chrysogenum	8082
Aspergillus niger	4373	Penicillium chrysogenum	8084
Aspergillus niger	5016	Penicillium chrysogenum	8085
Aspergillus niger	6050	Beauveria bassiana	10242
Aspergillus flavus	6224	Beauveria bassiana	10280
Aspergillus flavus	6441		
Aspergillus niger	6448		

showed total disappearance of humulene. Much consideration was given to the possibility that (i) the extraction process using ethyl acetate was not efficient enough, humulene and/or humulene-4,5-epoxide (8) being trapped within the tissue walls of the cultures, and (ii) even although humulene-4,5-epoxide (8) was produced by a particular culture, it might be too dilute to allow detection by analytical tlc. In an attempt to overcome the problem of efficient extraction, a mechanical device for breaking up the culture particles was employed but appeared to make very little difference. If anything, it would perhaps cause the release of other fungal products normally retained within the cell tissues. To investigate the second possibility, a series of experiments were run involving dosing of measured amounts of humulene-4,5-epoxide (8) in acetone solution to Candida zeylanoides 2661 such that a 20%, 10% and 5% conversion of humulene to its 4,5-epoxide (8) was simulated. This particular culture was chosen because it gave a fairly clean extract, no spots showing on analytical tlc in the area where the 4,5-epoxide (8) was expected. The experiments were carried out on a time-dependent study and it was seen that all levels of 4,5-epoxide (8) concentration were detectable by tlc. It can be assumed that as no culture out of the forty one chosen gave a spot on tlc with the same R_f value as the 4,5-epoxide (8), no culture engineered epoxidation of the $\Delta^{4,5}$ double bond of humulene.

Several cultures looked as though they might have produced humulene-1,2-epoxide (10) and so the fermentation was stepped up to a 500 ml shake flask size, the procedure remaining the same. The cultures used were Aspergillus niger 5065 and Cunninghamella elegans 2548. Of these two, 5065 showed most

promise and was run on a 5 litre scale using a 10 litre glass fermenter with paddle agitation and efficient aeration. The extract was run on glc and a comparison was made using an authentic sample of humulene-1,2-epoxide (10). From the traces obtained the extract gave a peak of practically identical retention time as that obtained from the 1,2-epoxide (10). However, a range of columns would need to be used before the peak could be absolutely identified as arising from humulene-1,2-epoxide (10). If sufficient extract was available, a sample of the compound could be isolated and an nmr spectrum would resolve the problem.

It would appear that much more time would need to be spent on varying media and cultures until possibly one could be found which would carry out the required microbial transformation. In light of the recent biological test results, the exercise would be fairly pointless.

EXPERIMENTAL

Melting points are uncorrected and were determined on a Kofler hot-stage apparatus; boiling points are not corrected. Kieselgel GF_{254} (Merck) silica was used for all preparative thin layer chromatography. Analytical tlc plates were eluted with a 10% ethyl acetate/petroleum ether solution unless otherwise stated and stained with iodine vapour and/or ceric ammonium sulphate followed by heating to approximately 120°C. Petroleum ether refers to the fraction of boiling range 60-80°C and all organic extracts were dried over magnesium sulphate unless otherwise stated. Where necessary, solvents were purified and dried in the recommended manner and reagents were either distilled or recrystallised.

Infra-red spectra were recorded on a Perkin-Elmer 557 grating infra-red spectrophotometer and, unless otherwise stated, were obtained from liquid films. Nuclear magnetic resonance spectra were recorded on a Hitachi Perkin-Elmer R24 (60 MHz) or a Perkin-Elmer R32 (90 MHz) nmr spectrometer using deuterated chloroform as solvent unless otherwise stated. Tetramethylsilane was employed as an internal standard and all spectral values are quoted in parts per million. Mass spectra were determined on a Jeol JMS D100 mass spectrometer combined with a Jeol JCS 20K gas chromatograph and using an Instem Data Mass Maxi data processing system. Microanalyses were carried out at the laboratories of ICI Pharmaceuticals Division, Alderley Park, Cheshire.

EXPERIMENTAL

Preparation of humulene trisepoxide (11)[8]

Humulene (1) (97% purity) (15g., 73.4 mmols.) was dissolved in dry dichloromethane (400 ml.) in a conical flask to which was then added aqueous sodium bicarbonate solution (0.5M, 600 ml.). The mixture was stirred magnetically at room temperature and to it was added slowly, to prevent excessive frothing, m-chloroperoxybenzoic acid (85% purity) (47.69g ≡ 0.235 mols.). On completion of the peroxy acid addition, the reaction mixture was stirred for 12 hours at room temperature. The two layers were separated and the organic layer was washed well with 1M aqueous sodium hydroxide solution (500 ml.) followed by water (500 ml.), and then dried. The solution was filtered and the solvent removed under vacuum to yield a yellow-green oil which was dissolved in a small amount of pet.ether. A white solid precipitated which was removed by filtration and washed with petroleum ether. The filtrate and washings were recycled through the process of solvent removal, precipitation, and filtration until 16.3g. (88%) of the trisepoxide (11) was obtained, m.pt. 114-118°C. Analytical tlc showed one spot R_f 0.06.

δ = 0.88 (s,3H), 1.10 (s,3H), 1.38 (s,6H), and 2.20-3.10 (m,4H).

Preparation of humulene-4,5-epoxide (8)[4]

Tetrahydrofuran (dried by 24 hour reflux over sodium, then 24 hour reflux over lithium aluminium hydride) (250 ml.) was distilled directly into a pre-dried 3-necked 500 ml. conical flask which was fitted with a magnetic follower and an alcohol thermometer carried in a thermometer pocket/pressure vent fitted

with a silica gel guard tube. Nitrogen, passed through Fieser's solution[92] and concentrated sulphuric acid was passed into the flask through a Subaseal stopper and a gentle flow of gas was maintained. With a glass stopper in the third port, the tetrahydrofuran was cooled to -70°C and stirred, maintaining that temperature with an acetone/Drikold bath. Resublimed tungsten hexachloride (15.78g., 0.04 moles.), previously packed in glass ampoules under nitrogen, was transferred to the flask from a glass reservoir when the solvent temperature had stabilised below -70°C., vigorous stirring being required to keep the solid mobile. Immediately on addition of the tungsten hexachloride, a Subaseal stopper was fitted to the third port and the mixture was stirred for 15 minutes at -70°C. n-Butyl lithium (1.6M in hexane, 74.6 ml., 0.12 mols.) was introduced through the Subaseal stopper by a hypodermic syringe, great care being taken to ensure that the reaction temperature did not rise above -65°C. On completion of the addition, the mixture was stirred for 10 minutes at -70°C then allowed to warm to room temperature where the colour changed from pea green to dark blue/black and all solids dissolved. The solution was cooled to 10°C and humulene trisepoxide (11) (4.37g., 17.3 mmols.) was added quickly. A reflux condenser was fitted to the centre port, the thermometer was transferred to the third port and the mixture stirred at room temperature for 5 minutes after which it was heated rapidly to 45-50°C using a pre-heated oil-bath (approximately at 80°C). After stirring at that temperature range for 45 minutes, the reaction mixture was cooled to 10°C, poured into a separating funnel containing ether (50 ml.) and an aqueous solution (1.5M

in sodium tartrate and 2M in sodium hydroxide, 500 ml.). The mixture was shaken vigorously, the ether layer separated, shaken again with aqueous sodium tartrate/sodium hydroxide solution (500 ml.), washed with brine, dried, filtered and the solvent removed under vacuum leaving a greenish-yellow oil, crude yield 4.3g. Analytical tlc showed predominantly the desired epoxide (8) R_f 0.49 with humulene R_f 0.68, humulene-1,2-epoxide (10) R_f 0.39, humulene diepoxides $R_f \sim 0.24$ and some unreacted trisepoxide (11) as the other significant components. The epoxide (8) was purified by high pressure column chromatography using Kieselgel HF_{254} (Type 60) (ratio 20:1) and eluting with 2% ether/pet.ether. A pale yellow oil was obtained (2.19g. 57%).

δ = 0.71 (s,3H), 1.05 (s,3H), 1.50 (s,3H), 1.60 (s,3H), 2.40-3.00 (m,2H), and 4.85 (m,2H).

Diol (14)[10]

After elution of the 4,5-epoxide (8) listed above, further elution with ether gave crystals on the column tip (0.46g. from 7.08g epoxide) m.pt. 109-111°C (after many recrystallisations from ether/petroleum ether (40-60°). The compound held water tenaciously. (Found for a sample dried overnight at RT over P_2O_5 and high vacuum: C, 75.4; H, 11.3. $C_{15}H_{26}O_2$ requires C, 75.6; H, 11.0%.)

The compound sublimed at 60°/0.1 mm. with some decomposition.

$\bar{\nu}_{max}$ (nujol): 3320, 1660, 1095, 1022, and 925 cm^{-1}.

δ = 0.36-1.09 (m,3H), 0.95 (s,3H), 0.95 (s,3H), 1.09 (s,3H), 1.63 (bs,3H), 2.78 (dd,1H), 2.95 (d,1H), and 5.26 (br.dd,1H).

m/e : 238 (M^+)

R_f 0.22 on a silica plate in 100% ethyl acetate.

Diacetate (17)[10]

Diol (14) (120 mg., 0.52 mmols.), was dissolved in pyridine/acetic anhydride, allowed to stand for 4 hours then was poured on to ice. Coagulation gave a flocculent mass, m.pt. 144°C (ether/petroleum ether (40-60°C) as chunky crystals). R_f 0.70 in 100% ethyl acetate.

Found: C, 70.7; H, 9.5. $C_{19}H_{30}O_4$ requires C, 70.8; H, 9.4%.

$\bar{\nu}_{max}$ (nujol) = 1725, 1240, 1035, 1018, and 950 cm^{-1}.

δ = 0.14-1.22 (m,3H), 0.95 (s,3H), 1.14(s,3H), 1.20 (s,3H), 1.74 (bs,3H), 4.23 (dd,1H), 4.65 (d,1H), and 5.45 (br.d,1H).

Diketone (15)[10]

Diol (14) (130 mg., 0.55 mmol.) was oxidised with pyridinium chlorochromate (255 mg., 1.38 mmol.). Sodium acetate was added as buffer and the reaction was monitored by tlc. Further oxidant was added twice to drive the reaction to completion. After 5 hours at room temperature, work-up gave 134 mg. of a colourless oil which partially crystallised overnight. Elution off a short silica column using 10% ether/petroleum ether (40-60°C) gave 113 mg. of chunky needles m.pt. 75-76°C. R_f 0.35 in 10% ethyl acetate/petroleum ether (40-60°C).

Found: C, 76.9; H, 9.7; $C_{15}H_{22}O_2$ requires C, 76.9; H, 9.5%.

$\bar{\nu}_{max}$ (nujol) = 1680, 1675, 1105, 1090, 1075, 975, and 930 cm^{-1}.

δ = 0.2 (m,2H), 1.17 (s,3H), 1.25 (s,3H), 1.37 (m,1H), 1.40 (s,3H), 1.76 (s,3H), 3.29 (m,2H), and 5.51 (bd,1H).

m/e = 234 (M^+)

Dihydrodiol (16)[10]

Diol (14) (103 mg., 0.43 mmol.) in ethanol was hydrogenated for 5 hours over Pd/C at atmospheric pressure and room temperature. Numerous recrystallisations (ether/petroleum ether (40-60°C)) and careful drying gave needles, m.pt. 81-83°C.

R_f 0.40 in 100% ethyl acetate.

Found: C, 75.2; H, 12.0. $C_{15}H_{28}O_2$ requires C, 75.0; H, 11.7%

$\bar{\nu}_{max}$ (nujol) = 3310, 1030, and 935 cm^{-1}.

δ = 0.3-0.7 (m,3H), 0.90 (d,3H), 0.92 (s,3H), 1.08 (s,3H), 1.15 (s,3H), 2.75 (m,1H), and 3.18 (d,J=7 Hz, 1H).

m/e = 240 (M^+).

Reaction of humulene-4,5-epoxide (8) with picric acid

Humulene-4,5-epoxide (8), (500 mg., 2.27 mmols.) was dissolved in redistilled, dry nitromethane (10 ml.) in a 100 ml. round bottom flask. Picric acid was sucked dry on a Buchner funnel then added (580 mg., 2.27 mmol.dry) to the reaction flask, the contents of which were then magnetically stirred at room temperature for 11 days, sampling at regular intervals for analytical tlc. The reaction mixture was worked up by adding saturated sodium bicarbonate solution and extracting with ether, the ether layer being dried, filtered under vacuum and the ether removed on the rotary evaporator. The crude product was a brown oil, yield 550 mg., from which a yellow solid, probably unreacted picric acid, precipitated. The brown oil was dissolved in ether/chloroform and multiple elution preparative tlc separation carried out to give a polar spot on analytical tlc, R_f 0.18 which developed rapidly on spraying with ceric ammonium sulphate and heating to give a strong purple-coloured spot.

$\bar{\nu}_{max}$ = 3400, 1380, 1360, 1070, 1050, 1025, and 1010 cm^{-1}

δ = 1.00 (s,3H), 1.10 (bs,6H), 1.65 (bs,3H), 3.20 (m,2H), and 5.30 (m,1H).

Reaction of humulene-4,5-epoxide (8) with boron trifluoride etherate in benzene

Humulene-4,5-epoxide (8), (500 mg., 2.27 mmols.) was dissolved in dry benzene (20 ml.), in a 100 ml. round bottom flask, and the flask and contents cooled to almost 0°C (until the benzene just began to freeze). Boron trifluoride etherate (280 µl, 2.27 mmols.), freshly distilled from calcium hydride, was quickly added by microsyringe and the reaction mixture stirred magnetically. After 15 minutes, the reaction mixture, which had turned dark brown, was worked up using saturated sodium bicarbonate and ether, the ether layer being separated, filtered and the ether removed by rotary evaporator. A yellow oil, crude yield 462 mg. was obtained. Preparative tlc using (i) 5% ethyl acetate/pet.ether (ii) 3% ethyl acetate/pet.ether x 3 multiple elution, gave the same alcohol as that obtained from the picric acid reaction.

Spectral characteristics were identical to the compound isolated from the picric acid reaction.

Reaction of humulene-4,5-epoxide (8) with boron trifluoride etherate in ether

Humulene-4,5-epoxide (8), (500 mg., 2.27 mmols.) was dissolved in anhydrous ether (10 ml.) and the solution stirred magnetically and cooled to -70°C using an acetone/Drikold bath. Freshly distilled boron trifluoride etherate (280 µl, 2.27 mmols.) was added quickly by microsyringe, the solution was stirred at -70°C for 1 hour, allowed to warm to room temperature and then stirred for a further 12 hours. The solution was worked up using saturated sodium bicarbonate solution and ether, the ether layer being dried, filtered and the solvent removed under vacuum to give a green-brown oil, crude yield 490 mg. Analytical tlc

showed a small amount of non-polar material, a small amount of unreacted starting material and a large polar spot R_f 0.18. This material was separated by high pressure column chromatography on Kieselgel HF_{254} (Type 60) (ratio 20:1) using 5% ether/pet.ether as eluant to give a pale yellow oil (322 mg.) which was subsequently separated into two compounds (29) and (35) by column chromatography using Hi-Flosil-Ag, 20% $AgNO_3$ support (60-200 mesh) (ratio 33:1) and eluting with 2% ethyl acetate/ pet.ether.

(29) m.pt. 63-64.5°C. Yield 146 mg. (32%).

$\bar{\nu}_{max}$ ($CHCl_3$) = 3610, 3060, 1050, 1030, and 1010 cm^{-1}

δ = 0.45-0.8 (m,3H), 0.90 (s,3H), 0.92 (s,3H), 1.00 (s,3H), 1.67 (bs,3H), and 3.23 (d, J = 9.5 Hz,1H).

ms M^+ (found) = 220.1816; M^+ (calc. for $C_{15}H_{24}O$) = 220.1827

p-Bromobenzoate derivative, m.pt. 53.5-55°C.

Crystal data: $C_{22}H_{27}BrO_2$, monoclinic, P $2_1/c$, Z = 4, a = 14.43, b = 5.85, c = 26.48 Å, β = 114.5°; 2283 unique reflections on layers h0-4ℓ (857 with $I > 3\sigma(I)$) were collected on a Stoe STADI-2 diffractometer (Mo-K_α radiation). The structure was solved using the SHELX-76 programme for crystal structure determination, G. M. Sheldrick, 1976, University of Cambridge, England with a present R factor of 7%.

(35) b.pt. 95-100°C/0.25 mm. Yield 180 mg. (39%).

$\bar{\nu}_{max}$ = 3610, 3060, 3040, 1650, 1055, 1030, 1012, and 825 cm^{-1}.

δ = 0.3-0.9 (m,3H), 1.02 (s,9H), 1.64 (bs,3H), 3.21 (d,J = 8 Hz,1H), and 5.33 (m,1H).

ms M^+ (found) = 220.1826; M^+ (calc. for $C_{15}H_{24}O$) = 220.1827.

Preparation of p-bromobenzoate of alcohol (29)

Alcohol (29) (25 mg., 0.1 mmol.) was dissolved in pyridine (2 ml.) and then p-bromobenzoyl chloride (30 mg., 0.14 mmol.) added. The solution was immediately cooled to 0°C and a fine white precipitate appeared. The flask was stoppered and allowed to stand in the fridge overnight. Analytical tlc showed that the reaction was incomplete so a further 10 mg. (0.05 mmol.) of p-bromobenzoyl chloride was added and the mixture warmed to room temperature to allow dissolution of the acid chloride. The flask was then cooled to 0°C and returned to the fridge. After 2 hours, analytical tlc showed the reaction was complete. The reaction was worked up by adding cold water and extracting well with ice-cold ether. The ether extract was then washed with ice-cold saturated copper sulphate solution (2 x 5 ml.), ice-cold saturated sodium carbonate solution (5 ml.), ice-cold half-saturated sodium carbonate solution (5 ml.) and ice-cold water (5 ml.). The ether extract was dried, filtered, and the solvent removed under vacuum to leave a solid residue with traces of oily liquid present. The oily liquid dissolved in chloroform leaving the solid residue. The liquors were purified by preparative tlc, eluting with 5% ethylacetate/pet.ether and a band removed which was eluted with ether and chloroform. After removal of solvent, a clear-oil remained, yield 39 mg. (85%) which crystallised on standing in the fridge. m.pt. 53.5-55°C.

δ = 0.30-0.80 (m,3H), 0.96 (s,6H), 1.10 (s,3H), 1.69 (bs,3H), 4.87 (m,1H), and 7.79 (ABq,4H).

Attempted isomerisation of alcohol (35)

(a) With trifluoroacetic acid

The trisubstituted olefinic alcohol (35), (5 mg.) was dissolved in dichloromethane (3 ml.) and stirred at -70°C (acetone/Drikold). One drop of trifluoroacetic acid (∿ 0.05 ml.) was added to the solution which was then stirred for 30 minutes. Analytical tlc showed no change so a further 0.15 ml. trifluoroacetic acid was added. After 30 minutes a large non-polar spot was observed. Certainly none of the tetrasubstituted olefinic alcohol (29) was seen.

(b) With p-toluenesulphonic acid

The alcohol (35), (5 mg.), dissolved in dry benzene (3 ml.) was treated with p-toluenesulphonic acid (5 mg.), the mixture being stirred, the temperature being kept below 10°C using a cold water bath. After 30 minutes, analytical tlc showed that no reaction had occurred so the temperature was allowed to rise and a further 10 mg. of acid was added. The mixture was stirred at room temperature for a further 5 hours but no reaction was observed.

(c) With boron trifluoride etherate

The alcohol (35), (5 mg.), ether (5 ml.) and boron trifluoride etherate (0.10 ml.) were stirred magnetically in a flask at room temperature. Analytical tlc monitoring over 21 hours showed no apparent reaction.

(d) With Amberlyst-15 (H^+ form) resin

The alcohol (35), (5 mg.), resin (200 mg.) and ether (3 ml.) were stirred magnetically for 15 minutes at room temperature. It was noted that a non-polar spot appeared on analytical tlc but none of the isomeric alcohol (29) was observed.

Attempted isomerisation of alcohol (29)

(a) With trifluoroacetic acid

The alcohol (29), (5 mg.) was dissolved in dichloromethane (3 ml.) and stirred at room temperature. Trifluoroacetic acid (0.1 ml.) was added and within 2 minutes the solution began to show a purple colouration. No trace of the isomeric alcohol (35) was observed by analytical tlc over 30 minutes.

(b) With perchloric acid

A solution of alcohol (29), (5 mg.) in dichloromethane (3 ml.), was cooled to -70°C in an acetone/Drikold bath and 70% perchloric acid (0.05 ml.) was added, the mixture being stirred magnetically. After 30 minutes, no reaction had occurred but on allowing to rise to room temperature, the reaction mixture became purple-coloured. Analytical tlc showed one non-polar spot, no starting alcohol (29) and no isomeric alcohol (35).

(c) With boron trifluoride etherate and silver tetrafluoroborate

The alcohol (29), (5 mg.), silver tetrafluoroborate (5 mg.), ether (3 ml.) and boron trifluoride etherate (0.15 ml.) were stirred at room temperature for 6 hours but analytical tlc showed that no isomerisation had occurred.

(d) Under hydrogenation conditions

The alcohol (29), (5 mg.) was dissolved in dry methanol (5 ml.) and, using 10% Pd./C as catalyst, was stirred in an atmosphere of hydrogen at room temperature and atmospheric pressure for 20 hours. No reaction was noted on monitoring by analytical tlc.

Preparation of keto-alcohol (44)[33].

(a) Acetylation of alcohol (29)

The alcohol (29) (393 mg., 1.79 mmols.) was dissolved in dry pyridine (0.5 ml.), acetic anhydride (219 mg., 2.15 mmols.) was added and the mixture was allowed to stand at 0°C overnight. The reaction was worked up in the usual manner, the ether extract being dried and the solvent removed under vacuum. The crude oil was purified by column chromatography using Kieselgel 60 (Art.9385) and eluting with 2% ether/pet.ether to give the ester (320 mg. 68%). R_f 0.42.

(b) Allylic oxidation of the acetate

Chromium trioxide (1.83g., 18.3 mmols.) was added to an ice-cold, rapidly stirred solution of dry pyridine (2.89g.) in distilled dichloromethane (30 ml.) under nitrogen. The deep burgundy solution was stirred for 5 minutes then allowed to warm to room temperature. The ester (320 mg., 1.22 mmols.) was added in dichloromethane solution and the reaction left for 24 hours. The reaction mixture was poured from the flask, the precipitate in the flask washed with ether and the washings combined with the organic layer. This was then washed with saturated sodium bicarbonate, saturated copper sulphate and then with brine. The organic layer was dried, filtered and the solvent removed under vacuum to give a crude oil. Purification by column chromatography using Kieselgel 60 (Art.9385) and 3-40% ether/pet.ether gave the acetoxyketone (90 mg., 27%).

m.pt. 78-79°C; R_f 0.18

λ_{max}^{EtOH} 240 nm (ε = 10,200)

$\bar{\nu}_{max}$ ($CHCl_3$) = 1730, 1700, and 1640 cm^{-1}

δ = 0.75-1.0 (m,3H), 0.8 (s,3H), 1.0 (s,3H), 1.03 (s,3H), 1.70 (bs,3H), 2.04 (s,3H), 2.25-2.8 (m,5H), and 4.7 (d,J=9Hz,1H).

(c) Saponification of the keto-ester

The keto-ester (80 mg., 0.29 mmols.) was dissolved in methanol (10 ml.) and potassium hydroxide solution (80 mg. in 4 mls. water) was added. The solution was stirred at 70°C for 1 hour in an oil bath. Analytical tlc showed that the ester had been hydrolysed. The methanol was removed on the rotary evaporator, a small amount of water was added to the solution and the solution was extracted with ether. The ether layer was dried, filtered and the solvent removed under vacuum. The keto-alcohol (44) was separated by preparative tlc using 50% ethylacetate/pet. ether to give 43 mg. (63%) of a colourless oil, R_f 0.23 which solidified.

$\lambda_{max}^{CCl_4}$ = 253 nm (ε = 12,200)

$\bar{\nu}_{max}(CCl_4)$ = 3430, 1685, 1630, and 1030 cm^{-1}

δ = 0.5-1.0 (m,3H), 0.72 (s,3H), 0.91 (s,3H), 1.02 (s,3H), 1.65 (bs,3H), and 3.22 (d,J=8 Hz.,1H).

Reaction of humulene-4,5-epoxide (8) with stannic chloride in benzene

Humulene-4,5-epoxide (8), (220 mg., 1 mmol.) was dissolved in benzene and cooled to almost 0°C (until benzene just began to freeze), stirring magnetically. Stannic chloride (115 µl., 1 mmol.) was quickly added by syringe and the mixture stirred at ~ 0°C for 20 minutes following which saturated sodium bicarbonate solution and ether were added. The organic layer was washed with brine after extraction and separation, dried, filtered, and solvent removed to give a green-yellow oil. Analytical tlc showed several polar spots as well as some starting material. Two purifications by column chromatography using (i) Kieselgel 60 (Art. 9385, 230-400 Mesh) (Ratio 40:1) with 5% then 10% ether/pet.ether as eluant, and (ii) Kieselgel HF_{254} (Type 60) (Ratio 80:1) with 4% ether/pet.ether as eluant,

gave a pale yellow oil, one spot by analytical tlc, R_f 0.43, whose structure was proposed to be either (47) or (48).

δ = 0.92 (s,3H), 1.09 (s,3H), 1.45 (s,3H), 1.64 (s,3H), 3.58-3.90 (m,2H) and 4.81 (m,2H).

ms: M^+ 256 and 258, 221 (M^+-35), 203 (M-(Cl + H_2O)).

Reaction of humulene-4,5-epoxide (8) with silver nitrate

To humulene-4,5-epoxide (8), (25 mg., 0.11 mmols.) dissolved in ether was added a 50% w/w silver nitrate/water solution (1 ml.). No precipitate was observed and it was noted that the reaction mixture was heterogeneous. In an attempt to homogenise the mixture, tetrahydrofuran (1 ml.) was added but this made no difference to the outcome of the reaction. Analytical tlc after 4 hours showed starting material.

Reaction of humulene-4,5-epoxide (8) with silver tetrafluoroborate

To humulene-4,5-epoxide (8), (24 mg., 0.11 mmols.) in anhydrous ether (4 ml.) was added silver tetrafluoroborate/ether solution (2 ml., 0.11 mmols.) and the solution was stirred magnetically at room temperature. Immediately a white precipitate appeared. The reaction was left in the fridge for 18 hours but analytical tlc showed no change, the starting epoxide still being present.

A similar reaction was run using benzene as solvent but no reaction at all occurred i.e. no precipitate formed.

Reaction of humulene-4,5-epoxide (8) with silver tetrafluoroborate and boron trifluoride etherate

To humulene-4,5-epoxide (8), (17mg., 0.08 mmols.), dissolved in anhydrous ether (1 ml.), was added a suspension of silver tetrafluoroborate (2 ml., 0.11 mmols.), in ether, a white precipitate immediately being observed. After standing for 15 minutes at room temperature, boron trifluoride etherate (9 µl., 0.08 mmols.) was added and the mixture allowed to stand for 18 hours at room temperature. A sample was then taken, worked up with saturated sodium bicarbonate solution and ether and analysed by tlc.

On comparison with an authentic sample of the alcohol (29), it was found that both gave the same R_f and that unreacted starting epoxide (8) was also present.

Preparation of sodium dicarbonylpentahaptocyclopentadienyl-ferrate (Na^+Fp^-)[41]

To pure mercury (100g.) warmed to 40°C was added sodium (1g., 43.4 mg. atoms) in 5 mm. cubes, introduced under the surface with care, a vigorous reaction taking place. When the amalgam had cooled, dry tetrahydrofuran (40 ml.) and dicarbonylpentahaptocyclopentadienyl iron dimer (3.14g., 8.7 mmol.) in tetrahydrofuran (20 ml.) were added and the mixture stirred vigorously under a flow of nitrogen at room temperature for 48 hours. The stirrer was then switched off and the reaction mixture allowed to stand for three days.

Preparation of the Fp-σ methallyl complex (52)[42]

The Fp^-Na^+ salt was stirred under nitrogen at room temperature and to it was added methallyl chloride (6.4 ml., 0.065 mmols.) by injection. The temperature was then raised to 40°C and the mixture stirred for 2 hours, after which the stirrer was switched off and the mixture allowed to stand overnight. The solvent was removed under vacuum (0.15 mm.) and a greyish-red-brown mass remained. This was extracted thoroughly with pet.ether (40-60°) resulting in a solution which contained a reddish-brown solid in fine suspension. This was filtered through a sinter at the pump, a dark yellow-orange oil filtering through as the solvent evaporated off. Eventually a dark red-brown liquid was obtained, yield 4.21g. The solvent was removed under vacuum and the red-brown oil distilled at 52°C/0.08 mm to give a fraction yield 3.10g. (76%). Great care was taken as this material is air sensitive. Spectral characteristics compared favourably with those of an authentic sample.

δ = 1.76 (bs,3H), 2.13 (bs,2H), 4.58 (bs,5H),

Preparation of Fp^+-π isobutene complex (53)[43]

To the σ-methallyl complex (52), (500 mg., 2.15 mmols.) was added a 1:1 hydrofluoroboric acid (40% aqueous)/acetic anhydride mixture (3 ml.) and the reaction mixture was allowed to stand at room temperature, with occasional swirling, for 2 hours. The dark red-brown liquor was poured into ether (40 ml.) and, upon stirring with a glass rod, traces of gummy material began to appear. Further dilution (40 ml.) and

trituration of the deposited gum gave a yellow-green solid which was filtered at the pump, yielding 350 mg. (51%) which was immediately placed under nitrogen and stored in the fridge. The nmr spectrum was identical to an authentic spectrum of (53).[45]

δ (CD_2Cl_2) = 1.84 (s,6H), 3.77 (s,2H), and 5.46 (s,5H).

Reaction of Fp^+-π isobutene complex (53) with humulene (1)[44]

The reaction was carried out in a 2-necked 25 ml. conical flask with a thermometer in one arm and a cork bung with a syringe needle stuck through it into the flask in the other. The Fp^+-π isobutene complex (53), (50 mg., 0.16 mmols.), humulene (1), (160 mg., 0.78 mmols.) and dichloroethane (10 ml.) were added together, the flask was heated to 65-70°C and held in that temperature range for 20 minutes. The flask was then cooled, the contents of which were transferred to a round-bottom flask. The solvent was removed under vacuum leaving a brown-green sludge, which was dissolved in the minimum of acetone and the solution diluted with ether. A pale fine precipitate began to appear and once this was complete, the solution was filtered, giving a pale beige solid. Further dilution of the filtrate gave a further precipitate. The combined solids were washed with ether to give a yield of 31 mg. The nmr spectrum was difficult to record because the complex did not dissolve readily in normal solvents. However an nmr spectrum recorded in CD_3CN compares with that from another preparation[45] although the peaks are difficult to assign.

Reaction of $[Fp^+ \leftarrow \text{Humulene}]BF_4^-$ with mercuric acetate

To the humulene complex (28 mg., 5.9×10^{-2} mmols.) was added 1% ethanol/tetrahydrofuran solution (12 ml.). The complex dissolved leaving a cloudy solution then mercuric acetate (38 mg., 11.9×10^{-2} mmols.) was added and the solution stirred magnetically for 22 hours sampling intermittently for analytical tlc. Sodium borohydride (15 mg., 0.39 mmols.) was added and the solution rapidly darkened. After being stirred for 30 minutes, 80% sodium iodide/acetone[93] solution (2 ml.) was added to the mixture; it was stirred for a further 1 hour, filtered and the filtrate washed with ether. This caused a copious white solid to precipitate, which was collected by filtration and the solvent was removed from the filtrate to give a green oil. Nmr spectral analysis showed a very complex set of signals but peaks identifiable with those seen in humulene could be observed.

Reaction of Fp^+-π isobutene complex (53) with humulene-4,5-epoxide (8)

Fp^+-π isobutene complex (53), (50 mg., 0.16 mmol.), humulene-4,5-epoxide (8), (170 mg., 0.78 mmol.) and dichloroethane (4 ml.) were heated together to 65-70°C at which temperature they were held for 20 minutes with stirring, the solution turning deep red in colour. The reaction mixture was allowed to cool, the solvent was removed under vacuum, the residues were dissolved in the minimum of acetone, filtered and then ether added (50 ml.). A slight brown precipitate was observed which on filtering gave only a yield of 10 mg. This was dissolved in CD_3CN but the nmr spectrum was inconclusive.

Preparation of copper(I) tetrafluoroborate[46]

Silver tetrafluoroborate (200 mg., 1.02 mmols.) was placed in a small glass tube and copper powder (1g.) and dry toluene (5 ml.) were added. The tube was stoppered and placed on a mechanical shaker for 2 days. The copper powder was removed by filtration and subsequently washed with toluene. The filtrate, greenish liquid, was stored in the fridge in a well-stoppered flask as the product is air-sensitive.

Reaction of humulene (1) with copper(I) tetrafluoroborate

To a magnetically stirred solution of humulene (1), (150 mg., 0.74 mmols.) in pentane (1 ml.), was added copper(I) tetrafluoroborate/toluene solution (2 ml.) and the flask stoppered. Immediately the solution became cloudy and traces of a white precipitate began to appear. Addition of a further volume of pentane (3 ml.) caused more precipitation. The mixture was stirred at room temperature for 30 minutes and then filtered at the pump. A white solid appeared on the filter paper but immediately disappeared. It was probably extremely air-sensitive.

Reaction of humulene-4,5-epoxide (8) with copper(I) tetrafluoroborate

To a magnetically stirred solution of humulene-4,5-epoxide (8), (50 mg., 0.23 mmol.), in n-pentane (2 ml.) at room temperature, was added copper tetrafluoroborate/toluene solution (2 ml.) and immediately the solution became cloudy.

After stirring for 15 minutes, the stirrer was stopped and it was noted that there was a pale green precipitate present. Boron trifluoride etherate (0.1 ml.) was added and the mixture stirred for 30 minutes, the colour changing from light green to dark purple. The reaction mixture was worked up by adding saturated sodium bicarbonate solution and ether. The ether extract was dried, filtered, and the solvent removed under vacuum to give a dark brown oil, yield 48 mg. Analytical tlc showed a heavy spot in the non-polar region but very little in the polar region. This reaction was not investigated further.

Hydrogenation of humulene with Adams' catalyst

Adams' catalyst ($PtO_2.H_2O$), (45 mg.) was added to the reaction flask then humulene (500 mg., 2.45 mmols.) dissolved in ethanol (10 ml.) was added. The system was well flushed out with hydrogen then the mixture was left stirring in an atmosphere of hydrogen at room temperature and atmospheric pressure. Using the arbitrary scale on the gas reservoir, previously calibrated using 2-methyl-2-butene, the rapid uptake of one molar equivalent of hydrogen was monitored. This having been achieved, water and ether were added to the reaction mixture. The ether layer was washed well with water, separated, dried and filtered through Celite. The Celite was washed with pet.ether (40-60°C) and the solvent was removed from the combined extracts under vacuum to give a brown oil, yield 490 mg. Purification by column chromatography using Hi-Flosil-Ag, 20% $AgNO_3$ support (60-200 mesh) (Ratio 30:1), eluting with 2% ethyl acetate/pet.ether gave a

fraction yield 209 mg., a pale oil, to which the structures (58) and (59) were assigned.

$\bar{\nu}_{max}$ = 1385, 1365, and 975 cm^{-1}

δ = 0.90 (s,3H), 1.08 (s,3H), 1.53 (bs,3H), and 4.50-5.55 (m,3H).

Monoepoxidation of 1,2-dihydrohumulenes (58) and (59) with m-chloroperoxybenzoic acid

To a solution of 1,2-dihydrohumulenes (58) and (59) (208 mg., 1.01 mmols.) in dichloromethane (40 ml.) was added sodium bicarbonate solution (0.5M, 50 ml.), the mixture being stirred at room temperature. m-Chloroperoxybenzoic acid (90%, 213 mg., 1.11 mmols.) was slowly added over 5 minutes and then the mixture stirred for 30 minutes. After testing with lead acetate paper, the dichloromethane layer was separated, washed with dilute sodium hydroxide solution followed by water, and dried. After filtration the solvent was evaporated off leaving a clear oil, yield 225 mg. Of this crude product, 125 mg. was purified on a Kieselgel HF_{254} (Type 60) column (Ratio 20:1) using 2% ether/pet.ether as eluant to give two fractions, yields 40 mg. and 14 mg. and R_f 0.41 and 0.38 respectively. The spot R_f 0.41 showed a double head on silver nitrate (20%) sprayed analytical tlc using 20% ethylacetate/pet.ether as eluant. A mixture of isomers (60) and (61) and their mirror images was assigned. The compound R_f 0.38 was assigned the structure (66).

(60)/(61) + mirror images: δ = 0.90 (d,3H), 1.04 (s,3H), 1.20 (s,3H), 1.26 (s,3H), 2.40-2.95 (m,1H), and 5.35 (m,2H).

It should be noted that the ratio of the 5.35 ppm to the 2.40-2.95 ppm multiplets is 2:1.

(66): δ = 0.89 (s,3H), 0.96 (s,3H), 1.11 (s,3H), 1.50 (bs,3H), 2.40-2.80 (m,1H), and 5.10 (bd,J = 10 Hz,1H).

Hydrogenation of humulene-4,5-epoxide (8) using Adams' catalyst

The procedure and apparatus used were exactly the same as that for the hydrogenation of humulene. Humulene-4,5-epoxide (8), (300 mg., 1.36 mmols.), Adams' catalyst (50 mg.) and ethanol (10 ml.) were all mixed together and stirred at room temperature in an atmosphere of hydrogen at atmospheric pressure until 1 molar equivalent of hydrogen was taken up. This was monitored on the gas reservoir scale, previously calibrated using cyclohexene (1.36 mmols.). The rate of hydrogen uptake was slower than that for humulene. The reaction mixture was filtered through Celite, which was then washed with ether. The combined extracts were washed thoroughly with water, the ether layer dried, filtered, and the solvent removed to give a brownish oil. 20% silver nitrate impregnated analytical tlc showed a double-headed spot less polar than the starting material. Two column separations using Hi-Flosil-Ag, 20% $AgNO_3$ support and eluting with pet.ether gave a mixture of the isomeric dihydro compounds (67) and (68) and their mirror images.

δ = 1.60 (bs,3H), 2.71-3.00 (m,1H), and 5.21 (bd,1H).

Subsequent attempts to repeat this reaction resulted in spontaneous rearrangement of the dihydroepoxides to give a complex mixture of alcohols which could not be separated. This rearrangement occurred at the column chromatography stage using $AgNO_3$ impregnated silica. Addition of D_2O to the nmr sample showed the disappearance of a hydroxyl peak in several fractions removed from the column. A very polar band, observed on preparative tlc, was removed and eluted with ethylacetate to give 60 mg. of a yellow oil.

$\bar{\nu}_{max}$ = 3600, 1380, 1370, 1102, and 1050 cm^{-1}

δ = 0.91 (s,3H), 1.04 (s,3H), 1.21 (s,3H), 1.58 (bs;3H), and 3.13 (bd,1H,J = 10 Hz).

$Eu(fod)_3$ lanthanide shift nmr proved to be inconclusive but indicated a mixture of the proposed structures (69), (70) and their mirror images.

Reaction of the 1,2-dihydro-humulene-4,5-epoxides (67), (68) and their mirror images with boron trifluoride etherate

The 1,2-dihydrohumulene-4,5-epoxides (67), (68) and their mirror images (16 mg., 7.0×10^{-2} mmols.) dissolved in anhydrous ether (2 ml.) were cooled to -70°C with stirring. Boron trifluoride etherate (9 µl., 7.0×10^{-2} mmols.) was added, the reaction mixture stirred for 30 minutes at -70°C, then allowed to warm to room temperature and stirred for a further hour. Analytical tlc showed polar spots with approximately the same R_f as those observed from the column rearrangement of the epoxides (67), (68) and their mirror images.

Reaction of humulene-4,5-epoxide (8) with pyridinium hydrobromide perbromide

Humulene-4,5-epoxide (8), (80 mg., 0.36 mmols.) was dissolved in dichloromethane (5 ml.) with magnetic stirring. The perbromide (115 mg., 0.36 mmols.) was added to the solution, thus imparting an orange colouration to the reaction mixture. After a few minutes stirring at room temperature, the solution lightened in colour slightly then a pale coloured precipitate appeared which quickly redissolved. After 15 minutes, analytical tlc revealed a multitude of spots.

Reaction of humulene-4,5-epoxide (8) with pyridine perbromide

To humulene-4,5-oxide (8) (20 mg., 0.09 mmols.) dissolved in dichloromethane (5 ml.) was added pyridine perbromide

(20 mg., 0.08 mmols.) and the mixture stirred at room temperature. After 20 minutes the pale yellow colour of the solution had disappeared. Analytical tlc showed a mass of products.

Reaction of humulene-4,5-epoxide (8) with bromine

To humulene-4,5-epoxide (8), (50 mg., 0.23 mmols.), dissolved in carbon tetrachloride (2 ml.) and stirred magnetically, was added bromine (11.9 µl., 0.23 mmols.) by microsyringe. The bromine colour instantly disappeared but analytical tlc showed a range of spots.

The same experiment was repeated except that it was carried out in ether at -78°C using an acetone/Drikold bath. The solution was stirred at -70°C for 15 minutes, allowed to warm to room temperature and stirred for 17 hours. Again a wide range of products was observed by analytical tlc.

Preparation of humulol (72)[50]

Humulene (2g., 9.80 mmols.) was mixed with 80% aqueous acetic acid (30 ml.) and the heterogeneous mixture stirred magnetically and heated at 100°C under reflux for 2 hours. The mixture was cooled, neutralised cautiously using saturated sodium bicarbonate solution, and then extracted with ether. The ether layer was washed with water, dried, filtered, and the solvent evaporated off to give a green-brown oil, yield 1.8g. 0.9g of the crude was purified using column chromatography (Kieselgel HF_{254}, ratio 20:1) and eluting with 3%, 5%, 10%, 15% ether/

pet.ether to give humulol (72), a pale yellow oil, yield 240 mg. (22%).

δ = 0.99 (s,3H), 1.02 (s,3H), 1.11 (s,3H), 1.52 (d,3H,J=1 Hz), and 4.55-5.48 (m,3H).

Attempted monoepoxidation of humulol (72) with t-butyl-hydroperoxide

To humulol (72), (135 mg., 0.61 mmol.), dissolved in dry benzene (4 ml.), was added vanadylacetoacetonate (5 mg), with magnetic stirring, the solution turning green. t-Butyl-hydroperoxide (70%, 12.6 mg., 0.98 mmols.) dissolved in benzene (1.5 ml.) was then added slowly over 10 minutes, the solution turning blood red. After the addition, the solution was stirred at room temperature for 12 days, then the reaction mixture was worked up with aqueous sodium metabisulphite and ether, the organic layer being dried, filtered, and the solvent removed to give a yellow viscous oil, yield 119 mg. Column chromatography and preparative tlc gave two fractions yields 14 mg. and 9 mg. respectively, but the nmr spectra proved to be totally inconclusive.

Monoepoxidation of humulol (72) with m-chloroperoxybenzoic acid

Humulol (72) (200 mg., 0.90 mmols.) was dissolved in dichloromethane (10 ml.) and to this was slowly added m-chloroperoxybenzoic acid (85%, 200 mg., 0.99 mmols.) at room temperature, the solution being stirred magnetically. After 30 minutes water was added and the mixture was extracted with ether. The organic layer was washed with dilute sodium

bicarbonate solution, brine, dried, filtered, and the solvent removed leaving a yellow viscous oil, yield 195 mg. Separation by column chromatography using Kieselgel HF_{254} and 30% ether/pet.ether as eluant gave a fraction which solidified to give a white solid (75), yield 70 mg. (33%).

Recrystallisation was difficult.

$\bar{\nu}_{max}$ ($CHCl_3$) = 3610, 1380, 1365, 1130, 1090, and 1075 cm^{-1}

δ = 1.05 (s,3H), 1.17 (s,3H), 1.29 (s,6H),
2.72-2.90 (dd,J=10Hz and 2Hz,1H), and 5.21-5.81 (m,2H).

Preparation of humulol methylthiomethyl ether (77)[56]

Humulol (72), (327 mg., 1.47 mmols.) was dissolved in dimethylsulphoxide (7 ml.) and acetic anhydride (7 ml.) and stirred magnetically at room temperature. After 20 hours, the solution was evaporated at reduced pressure (0.2 mm.) using a condenser and an acetone/Drikold cooled receiving vessel to trap the dimethylsulphoxide/acetic anhydride as it distilled off. A dark yellow oil, which remained in the flask, was dissolved in benzene, washed twice with saturated sodium bicarbonate solution, once with brine, four times with water then the benzene extract dried over anhydrous sodium sulphate. The extract was filtered and the solvent removed under vacuum to give a yellow-brown oil, yield 170 mg. The crude product was purified by high pressure column chromatography using Kieselgel HF_{254} (Type 60) (Ratio 20:1) and 2% ether/pet.ether (60-80°) as eluant. The fraction isolated from the column was then distilled under vacuum, the fraction boiling at 127-130°C/0.1 mm. being collected. Yellow oil, yield 53 mg. (13%).

δ = 1.04 (s,3H), 1.07 (s,3H), 1.18 (s,3H), 1.60 (s,3H),
2.18 (s,3H), 4.50 (s,2H), 4.85-5.50 (m,3H).

Reaction of humulol methylthiomethyl ether (77) with mercuric trifluoroacetate

Humulol methylthiomethyl ether (77), (40 mg., 0.14 mmols.) was dissolved in dry ether (3 ml.) and cooled to -78°C using acetone/Drikold, whilst stirring magnetically. Mercuric trifluoroacetate (72 mg., 0.17 mmols.) was added and the mixture stirred at -70°C for 1 hour after which brine then solid sodium bicarbonate were added to the flask. The mixture was extracted with chloroform, the chloroform layer dried, filtered, and the solvent removed under vacuum to give a clear oil, yield 44 mg. Nmr spectroscopy shows this to be humulol (72).

Reaction of humulene-1,2-epoxide (10) with mercuric trifluoroacetate

Mercuric trifluoroacetate (98 mg., 0.23 mmols.) dissolved in dry methanol (2 ml.), was stirred magnetically at room temperature. Humulene-1,2-epoxide (10), (50 mg., 0.23 mmols.) dissolved in methanol (1 ml.) was added to the solution and the mixture stirred for 1.5 hours. Analytical tlc showed mostly unreacted starting material so the reaction mixture was heated to 60°C and held at that temperature for 3 hours. Analytical tlc showed a multitude of polar spots.

Reaction of humulene-1,2-epoxide (10) with mercuric acetate

To mercuric acetate (145 mg., 0.45 mmol.) in a flask was added 50% tetrahydrofuran/water (7 ml.) and an immediate bright yellow precipitate was observed. The mixture was stirred magnetically and the 1,2-epoxide (10) (100 mg., 0.45 mmols.)

added, dissolved in 50% tetrahydrofuran/water (2 ml.). After stirring at room temperature for 3 hours, no reaction had occurred on monitoring by analytical tlc.

Reaction of humulene-cis-1,2:4,5-diepoxide (64) with boron trifluoride etherate

The diepoxide (64), (18 mg., 7.6×10^{-2} mmols.) was dissolved in anhydrous ether (2 ml.) and boron trifluoride etherate (9 µl, 7.6×10^{-2} mmols.) added to the stirred solution. After stirring the mixture for 18 hours at room temperature, no reaction was observed by analytical tlc. A further 30 µl boron trifluoride etherate was added and the mixture stirred for 3 days. Analytical tlc showed a large range of polar products.

This reaction was repeated using benzene as solvent at room temperature and a ratio of 1:1 substrate/Lewis acid. After 24 hours stirring at room temperature, a multitude of spots was seen.

Reaction of humulene-cis-1,2:4,5-diepoxide (64) with anhydrous copper sulphate/acetone[60]

The diepoxide (64), (78 mg., 0.33 mmoles.), anhydrous copper sulphate (50 mg.) and acetone (500 µl.) were added together and stirred magnetically at room temperature for 24 hours, after which the mixture was extracted with ether and the solvent removed under vacuum. A clear colourless oil remained which by nmr spectroscopy proved to be unreacted starting material.

Preparation of humulen-5-ol (49)[4]

Humulene-4,5-epoxide (8) (350 mg., 1.59 mmols.) was dissolved in anhydrous ethylene diamine (20 ml.) and with magnetic stirring, strips of lithium metal (300 mg., 42.5 mg.atoms) were added. After about 25 minutes, the solution became fairly viscous, turned dark blue and effervesced briskly for a few minutes. The mixture was stirred for a total of 1.5 hours, the colour gradually lightening from dark blue to grey. A large volume of water was carefully added (100 ml.) and the mixture extracted with ether, the organic layer being dried, filtered and the solvent removed under vacuum. The crude product, a clear oil which solidified on standing, yield 315 mg., was purified by high pressure column chromatography using Kieselgel HF_{254} (Type 60) (Ratio 20:1), 5% ethyl acetate/pet.ether being used as eluant. A fraction from the column gave a white solid, yield 269 mg. (76%), R_f 0.25, m.pt. 92-93°C.

$\bar{\nu}_{max}$ ($CHCl_3$) = 3620, 1380, 1365, 1060, 1045 cm^{-1}

δ = 0.81 (s,3H), 1.05 (s,3H), 1.40 (bs,3H), 1.57 (bs,3H), 3.46 (bt,1H), and 4.83 (m,2H).

ms: M^+ (found) 222.1986; M^+ (calculated for $C_{15}H_{26}O$) = 222.1984.

Preparation of humulen-5-one (79)

Pyridinium chlorochromate[63] (200 mg., 1.08 mmols) was suspended by magnetic stirring in dichloromethane (10 ml.). Humulen-5-ol (49), (130 mg., 0.59 mmols.), dissolved in dichloromethane (2 ml.) was added to the suspension and immediately the colour of the reaction mixture darkened. After 10 minutes a black residue in the bottom of the flask began to appear. The reaction

mixture was stirred at room temperature for 1 hour then worked up by adding 5 x volume of ether and the mixture filtered through a Florisil column. The solid residue in the flask was washed twice with ether, as was the Florisil, and then the ether was removed from the filtrate under vacuum to give a white solid, yield 110 mg. (85%) which was recrystallised from pet.ether. M.pt. 83-84°C.

$\bar{\nu}_{max}$ ($CHCl_3$) = 1698, 1385, 1365 cm^{-1}

δ (ppm) = 1.19 (s,6H), 1.45 (s,3H), 1.60 (s,3H), 4.80 (m,2H).

ms: M^+ (found) = 220.1855; M^+ (calculated for $C_{15}H_{24}O$) = 220.1828.

Found: C, 81.1; H, 11.1. $C_{15}H_{24}O$ requires C, 81.8; H, 11.0%.

Reaction of humulen-5-one (79) with lithium N-isopropylamide/diphenyl disulphide[66]

Dry tetrahydrofuran (10 ml.) was cooled to -78°C, under nitrogen, using an acetone/Drikold bath. Stirring magnetically, N-isopropylamine (36 mg., 0.36 mmols.) was added by syringe through a rubber septum, followed by the careful addition of n-butyl lithium (1.6M in hexane, 0.23 ml., 0.38 mmols.) in like manner. The solution was allowed to stir at -70°C for 15 minutes then the ketone (79), (80 mg., 0.36 mmols.) dissolved in tetrahydrofuran (2 ml.) was added, maintaining the temperature at -70°C. After stirring for 20 minutes, the solution temperature was allowed to rise to -25°C by immersing the flask in a carbon tetrachloride/Drikold bath. Diphenyldisulphide (90 mg., 0.41 mmols.), dissolved in hexamethylphosphorustriamide (5 ml.), was added by syringe, the temperature then allowed to rise to room temperature and the mixture stirred for 16 hours.

The reaction mixture was shaken with saturated potassium tartrate solution/pet.ether (40-60°), the pet.ether layer was washed well with water and separated. After drying and filtration, the solvent was removed under vacuum. A pale yellow solid, yield 100 mg. (84%) was obtained which corresponded by nmr spectroscopy to the structure (81).

nmr Methyl region difficult to assign; δ= 3.96 (dd,J=10 Hz and 4Hz,1H), and 7.16 (bs,5H).

Reaction of (81) with tosylhydrazine

The crude ketone product (81), (100 mg., 0.31 mmols.) was dissolved in ethanol (5 ml.) and then tosylhydrazine (74 mg., 0.4 mmols.) added, the mixture being stirred magnetically for 18 hours at room temperature. Analytical tlc showed unchanged starting material.

Reaction of humulen-5-one (79) with isoamyl nitrite[67]

Potassium t-butoxide (19 mg., 0.17 mmols.) was dissolved in t-butanol (5 ml.) at room temperature with magnetic stirring under a flow of nitrogen. Isoamyl nitrite (13 mg., 0.11 mmols.) was introduced into the reaction flask via a rubber septum using a syringe. The solution was stirred for 5 minutes then the ketone (79), (25 mg., 0.11 mmols.), dissolved in a small amount of t-butanol (1 ml.), was added by syringe and the mixture stirred at room temperature for two days, during which time no reaction was observed by monitoring with analytical tlc.

Reaction of humulen-5-one (79) with lithium diisopropylamide/ isoamyl nitrite

Dry tetrahydrofuran (3 ml.) was cooled to -78°C under nitrogen using an acetone/Drikold bath. With magnetic stirring, diisopropylamine (38 µl., 0.34 mmols.) was added by microsyringe and the solution stirred for 5 minutes. n-Butyl lithium (1.5M in hexane, 230 µl., 0.34 mmols.) was slowly added, the mixture then being stirred for 20 minutes. The ketone (79), (75 mg., 0.34 mmols.), dissolved in a small amount of tetrahydrofuran (1 ml.) was added to the reaction mixture which was then stirred for 30 minutes at -70°C. The temperature was then allowed to rise to 0°C and isoamyl nitrite (68 µl., 0.51 mmols.) added by syringe. There was an immediate darkening of the solution to an orange colour, the mixture was stirred at 0°C for 1.5 hours and then allowed to warm to room temperature. Ice water was added and the mixture extracted with ether. The aqueous layer was cooled to 0°C, neutralised carefully with concentrated hydrochloric acid in the presence of chloroform, the aqueous phase separated and further extracted with chloroform until colourless. After drying the chloroform layer and removing the solvent under vacuum, no product was found. The ether layer was dried, filtered and the solvent removed to give a yellow-brown oil which by analytical tlc and nmr spectroscopy proved to be a mixture of starting ketone (79), diisopropylamine and isoamyl nitrite. No trace of the expected oxime (83) was found.

Preparation of humulene-trans-1,2-8,9-diepoxide (57)

To a solution of humulene (1.02g, 5 mmol.) in dichloromethane (100 ml.) was added aqueous sodium bicarbonate solution (0.5M, 100 ml.). The mixture was stirred at room temperature and

to it was added slowly M-chloroperoxybenzoic acid (85% purity, 2.15g., 10 mmol.). After addition was complete, the mixture was stirred at room temperature for 3 hours and the two layers were separated. The organic layer was washed well with 1M aqueous sodium hydroxide solution (50 ml.) followed by water (50 ml.) and then dried. The solution was filtered and the solvent removed under vacuum to give a yellow viscous oil which was dissolved in pet.ether. The white solid diepoxide (57) crystallised out on standing to give a yield of 0.52g (44%). δ = 1.05 (s,3H), 1.17 (s,3H), 1.22 (s,6H), and 5.30 (m,2H).

Hydroboration/acetylation of humulene-trans-1,2-8,9-diepoxide (57)

A 25 ml. 3-necked flask fitted with 2 rubber septums, an air-condenser, a magnetic stirrer and a nitrogen line was flame dried and cooled under a flow of nitrogen. The diepoxide (57), (1g, 4.23 mmols.) was dissolved in dry tetrahydrofuran (7 ml.) and injected into the flask. With magnetic stirring, the borane/dimethylsulphide complex[74] (neat, 420 µl, 4.23 mmols.) was added by syringe. An immediate effervescence occurred which ceased after about 1 minute, then the reaction mixture was stirred for 1 hour. This was followed by adding 10% sodium hydroxide solution (12 ml.), 30% hydrogen peroxide (7 ml.), heating the solution to 40°C and holding for 1 hour. At the end of the holding period, the mixture was extracted with ether, the ether layer washed with brine, dried and filtered. The solvent was removed under vacuum to give a light brown viscous oil which was immediately dissolved in dry pyridine (7 ml.) and then acetic anhydride (2 ml.) was added. The mixture was stirred for 22 hours

at room temperature then worked up in the usual manner to give a reddish brown viscous oil which began to crystallise. 25% Ethylacetate/pet.ether (20 ml.) was added and a white solid precipitated which was filtered and washed with the same solvent. The filtrate was retreated as above until 550 mg. white solid were obtained (44%). Flash column chromatography using Kieselgel 60 (Art. 9385, 230-400 mesh) (Ratio 20:1) and eluting with 10%, 15% ether/pet.ether and ether gave the products (93) and (94) and their mirror images.

(93), δ = 0.97 (s,3H), 1.07 (s,3H), 1.25 (s,3H), 1.29 (s,3H), 1.99 (s,3H), 2.71-3.11 (m,2H), and 4.91-5.20 (m,1H).

(94), δ = 1.02 (s,3H), 1.05 (s,3H), 1.35 (s,3H), 1.46 (s,3H), 2.05 (s,3H), 2.76-2.98 (m,2H), and 4.88-5.2 (m,1H).

Deoxygenation of the humulene-trans-1,2:8,9-diepoxy-4-acetates (93), (94) and their mirror images

Using the diepoxy-4-acetate mixture (1.9g., 6.41 mmols.), tungsten hexachloride (6.39g., 16.11 mmol.), n-butyl lithium (1.6M in hexane, 21.03 ml., 33.65 mmols.) and dry tetrahydrofuran (400 ml.), the same procedure as that described for the deoxygenation of humulene trisepoxide (11) was followed. Purification of the crude product by high pressure column chromatography using Kieselgel HF_{254} Type 60 (Ratio 20:1) and eluting with 2% ether/pet.ether gave humulene-4-acetate (91) (1.20g, 71%), R_f 0.43.

δ = 0.88 (s,3H), 0.96 (s,3H), 1.49 (s,3H), 1.69 (s,3H), 1.96 (s,3H), 4.50 (m,1H), and 4.90 (m,2H).

ms: M^+ (found) = 264.2105; M^+ (calculated for $C_{17}H_{28}O_2$) = 264.2090.

It should be noted that in this particular reaction,

a second compound was isolated from the column, a white solid, yield 468 mg., on eluting the column with 5% ether/pet.ether. Analytical tlc showed only one spot, R_f 0.35. The solid was recrystallised from n-hexane to give white needles, m.pt. 113.5-114°C. The structure is proposed as either (98) or (99).

$\bar{\nu}_{max}$ ($CHCl_3$) = 1725, 1380, 1370, 1105, 1045 and 1015 cm^{-1}.

δ = 0.99 (s,3H), 1.05 (s,3H), 1.21 (s,3H), 1.50 (s,3H), 2.00 (s,3H), and 3.65 (bd,J=10 Hz., 1H).

ms: M^+ (found) = 316.1817; M^+ (calculated for $C_{17}H_{29}ClO_3$) = 316.1806.

Found: C, 64.8; H, 9.5; Cl, 11.3. $C_{17}H_{29}ClO_3$ required C, 64.7; H, 9.2; Cl, 11.2%.

Reduction of humulene-4-acetate (91)[4]

The acetate (91), (85 mg., 0.32 mmols.) was dissolved in anhydrous ether then lithium aluminium hydride (100 mg., 2.63 mmols.) was added, the mixture being stirred magnetically at room temperature for 20 minutes. The flask was then cooled in an ice-bath, cold brine (40 ml.) cautiously added and the mixture extracted with ether. The ether layer was dried over anhydrous sodium sulphate, filtered and the solvent removed under vacuum to give a viscous clear oil (50), in quantitative yield.

$\bar{\nu}_{max}$ = 3345, 1380, 1365, and 1050 cm^{-1}

δ = 0.98 (s,3H), 1.08 (s,3H), 1.46 (bs,3H), 1.61 (bs,3H), 3.40-3.60 (m,1H), and 4.90 (m,2H).

ms: M^+ (found) = 222.1968; M^+ (calculated for $C_{15}H_{26}O$) = 222.1983.

Preparation of humulene-4-tosylate (92)

Humulen-4-ol (50), (40 mg., 0.18 mmol.) was dissolved in dry pyridine (1 ml.), cooled to 0°C in an ice-bath and recrystallised p-toluene sulphonyl chloride (45 mg., 0.25 mmol.) added, the solid dissolving. The solution was placed in the fridge overnight and checked by analytical tlc, some unchanged starting material still being observed. The flask was flushed with a nitrogen blanket and allowed to stand at room temperature for 1.5 days. The reaction was worked up in the usual manner, care being taken to ensure that the washes were kept cold. The ether extract was dried, filtered and the solvent removed under vacuum to give a yellow oil, yield 25 mg. which by nmr proved to be humulene with traces of starting alcohol (50) and another minor product.

Attempted preparation of humulen-5-one ketal (105)

The ketone (79), (50 mg., 0.23 mmols.) was dissolved in dry benzene (6 ml.). Ethylene glycol (3 ml.) was added then p-toluenesulphonic acid (catalytic) and the mixture refluxed for 7 days using a Dean and Stark apparatus, the side-arm being filled with silica gel (self-indicating). The reaction mixture was poured into ether and water, the ether layer washed well with water, with saturated sodium carbonate solution, dried over anhydrous sodium sulphate, filtered and the solvent removed under vacuum. The product, a yellow oil, yield 40 mg., was examined in the crude state by spectral methods.

$\bar{\nu}_{max}$: 1380, 1360, 1110, and 1080 cm^{-1}.

δ = 0.95 (s,6H), 1.59 (bs,3H), and 3.81 (bs,4H).

Hydrolysis of crude product from attempted ketalisation of humulen-5-one (79)

The crude product (40 mg.) was dissolved in ethanol (10 ml.), with magnetic stirring, then concentrated sulphuric acid (0.5 ml.) was added. The mixture was stirred at room temperature for 24 hours then ether and saturated sodium bicarbonate solution were added. The ether layer was washed with water, dried, filtered and the solvent removed under vacuum. The product, a yellow oil, yield 35 mg. was obtained.

$\bar{\nu}_{max}$: 1702, and 1080 cm^{-1}

δ = 1.08 (s,6H), 1.14 (s,3H), and 1.63 (bs,3H).

Oxidation of humulen-4-ol (50)

Pyridinium chlorochromate (150 mg., 0.81 mmols.) was suspended, with magnetic stirring, in dichloromethane (20 ml.) at room temperature. Humulen-4-ol (50), (70 mg., 0.31 mmols.), dissolved in dichloromethane (1 ml.) was added to the suspension and the mixture stirred at room temperature for 1 hour. A five times volume of ether was added and the mixture filtered through a Florisil column, the solid residue in the flask being well washed with ether. Removal of the solvent under vacuum gave a pale yellow oil (80) in quantitative yield which slowly crystallised to give a solid m.pt. 32-33°C.

$\bar{\nu}_{max}$ ($CHCl_3$) = 1702, 1369, and 1350 cm^{-1}

δ = 1.13 (s,6H), 1.42 (bs,3H), 1.65 (bs,3H), 2.41 (bs,2H), 2.85 (bs,2H), and 4.72-5.20 (m,2H).

ms: M^+ (found) = 220.1819; M^+ (calculated for $C_{15}H_{24}O$) = 220.1827

Attempted preparation of humulen-4-one ketal (106)

Humulen-4-one (80), (67 mg., 0.30 mmols.) was dissolved in dry benzene (25 ml.) and a catalytic amount of p-toluenesulphonic acid was added. Ethylene glycol (2 ml.) was then added and the mixture refluxed for 8 hours. The reaction was worked up by adding saturated sodium bicarbonate solution and ether. The ether layer was washed well with water, dried, filtered and the solvent removed to give a yellow oil, yield 70 mg. Column chromatography could not separate the starting ketone (80) from the product.

δ = Appearance of peak at 3.86 (s,4H)

Diminution of peaks at 2.41 and 2.85.

Attempted preparation of humulen-4-one ketal (115)

Humulen-4-one (80), (50 mg., 0.23 mmols.) was dissolved in dry benzene (25 ml.). p-Toluenesulphonic acid (catalytic) and 2,2-dimethyl-propan-1,3-diol (40 mg., 0.38 mmols.) were added and the mixture was refluxed in a Dean and Stark apparatus, the side arm of which was filled with self-indicating silica gel. After 18 hours, saturated sodium bicarbonate solution and ether were added, the ether extract being dried, filtered and the solvent removed to give a yellow oil. Flash column chromatography using Kieselgel 60 (Art. 9385, 230-400 mesh) (Ratio 20:1), with pet.ether and 2% ether/pet.ether as eluant, gave a yellow oil, yield 49 mg. The nmr spectrum showed no olefinic protons but ketal formation was observed.

δ = 2.98-3.63 (AB system,4H).

Alcohol (116)

Alcohol (35) was hydrogenated (10% Pd/C, petroleum ether, RT, atmospheric pressure) for 6 hours to give (116), m.pt. 67°C (from petroleum ether (40-60°C) after a number of recrystallisations). R_f 0.58 on a silver nitrate plate (40% ethyl acetate/petroleum ether).

Found: C, 80.7; H, 11.8. $C_{15}H_{26}O$ requires C, 81.0; H, 11.8%.

$\bar{\nu}_{max}$ (nujol) = 3380, 3040, 1055, 1038, 1020 and 1005 cm^{-1}.

δ = 0.96 (d,3H), 0.99 (bs,9H), and 3.16 (d,J=7Hz, 1H).

Ketone (117)

Alcohol (29), on oxidation with pyridinium chlorochromate in dichloromethane, gave a colourless mobile oil, R_f 0.35.

Found: C, 81.4; H, 10.6. $C_{15}H_{22}O$ requires C, 82.5; H, 10.2%.

$\bar{\nu}_{max}$ = 1695, 1660, 1088, and 925 cm^{-1}

δ = 0.99 (s,3H), 1.01 (s,3H), 1.22 (s,3H), and 1.66 (bs,3H).

m/e = 218 (M^+).

Alcohol (118)

Ketone (117) was reduced with sodium borohydride in methanol at 0°C for 1 hour. The reaction mixture was allowed to warm to room temperature, was poured into aqueous ammonium chloride and extracted into ether to give (118) as a colourless oil eluting in 2-5% ether/petroleum ether (40-60°C) from a short column. R_f 0.35.

Found: C, 82.4; H, 11.5. $C_{15}H_{24}O$ requires C, 81.8; H, 11.0%.

$\bar{\nu}_{max}$ = 3430, 1048, 1008, and 902 cm^{-1}

δ = 0.4-0.7 (m,3H), 0.92 (bs,6H), 1.10 (s,3H), 1.63 (s,3H), and 3.86 (d,J=2Hz,1H).

m/e = 220 (M^+).

Preparation of bis-cyclopropyl-t-butyl-carbinol (121)

A 100 ml. three-necked flask fitted with reflux condenser, dropping funnel and nitrogen inlet and containing magnesium turnings (200 mg., 8.3 mg. atoms) was flame-dried and allowed to cool under a flow of nitrogen. A solution of t-butyl bromide (1g., 7.3 mmol.) in dry tetrahydrofuran (10 ml.) was slowly added via the dropping funnel while the reaction mixture was slowly heated. The reaction began at about 45°C and when all the reagent had been added (∿ 15 mins.), the mixture was heated to reflux temperature and held for 3 hours. After the reflux period, dicyclopropylketone (800 mg., 7.3 mmol.) in dry tetrahydrofuran was added via the dropping funnel and then the reaction mixture refluxed for 18 hours. The resultant dark brown solution was hydrolysed by adding crushed ice, stirring then adding 3-4 ml. of 4M hydrochloric acid, a little ether and extracting. The ether layer was separated, washed with saturated sodium bicarbonate solution, dried, filtered and the ether removed under vacuum to give a yellow-brown liquid, yield 500 mg. Chromatography of the crude product on 10g. of Hi-Florisil-Ag 20% using 10% ethyl acetate/pet.ether as eluant gave a fraction which was further purified using preparative tlc with 5% ethyl acetate/pet.ether as eluant. The edges of the plate were sprayed with ceric ammonium sulphate and a band scraped off which, on elution with chloroform, gave a pale yellow oil, yield 126 mg. (121) showing one spot, R_f 0.38 by analytical tlc. The oil was distilled at 50-55°C/0.3 mm.

$\bar{\nu}_{max}$ = 3600, 3080, and 1030 cm^{-1}

δ = 1.01 (s,9H)

Found: C, 78.1; H, 12.0. $C_{11}H_{20}O$ required C, 78.5; H, 12.0%.

Preparation of Cycloheptanone-2-phenylthioether (126)[68]

Into a dry 2-necked flask was added diisopropylamine (6.06 ml., 53.58 mmols.) in dry tetrahydrofuran (30 ml.). Under a nitrogen flow, the solution was cooled, with magnetic stirring, to -78°C using an acetone/Drikold bath. n-Butyl lithium (1.6M in hexane, 33.48 ml., 0.05 mmols.) was added using a syringe and the mixture stirred for 30 minutes. Cycloheptanone (125), (6.10 ml., 51.88 mmols.) was dissolved in dry tetrahydrofuran and added slowly to the reaction mixture, maintaining the temperature below -70°C. After addition was complete, the mixture was stirred at -78°C for 30 minutes then, using a flexi-needle, was blown under nitrogen pressure into a round bottom flask containing diphenyldisulphide (13.44g., 61.62 mmols.) in dry tetrahydrofuran (50 ml.), stirred magnetically at room temperature. When the mixture was all blown over, the flask contents were stirred for 1 hour, water added and the mixture allowed to stand overnight. Ether extraction, drying of the ether layer, filtration and removal of the ether under vacuum gave a yellow mobile liquid, yield 16.4g. An aliquot (6g.) was purified by flash column chromatography using Kieselgel 60 (Art. 9835, 230-400 mesh) (Ratio 25:1) and ethyl acetate as eluant to give a yellow liquid, yield 3.25g (77%). (It should be noted that the bulk of the product was used crude for the next stage on the assumption that the ratio of thioether: starting ketone = 2:1).

δ = 2.30-2.90 (m,2H), 3.78 (dd,J=4Hz and 2Hz,1H), and 7.30 (m,5H).

ms: M^+ (found) = 220.0910; M^+ (calculated for $C_{13}H_{16}OS$) = 220.0922

Preparation of (127)[80]

Sodium hydride (100%) (39 mg., 1.61 mmols.) was quickly weighed into a dry, 2-necked 50 ml. flask under a nitrogen flow. Cycloheptanone-2-phenyl thioether (126), (100 mg., 0.46 mmol.) was dissolved in dry tetrahydrofuran (5 ml.) and added to the flask using a syringe *via* a Subaseal stopper. Immediately an effervescence was noted and with magnetic stirring the reaction mixture was cautiously heated to 60°C and held for 30 minutes during which time the solution became bright orange in colour. The flask was cooled to 0°C and methyl iodide (58 µl., 0.92 mmols.) was slowly added using a microsyringe after which the solution was stirred at 0°C for 1 hour. Water was added carefully and the crude product extracted with ether, the ether layer being dried, filtered and the solvent removed under vacuum. The crude product, a yellow oil yield 91 mg. was purified using preparative tlc, 6% ethyl acetate/pet.ether being used as eluant to give a pale yellow oil, yield 70 mg. (66%), R_f 0.30 on analytical tlc, visible under U.V. light (254 nm.). It should be noted that larger scale preparations gave considerably lower yields (40-45%).

$\bar{\nu}_{max}$ = 3070, 1695, 1075, and 1060 cm^{-1}

δ = 1.22 (s,3H), 2.50 (m,2H), and 7.30 (s,5H).

ms: M^+ (found) = 234.1088; M^+ (calculated for $C_{14}H_{18}OS$) = 234.1079

Found: C, 72.0; H, 7.9; S, 13.9. $C_{14}H_{18}OS$ requires C, 71.8; H, 7.8; S. 13.7%.

Preparation of 2,2-dimethylcycloheptanone (128)[81]

Liquid ammonia (400 ml.) was distilled into a flask from a cylinder, was dried by adding a piece of sodium and then was distilled into a 3-necked flask fitted with a stirrer bar and a pressure-equilibrating dropping funnel fitted with a calcium chloride guard tube. Strips of lithium metal (1.53g., 216.9 mg. atoms = 10 mol.eq.) were added to the ammonia which immediately turned blue and the mixture was stirred for 15 minutes. The thioether (127), (5g., 21.69 mmols.) was quickly dissolved in ether (50 ml.), previously dried by refluxing over lithium aluminium hydride, and the solution added dropwise over 10 minutes to the reaction mixture via the dropping funnel. After stirring for 15 minutes, methyl iodide (20 ml., 320 mmol. = 15 mol.eq.), previously dried by passing down an alumina column, was added to the reaction mixture dropwise via the dropping funnel. The blue colouration disappeared after about one-third of the methyl iodide had been added. The reaction mixture was stirred for 20 minutes after addition of the methyl iodide then ammonium chloride was added until any unreacted lithium was destroyed. The flask was allowed to stand at room temperature until all ammonia had evaporated, water was added and the ether layer separated after extraction. After drying and filtering, the ether was removed under vacuum to give a brown oil, yield 3.7g. The crude product was purified by flash column chromatography using Kieselgel 60 (Art. 9835, 230-400 mesh), gradient elution being employed - pet.ether, 2% ethyl acetate, 5% ethyl acetate/pet.ether. A pale yellow oil, yield 1.47g. (49%),

R_f 0.32 by analytical tlc, was obtained.

$\bar{\nu}_{max}$ = 1702, 1382, 1372, 1122, and 1060 cm^{-1}

δ = 1.08 (s,6H), 1.58 (bs,6H), and 2.48 (m,2H).

ms: M^+ (found) = 140.1195; M^+ (calculated for $C_9H_{16}O$) = 140.1201

Preparation of (129)[82]

To a solution of the ketone (128), (1g., 7.14 mmol.) in dry tetrahydrofuran (5 ml.) at 0°C was cautiously added bromine (0.36 ml., 7.14 mmol.) in dichloromethane (5 ml.). After the addition, the solution was stirred for 5 minutes at 0°C then heated to reflux and held for 1.5 hours, after which the solution was diluted with 10% aqueous sodium bicarbonate solution (10 ml.) and then poured into cold water (10 ml.). The solution was extracted with dichloromethane and the extract washed with water and dried. Concentration gave a brown oil yield 1.3g. which was dissolved in dimethylformamide (10 ml.), added to a stirred suspension of magnesium oxide (0.5g., 12.50 mmols.) in dimethylformamide (10 ml.) at 140°C. and held at that temperature for 4 hours. The mixture was cooled in an ice-bath as dilute hydrochloric acid (200 ml.) was added. After all the magnesium oxide had dissolved, the mixture was diluted with ice-water (200 ml.), extracted several times with ether, the ether extracts washed with aqueous sodium chloride solution, saturated sodium bicarbonate solution and brine, dried, filtered and the solvent removed under vacuum. A brown oil, yield 800 mg., was obtained which was purified by flash column chromatography using Kieselgel 60 (Art. 9385, 240-400 mesh) and eluting with 0.5% ethyl acetate/pet.ether. A pale yellow oil, yield 220 mg.

(22%) was obtained, R_f 0.34 by analytical tlc.

$\bar{\nu}_{max}$ = 1658 cm^{-1}

δ = 1.05 (s,6H), and 5.91 (m,2H).

ms: M^+ (found) = 138.1033; M^+ (calculated for $C_9H_{14}O$) = 138.1044

Cyclopropanation of (129)[83]

Sodium hydride (50% suspension in oil, 100mg., 2.25 mmols.) was quickly weighed into a 3-necked flask equipped with a magnetic stirrer and under a gentle flow of nitrogen. Pet.ether (40-60°) was added sufficient to cover the sodium hydride and the contents of the flask were stirred for a short time. The excess pet.ether was then carefully removed by Pasteur pipette and the process repeated another twice. Finally the pet.ether was blown off by increasing the nitrogen flow. Dimethylsulphoxonium iodide (270 mg., 2.25 mmols.) was added as a fine powder, then dimethylsulphoxide (3 ml.), distilled from calcium hydride, was slowly added using a syringe. Instantaneous effervescence was noted which continued for 15 minutes, leaving a cloudy suspension. The enone (129), (103 mg., 0.75 mmols.) was dissolved in dimethylsulphoxide (1 ml.) and added to the reaction mixture using a syringe. The mixture turned very dark and on completion of addition was stirred for 2 hours at room temperature then 1 hour at 50°C. After the holding period, the mixture was poured into cold water (25 ml.) and extracted with ether, the ether extract being washed three times with water, dried, filtered and the ether removed under vacuum. A yellow oil, yield 45 mg. (40%) was obtained, but attempts to purify this were completely unsuccessful. The yield is calculated on a crude basis. Crude nmr infers structure (130).

δ = 0.3-0.9 (m,4H) and 1.04 (s,6H).

MICROBIAL CONVERSION PROCEDURE

1. Shake flasks

(a) 100 ml. flask with 19 ml. medium.

(b) 500 ml. flask with 200 ml. medium. These were incubated at 25°C on a rotary shaker for 3 days after inoculation.

2. Fermenter

This consisted of a 10 litre glass vessel with a paddle stirrer (712 rpm) and an aeration rate of 2.5 litres per minute.

5 litres of medium were added and incubated at 25°C for 3 days after inoculation.

3. Inoculation

A suspension of fungal spores from each culture was prepared using sterile water and 0.1 ml. of the relevant culture suspension added to each shake flask.

4. Substrate

The standard dosage of substrate for this type of experiment is 200 µg. substrate per 1 ml. medium and the following stock solutions of humulene in acetone were used:-

(a) 400 mg. humulene → 100 ml. with acetone such that 1 ml. solution ≡ 4 mg. humulene. This was used for the 100 ml. shake flasks, bringing the total volume up to 20 ml.

(b) 4g. humulene → 100 ml. with acetone such that 1 ml. solution ≡ 40 mg. humulene. This was used for the 500 ml. shake flasks bringing the total volume up to 201 ml.

(c) 1g. humulene → 25 ml. with acetone. This was used for the 5 litre batches.

In the case of the humulene-4,5-epoxide (8) detection experiment, this was carried out using 100 ml. shake flasks and working from a stock solution made up as follows: 8.6 mg. 4,5-epoxide (8) in 5 ml. acetone. The shake flasks were made up as shown in Table 6.

TABLE 6

	20% conversion simulation	10% conversion simulation	5% conversion simulation
Vol. of medium (ml)	19	19	19
Vol. of stock 4,5-epoxide soln. (ml)	0.5	0.25	0.125
Vol. of stock humulene soln. (4 mg./ml.) (ml)	0.5	0.5	0.5
Vol. of acetone (ml)	-	0.25	0.375

5. Media

The two media used throughout were glucose-tartrate based NPT1 and NPT2, buffered to pH 5.5 for fungi. Table 7 shows the composition of each medium.

TABLE 7

	NTP1	NTP2
Cerelose %	3.0	1.0
Ammonium tartrate %	0.75	0.2
Potassium di-hydrogen phosphate %	0.2	0.1
Magnesium sulphate (7 H_2O) %	0.05	0:05
Minor elements	0.1	0.1
Yeast extract	0.1	0.1

6. Extraction

In the case of the 100 ml. shake flasks, 10 ml. of ethyl acetate was added when the incubation period was complete. The flask was then returned to the shaker for a further 1 hour, removed, allowed to settle, 5 ml. of the ethyl acetate layer drawn off by pipette, vacuumed down on the rotary evaporator, the residue redissolved in 0.1 ml. ethyl acetate and each extract run on analytical tlc.

In the case of the 500 ml. shake flasks, 50 ml. of ethyl acetate was added, the flask shaken for 1 hour, the ethyl acetate/culture mixture centrifuged, the organic layer decanted into tubes, 20 ml. of the extract sampled, concentrated, taken up in 0.1 ml. ethyl acetate and run on analytical tlc.

Many cases arose where emulsions were formed and this was combatted by filtering the culture + ethyl acetate through Hiflosil and then pipetting a sample from the organic layer.

In the case of the 5 litre fermentation, 1 litre of ethyl acetate was added, stirred for 10 minutes, and then the organic layer separated using a combination of Hiflosil filtration and separating funnel. The extract was dried, filtered, and the solvent removed under vacuum. The extract was then dissolved in ether, filtered through Celite to remove some of the solid rubbish and the solvent removed under vacuum to give approximately 1 gram of extract.

Experimental details

Humulene conversion

The cultures 1346, 1868, 1870, 2548 and 5065, as seen in Table 5, were run during the first week in 100 ml. flasks.

After the addition of the substrate, the flasks were extracted after 1, 2, 3, and 4 days by the procedure previously described. Control flasks containing (a) medium and culture and (b) medium, culture and acetone, were also run such that the total volume of the solution was always 20 ml. In subsequent weeks, after it had been shown that acetone did not have a toxic effect upon the cultures, (b) was omitted and the flasks were only extracted after 2 and 4 days respectively.

In the case of the 500 ml. shake flask fermentations, controls (a) and (b) were both used, extraction being carried out on days 2 and 4.

The 5 litre fermentations were run for 6 days.

Humulene-4,5-epoxide (8) detection

The experiment was carried out in 50 ml. shake flasks, the two media being used and the flasks extracted after 30 minutes, 1 hour and 2 hours respectively on the shaker, comparison being made with culture controls run for a similar period.

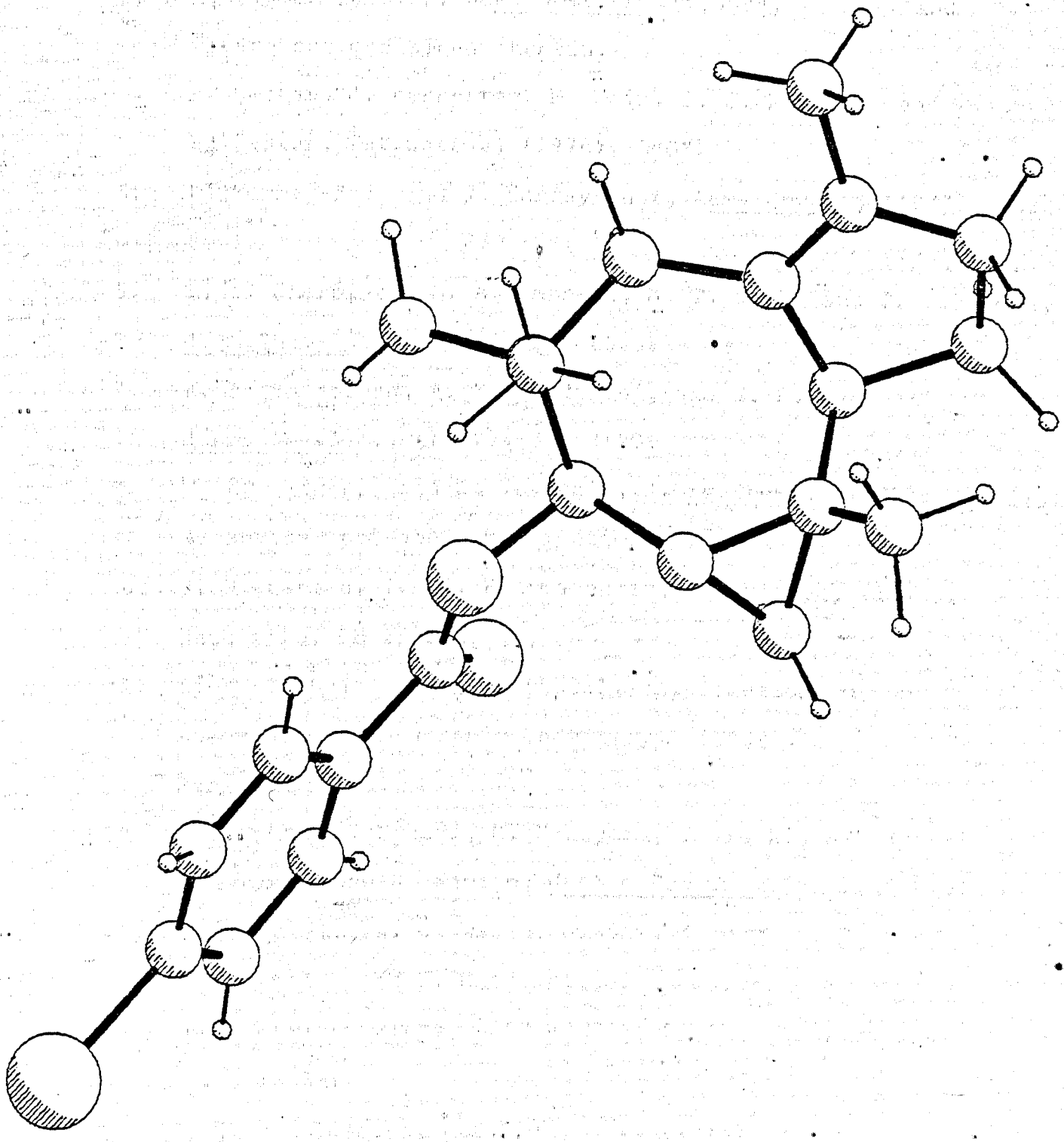
Glc of 5 litre fermentation extract

A sample of the extract was compared with an authentic sample of humulene-1,2-epoxide (10) using a Pye Series 104 Gas Chromatograph fitted with a 5% Carbowax 20M on Gas Chrom Q column, carrier gas flow (N_2) 60 ml./min, temperature 180°C. A retention time of 4.4 minutes was observed for both samples.

APPENDIX

p-Bromobenzoate of alcohol (29)

JR04



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