TRANSFORMATION OF TRITERPENES

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By
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A C K N O W L E D G E M E N T S

The present thesis embodies the results of research carried out by the author at the Department of Chemistry, University of North Bengal, Dist. Darjeeling.

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S U M M A R Y

The work embodied in the present thesis has been divided into three parts:

A. The first part (Part I) consists of the autoxidation studies on β-amyrone and investigations on the stereochemistry of 2-acetoxy-3-keto-β-amyrin and 3-acetoxy-2-keto-β-amyrin.

B. The second part (Part II) describes the synthesis of isomeric 2,3-diols of isohopane (moretane) (Section B) and isomeric 2, 3-diols of methyl olean-12-en-28-oate. (Section C)

C. The last part (Part III) deals with allylic oxidation and bromination studies with NBS on taraxeryl acetate.

A. Part I:

Chapter II deals with the studies on the stereochemistry of the 2-acetoxy group in 2-acetoxy-3keto-β-amyrin D and 3-acetoxy group in 3-acetoxy-2-keto-β-amyrin G from NMR spectra, O.R.D. spectra and chemical evidences.

β-amyrone A on autoxidation gave the diosphenol B which on acetylation followed by hydrogenation gave 2-acetoxy-β-amyrone D, m.p. 158-60°. NMR spectrum of the compound indicated that it may equally explain both for the 2α-equatorial acetoxy group with the chair conformation of ring A and 2β-acetoxy group with the boat conformation of ring A. The ORD spectra of α-acetoxy ketones have been studied recently by Bull and Enslin and also

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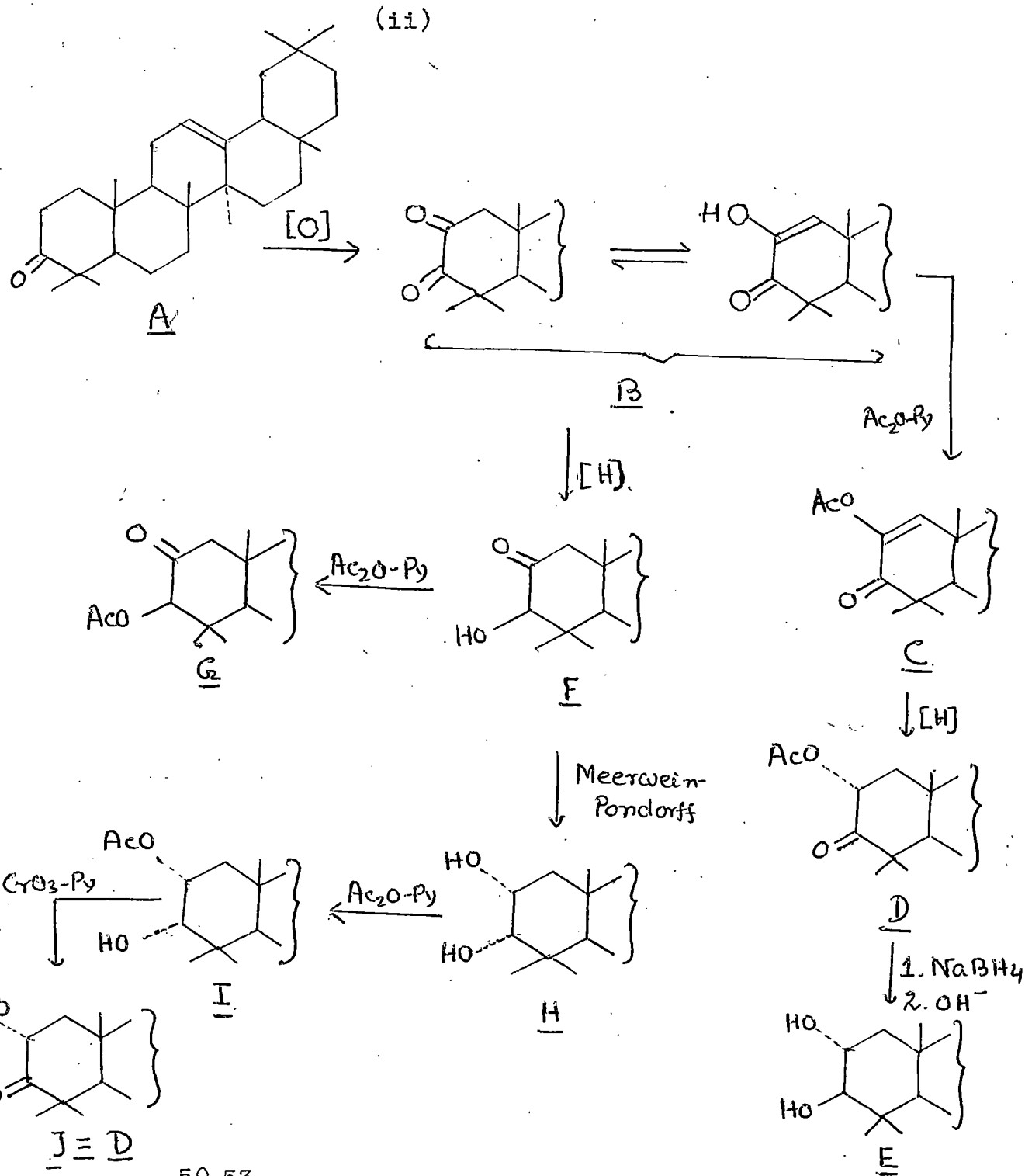


by Klyne et al[50,53]. From their studies it appears that in many cases (though not in all) the effect of an acetoxy to the carbonyl chromophore is anti-octant. The ORD studies revealed that in β-amyrone

(parent ketone) A, the conformation of ring A is a flattened chair (to relieve the diaxial interaction between the 10β-methyl and 4-β methyl groups) and this leads to a positive Cotton effect (Fig. 17). The same conformation is possible for the 2α-acetoxy derivative D but as a consequence of the flattening of ring A, the 2α(equatorial) acetoxy group does not lie in the nodal plane of the carbonyl group but protrudes into the back upper right octant. An alkyl group in that situation would make a negative contribution to the Cotton effect but the acetoxy group shows an '<u>anti-octant</u>' effect and makes a positive contribution. Therefore, as the ORD studies indicate (Fig. 18), if the compound D has the 2α-acetoxy configuration, we would expect it to have a more positive Cotton effect, than the parent ketone. The alternative 2β-acetoxy configuration with the boat conformation of ring A would lead to a small negative Cotton effect. In the ORD curve of the compound D the amplitude is greater than the amplitude in the corresponding parent ketone A. Thus NMR and ORD spectral evidences lead us to conclude that the 2-acetoxy group in D has the α-equatorial configuration with ring A in the chair conformation.

In order to afford further chemical evidences we have prepared Δ^{12}-oleanene-2α, 3β-diol, E m.p. 202-4°, $(\alpha)_D$ 60° from D by $NaBH_4$ reduction followed by hydrolysis. The latter has been found to be identical with an authentic sample of the 2α, 3β-diol[65], indicating α-equatorial orientation of the 2-acetoxy

group in the original compound D .

Further we have also prepared 2α, 3α-diol H m.p. 278-80°, $(\alpha)_D$ 71.28° from 2-keto β-amyrin F. The α, α-configuration is based on NMR spectral data. The monoacetate I, obtained by partial acetylation of H, on CrO_3-pyridine oxidation gave a crystalline solid J, m.p. 158-60°, $(\alpha)_D$ 108.5°, identical with 2α-acetoxy-β-amyrone D obtained by hydrogenation of the diosphenol acetate C. This experiment provided a further support for the assignment of 2α-acetoxy equatorial configuration to the 2-acetoxy group in D.

ORD measurement of 2-keto-3-acetoxy β-amyrone G was also carried out. The compound G with chair conformation of ring A and a 3β-equatorial acetoxy group would be expected to exhibit a positive Cotton effect. This is in accordance with the ORD experimental results. Thus the assignment of stereochemistry as shown in G is consistent with the ORD and NMR measurements.

Above work was presented in the Convention of Chemists held in Madras, India 1970.

B. Part II:

Chapter II describes the synthesis of all the isomeric 2,3-diols of isohopane (moretane) (Section B) and three out of the four possible isomeric 2,3-diols of methyl olean-12-en-28-oate (Section C).

Section B:

Isohopanone (moretanone) on autoxidation gave the diosphenol, m.p. 190-92°, $(\alpha)_D$ 40°. Acetylation of the diosphenol followed by hydrogenation gave the corresponding 2α-acetoxy isohopanone, m.p. 179-81°, $(\alpha)_D$ 86.31°, γ_{max} 276 mμ (ε, 82). Sodium borohydride reduction of the latter at pH 8 gave the 2α-acetoxy-3β-hydroxy isohopane, m.p. 199-200°. The latter on acetylation gave the 2α, 3β-diacetate, m.p. 228-30° $(\alpha)_D$ 50.60°, which on hydrolysis furnished 2α, 3β-dihydroxy isohopane, m.p. 240-2°, $(\alpha)_D$ 82.86°.

Meerwein-Pondorff reduction of 2-keto moretanol, m.p. 181-3°, $(\alpha)_D$ 29.41° (obtained by 1,4 addition of hydrogen during palladium-charcoal hydrogenation of the above diosphenol) furnished a mixture of diols consisting of 5% of the 2α, 3β diol described above and 92% of a diol, m.p. 250-51°, $(\alpha)_D$ 9.37°. The new diol has been assigned 2α, 3α-configuration from its NMR spectrum. It was also found to be identical with an authentic sample of 2α, 3α-diol prepared by osmylation of Δ^2-moretane. The product in the osmylation reaction also gave 2β, 3β-diol as a minor product (8%).

Sodium borohydride reduction of the diosphenol gave 2β, 3β-diol, m.p. 262-4°, $(\alpha)_D$ 23.68°, which on acetylation afforded the 2β, 3β-diacetate, m.p. 214-5°, $(\alpha)_D$ 31.25°.

Performic acid oxidation of Δ^2-moretane by the method reported in literature[1,2,3] afforded the 2β, 3α-diol, m.p. 221-4°, $(\alpha)_D$ 21.18°, acetate m.p. 145-7°.

The structures of the diols were established on the basis of NMR spectra. Acetonide derivatives of two out of the four isomeric diols have been prepared. All the diols described above are new and have not hitherto been reported in the literature.

Section C:

Methyl oleanonate on autoxidation furnished the diosphenol (2,3-dioxo-olean-12-en-28-oate), m.p. 130-35°, $(\alpha)_D$ 104.4°. Hydrogenation of the latter gave methyl-3β-hydroxy-2-keto-olean-12-en-28-oate, m.p. 129-31°, $(\alpha)_D$ 109.09° which on Meerwein-Pondorff reduction gave the methyl 2α, 3α-dihydroxy olean-12-en-28-oate, m.p. 286-7°, $(\alpha)_D$ 71.11°, identical with an authentic sample (supplied by Dr. H.T.Cheung). The diacetate, m.p. 226-8°, $(\alpha)_D$ 95.20° and the acetonide derivative, m.p. 235-9° have also been prepared.

The diosphenol on sodium borohydride reduction gave methyl 2β, 3β-dihydroxy-olean-12-en-28-oate, m.p. 269-72°, $(\alpha)_D$ 88.88°, which on acetylation furnished the diacetate m.p. 220-22°, $(\alpha)_D$ 86.20°. It afforded an acetonide derivative sintering at 75-80°.

Methyl-3-keto-2α-acetoxy olean-12-en-28-oate (obtained by 1,2-addition of hydrogen during palladium-charcoal hydrogenation of diosphenol acetate) on $NaBH_4$ reduction at pH 8 furnished methyl 2α-acetoxy-3β-hydroxy-olean-12-en-28-oate m.p. 199-204°, $(\alpha)_D$ 28.9°. The latter on hydrolysis afforded the 2α, 3β-diol (methyl crategolate) m.p. 220-22°, $(\alpha)_D$ 36°, identical with an authentic sample of methyl crategolate provided by Prof. P.Sengupta.

All the assignments are based on spectral evidences (IR, NMR, UV).

The above work described in Part II was presented in the Convention of Chemists held in Bombay, India.(1971.)

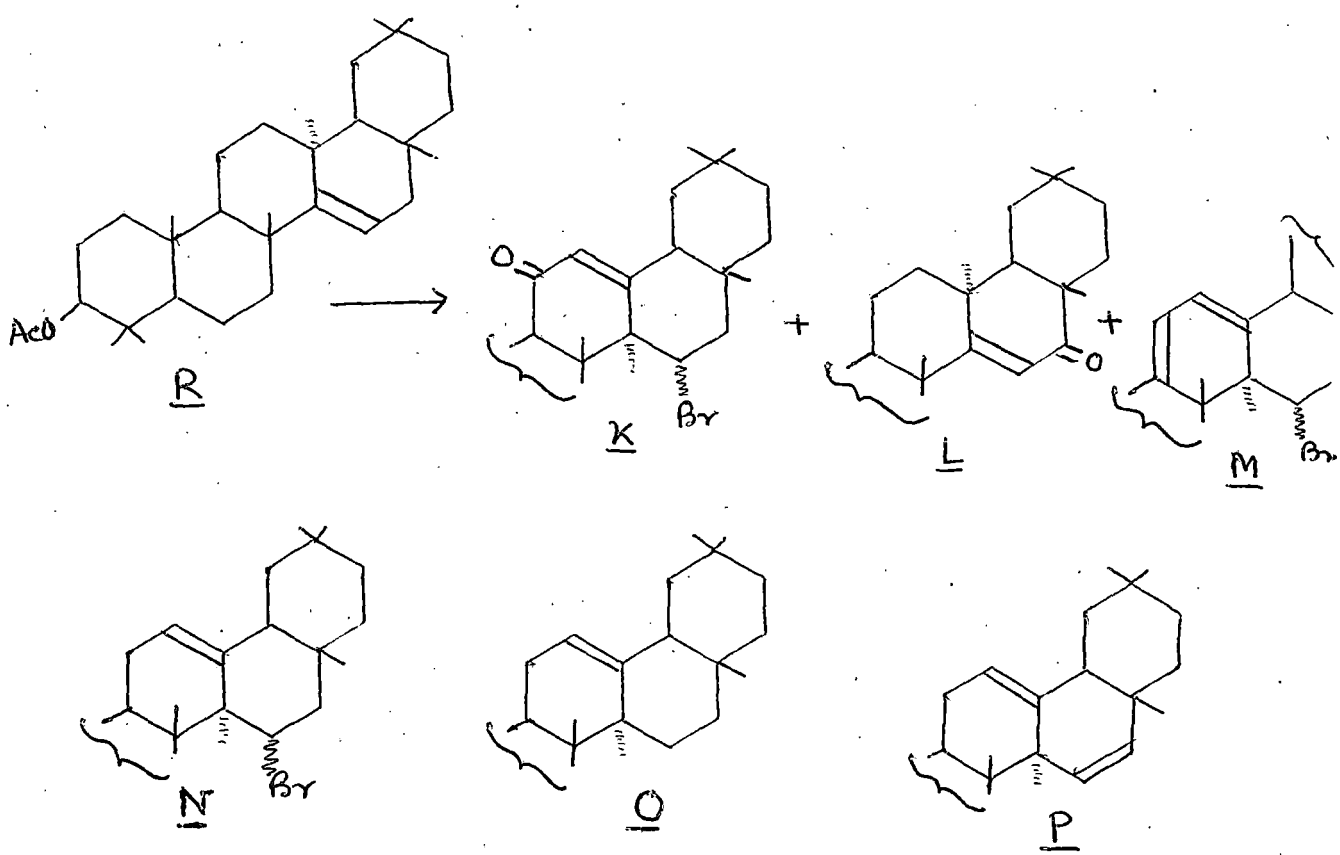
C. Part III

Finucane and Thomson (Chem.Comm. 20, 1220, 1969; J.Chem.Soc. 1856, 1972) recently described a method for allylic oxidation of taraxeryl acetate, β-amyrin acetate etc. using NBS-$CaCO_3$ in aq. dioxan in presence of visilbe light and claimed the isolation of the corresponding $\alpha\beta$-unsaturated ketones in high yield. With a view to preparing 16-OH β-amyrin derivatives oxidation of taraxeryl acetate by their method was taken up. The results were widely different from those recorded by Finucane and Thomson. The reaction product yielded three products after chromatography and crystallisation.

The first compound K, m.p. 238-40°, $C_{32}H_{49}O_3Br$, $(\alpha)_D$ 88.07°,

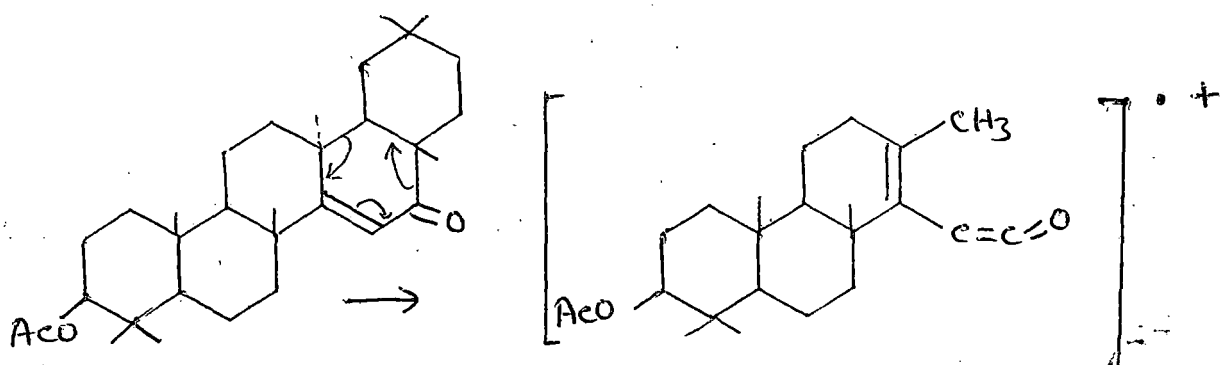
λ_{max} 249.5 mμ (ε, 11,000) was found to be identical with 15-bromo-β-amyrenonyl acetate, established by UV, IR, NMR and its partial synthesis from taraxeryl acetate R. Taraxeryl acetate R on treatment with NBS-DMSO in chloroform gave 15-bromo-β-amyrin acetate N, m.p. 180-2°, $(\alpha)_D$ 47.37°. The latter on treatment with Zn-HOAc gave β-amyrin acetate O indicating that it was a β-amyrin derivative. IR, UV, NMR and mass fragmentation established its structure as depicted in N. Oxidation of 15-bromo-β-amyrin acetate N with CrO_3-HOAc gave 15-bromo-β-amyrenonyl acetate identical with K (m.m.p. and I.R. comparison).

Solvolysis of 15-bromo-β-amyrin acetate N with K-acetate in HOAc gave olean-12, 15-dien-3β-yl-acetate P, m.p. 199-200°. The latter was also obtained when N was treated with dimethyl aniline (IR and m.m.p.).



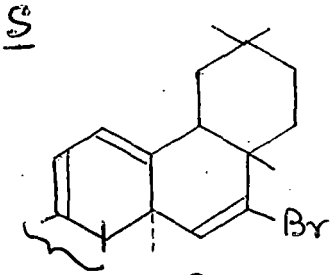
Probable mechanisms for the formation of 15-bromo-β-amyrenonyl acetate K and 15-bromo-β-amyrin acetate N, have been discussed.

The second compound L, $C_{32}H_{50}O_3$, m.p. 280-82°, $(\alpha)_D$ -38.71°, UV λ_{max} 245 mμ (ε, 10,500) was assigned as 16-oxo taraxeryl acetate from its UV, IR, NMR and mass spectral studies. It showed a mass peak at M^+ 482. Moreover, an abundant base peak at 358 was observed. The appearance of this peak may be explained by assuming that it arises from a 16-oxo taraxeryl acetate by the following genesis S. Our compound was widely different from the sample of 16-oxo taraxeryl acetate, m.p. 251-52°d procured from Dr. Thomson. Their sample was found to give positive bromine test. All physical and chemical evidences prove that our compound m.p. 280-82° is the correct 16-oxo taraxeryl acetate. Attempts to reduce 16-oxo



S

$C_{23}H_{34}O_3$, m/e, 358



Q

taraxeryl acetate to give 16-OH taraxeryl acetate proved futile.

The third compound, M, $C_{32}H_{49}O_2Br$, m.p. 176-8°, $(\alpha)_D$ 249.12°, λ_{max} 276 mμ (ε, 6000) has been assigned as 15-bromo-9(11), 12-olean-diene, on the basis of UV, NMR and mass spectral studies. A probable mechanism for formation of M has also been suggested.

With the expectation of obtaining a 16-bromo taraxeryl acetate we extended our studies on allylic bromination of taraxeryl acetate using NBS in dry CCl_4 in presence of light and benzoyl peroxide as the initiator. The results obtained during these studies were interesting and is summarized below.

When taraxeryl acetate R was refluxed with 2 mole equivalents of NBS in dry CCl_4 using visible light, furnished 16-bromo-olean-9(11), 12,15-trien-3β-yl-acetate Q, $C_{32}H_{47}O_2Br$, m.p. 240°, $(\alpha)_D$ 267.53°, λ_{max} 279 mμ (ε, 6000). The bromine atom in ring D was resistant to reactions (1) Zn-HOAc (2) H_2/Pd-C (3) H_2-PtO_2 (4) Li_2CO_3-LiBr (5) anhydrous KOAc-HOAc (6) $C_6H_5N(CH_3)_2$, indicating that it contained a vinyl bromine atom as shown in structure Q. The mechanism for its formation has also been discussed.

However, with 1 mole equivalent of NBS the product was a bromine free compound, m.p. 199-200°, olean-12,15-dien 3β-yl acetate P, identical with product obtained by solvolysis of 15ξ-

bromo β-amyrin acetate (m.m.p. and IR comparison). The mechanism for the formation of P has been discussed.

This work was presented in the Convention of Chemists, held in Calcutta in 1973.

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PART-I

AUTOXIDATION STUDIES ON β -AMYRONE: INVESTIGATIONS ON THE STEROCHEMISTRY OF 2-ACETOXY-3-KETO- β -AMYRIN AND 3-ACETOXY-2-KETO- β -AMYRIN.

PART-I

CHAPTER-I

A review on optical rotatory dispersion and its application in stereochemical assignments in Organic Chemistry.

Section A: Optical Rotatory Dispersion:

A wave of plane -polarised light may be considered to be made up of two types of "circularly polarised light": one right circularly polarised wave and one left circularly polarized wave[1]. A "circularly polarized wave" is one whose plane of polarisation rotates continuously and in the same sense around the axis of propagation of the wave. Thus the electric field of a right circularly polarised wave may be described as a right-handed screw or helix twisting round the direction of propagation (Fig. 1), whereas a left circularly polarised wave describes a left-handed screw. Fig. 2 shows how the electric vector of a right circularly polarised wave (E_R) and that of a left circularly polarised wave (E_L) combine to give the vector of a plane-polarised wave (E), which starting out with a maximum value, decreases to zero and then to a minimum and grows again to zero and back to the maximum. Circularly polarised light may actually be produced by passing plane polarised light through a specially cut glass prism known as "Fresnel's rhomb".

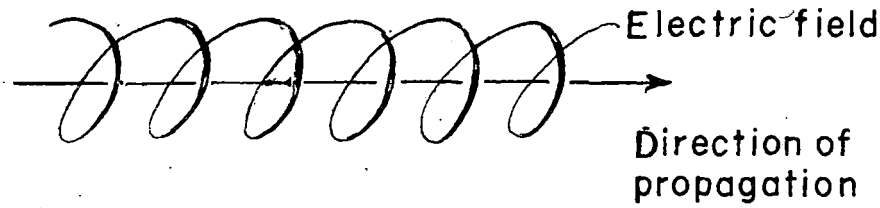


Fig-1. Right circularly polarized light wave

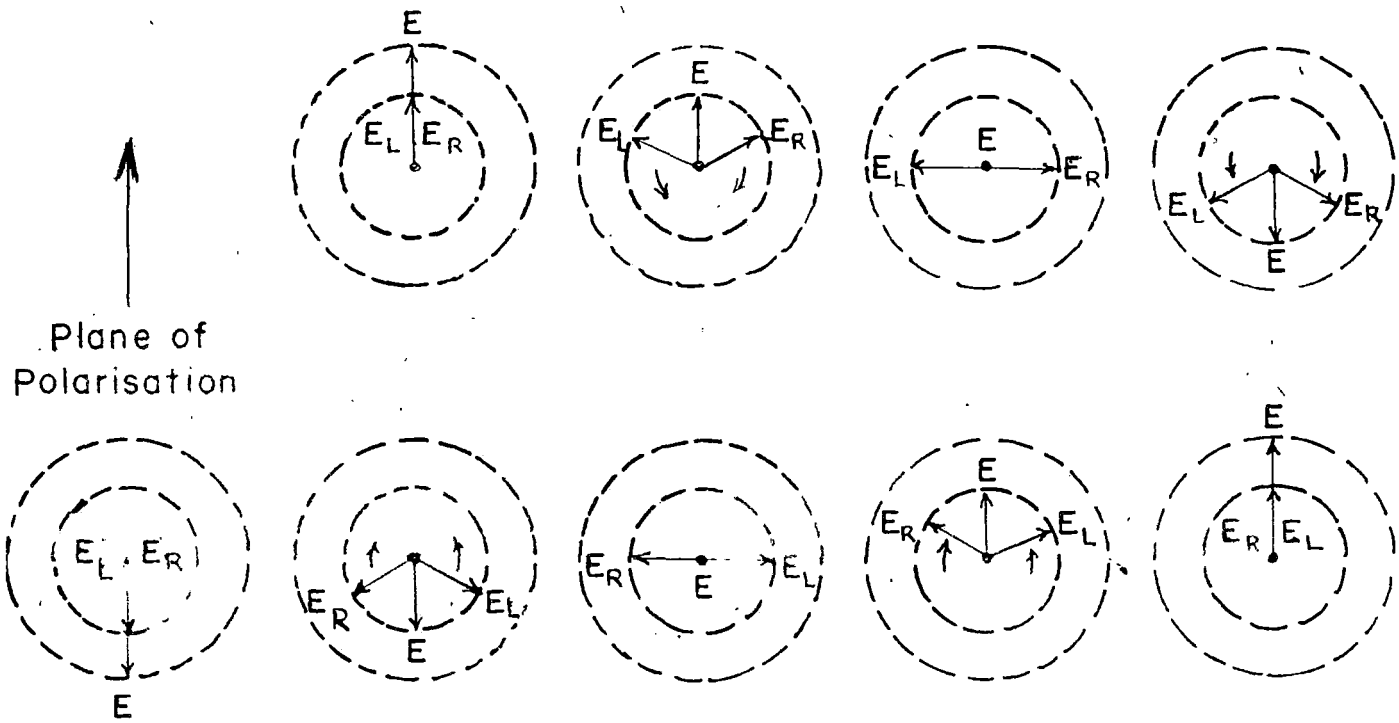


Fig-2.

The important feature of an optically active substance is that it is "circularly birefringent" i.e. that it has unequal refractive indices for right and left circularly polarised light. Since the velocity of light in a medium is given by $v = c/n$, where c is the velocity of light in vacuo and n is the refractive index of the medium, the result of circular birefringence is an unequal velocity of propagation of the left and right circularly polarised rays. If the right circularly polarised ray E_R travels faster than the left circularly polarised one E_L, the result will be as shown in Fig. 3. The resultant wave will still pulsate in a plane i.e. be plane-polarised but the plane of polarisation will no longer be the x-plane but will make an angle α' with the x-plane; in other words the circularly birefringent medium has rotated the plane of polarisation by an angle α'. If the right circularly polarised wave travels faster, α' is positive and the medium is dextrorotatory, whereas if the left circularly polarised wave is faster, α' is negative and the medium is levorotatory.

The variation of optical activity with the wave length gives an optical rotatory dispersion curve. For a compound containing no chromophore (a substance which does not absorb light in the region of wavelength in which it is being examined) the optical activity progressively decreases in magnitude as the wave length increases. A plain positive or plain negative dispersion curve is obtained, depending upon whether it rises or falls with decreasing wave length. For a compound presenting one or several optically

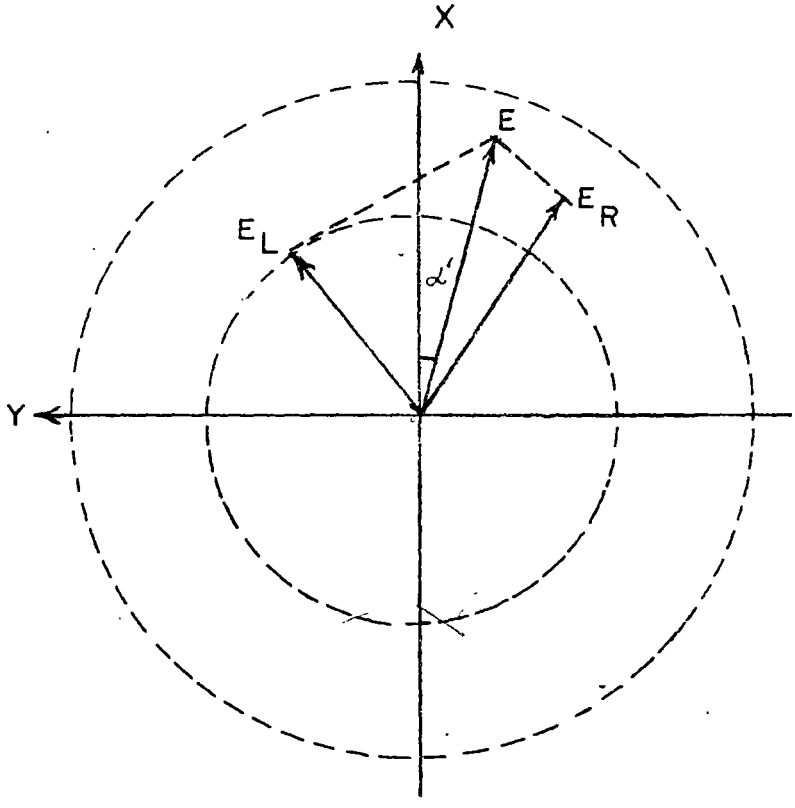


Fig – 3

active absorption bands within the spectral range under experimental observation, the dispersion curve is anomalous and shows one or several "extrema" (peaks or troughs) in the spectral region in which the chromophore absorbs.

Section B: Circular Dichroism:

After passing through an optically active medium, both constituents (E_L and E_R) of a circularly polarised ray not only show circular birefringence, but they are also differentially absorbed. As a result, the two vectors E_L and E_R (Fig 2) are unequal in length and their resultant, instead of decreasing or increasing in a given plane (the plane of polarisation) actually sweeps out in an elongated ellipse. The plane polarised light passing through a dissymmetric medium therefore becomes elliptically polarised. This phenophenon is known as "Circular Dichroism"[2].

Section C : Cotton Effect:

The combination of unequal absorption and unequal velocity of transmission of left and right circularly polarised light in the region in which optically active absorption bands are observed is a phenomenon called the "Cotton Effect". Cotton[3,4] observed that an optically active compound showed in this spectral region an abnormal behaviour of its rotatory power. Thus the basic information which can be deduced from rotatory dispersion and circular

dichroism curves is obtained most easily in the immediate vicinity of the spectral region of maximal absorption.

According to Moscowitz[1,5-8] optically active chromophores can be classified into two extreme types: (a) the inherently dissymmetric chromophore and (b) the inherently symmetric, but assymmetrically perturbed, chromophore.

The optical activity of compounds belonging to the first class is inherent in the intrinsic geometry of the chromophore, as for example hexahelicene[5] and twisted biphenyls[8,9]. In these compounds the molecular amplitude of the O.R.D. curve and the maximum value of the c.d curve are generally quite high in comparison with the corresponding quantities observed for the second type of chromophore.

A typical example of the second class is the carbonyl function. For an isolated carbonyl there are two orthogonal reflection planes of symmetry, and to a first approximation the chromophore should be optically inactive, as indeed it is in formaldehyde. Only when the chromophore is placed in some dissymmetric molecular environment e.g. in a terpene or steroid, do its transitions becomes optically active. Since this optical activity is induced in the chromophore by its environment, rather than being inherent, the magnitude of the associated Cotton effect is often considerably smaller than in the first type of chromophore.

The optical activity associated with the type (b) chromophore is typified by the presence of an asymmetric carbon atom.

In such instances, the inherently symmetric chromophore acts like a molecular probe with which to explore the extra chromophoric geometry, since the magnitude of the induced optical activity depends on the geometry of the extra chromophoric portion of the molecule relative to the symmetry elements of the chromophore. In conclusion, the Cotton effect associated with an optically active absorption band of a given compound manifests itself by a c.d. curve and an anomalous o.r.d. curve.

The rotatory dispersion curves can be divided into three different groups: plain curves, single Cotton effect curves, and multiple Cotton effect curves. Plain dispersion curves are called positive or negative according to their tendency toward more positive or more negative values with decreasing wavelength. When the peak occurs at higher wavelength than the trough, the curve is a positive Cotton effect curve. Conversely when the trough occurs first, the curve is a negative Cotton effect curve. The vertical distance between the peak and trough is called the molecular amplitude[10] a

$$a = \frac{[\phi]_1 - [\phi]_2}{100}$$

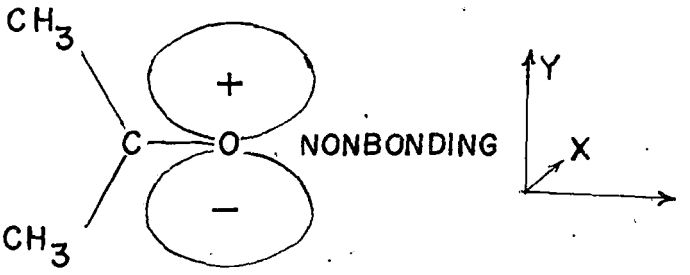
, where $[\phi]_1$ is the molecular rotation at the extremum (peak or trough) of longer wavelength and $[\phi]_2$ molecular rotation at the extremum of shorter wavelength. The third type of dispersion curve is more complicated in the region of ultraviolet absorption in that it possesses two or more peaks with a corresponding number of troughs and therefore is called a multiple

Cotton effect curve. Such features are normally observed with $\alpha\beta$ -unsaturated ketone[11] and in these cases it is also necessary to distinguish between broad peaks and troughs, as well as between shoulders and inflections. Fig. 4

Section D : The Octant Rule:

The Octant Rule[12] relates the Cotton effects due to the $n \longrightarrow \pi^*$ transitions of saturated carbonyl compounds to the stereochemistry of the surroundings of the chromophore. The 290 mμ transition of the carbonyl group (in acetone) involves[13,14] roughly speaking the promotion of an electron from a non-bonding 2 p orbital situated on the oxygen atom to an anti-bonding π orbital. Concerned with both the carbon and oxygen atoms of the carbonyl chromophore (Fig. 5). Whatever the perturbations the rest of the molecular framework may induce on the chromophoric electrons, they are not significant for the dipole strength of the transitions. However, while the rotational strength of a n-π^* transition must be zero in a symmetrical molecule like acetone, this strength has some non-zero value when the carbonyl is asymetrically surrounded, as in a steroid or a terpene. Hence the rotational strength of a carbonyl function is quite sensitive to molecular environment and will reflect quantitatively the asymmetry around the chromophore[15,16].

As a result of the above concept, the carbonyl group or any symmetric chromophore whose associated optically active transitions are readily amenable to investigation, becomes an ideal


CH3
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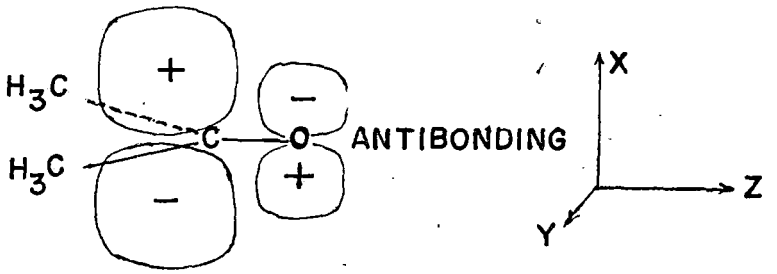
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Z
Y

Fig. 5

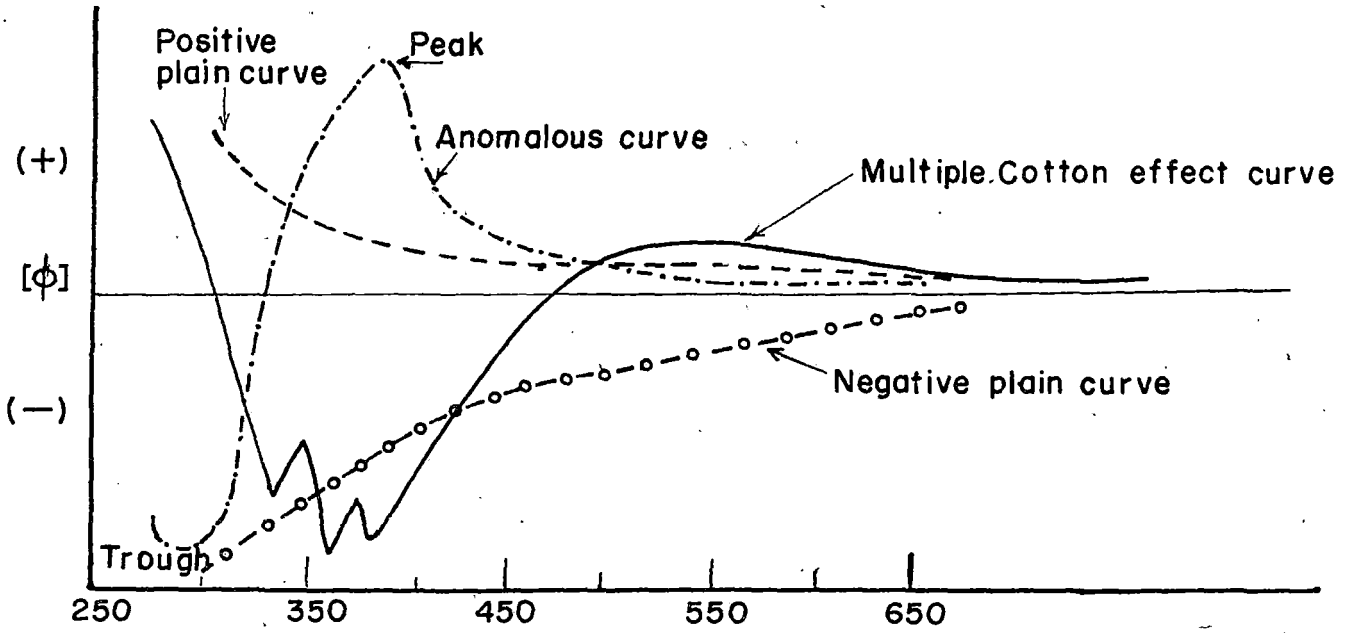

Positive plain curve
Peak
Anomalous curve
Multiple Cotton effect curve
(+)
[ϕ]
(−)
Negative plain curve
Trough
250
350
450
550
650

Fig - 4

probe with which to search out structural, configurational and conformational subtleties of a particular molecular framework.

It will be observed that when a carbonyl function is situated in a cyclohexane ring in the chair conformation, the $n \longrightarrow \pi^*$ transition of the chromophore will result in a Cotton effect which is directly dependent upon the spatial orientation of the substituents in the ring system. The Octant rule[12] which is obtained from the symmetry principles, relates the sign and amplitude of the Cotton effect exhibited by an optically active saturated ketone to the spatial orientation of atoms about the carbonyl function. As a consequence, this rule permits prediction of the sign and semiquantitatively, the intensity of the Cotton effect exhibited by saturated ketones.

Taking the carbonyl chromophore as the reference point, a cyclohexane can be divided into eight Octants by means of three mutually perpendicular planes[12,17,18]. These are nodal and symmetry planes of the orbitals involved in the $n-\pi^*$ transition associated with the absorption of the carbonyl group. Here, cyclohexanone ring is used as an example as it is easy to visualise and discuss. However, the same concept is applicable to any ring system or side chain carrying a carbonyl function.

In figure 6 plane A is vertical passing through C-1 and C-4. The only substituents in this plane are the ones attached to C-4. The Plane B is horizontal and encompasses the carbon atom bearing the carbonyl group (C-1) and its two adjacent atom (the C-atom C-2 to the right called R_2 and the carbon atom C-6 to the

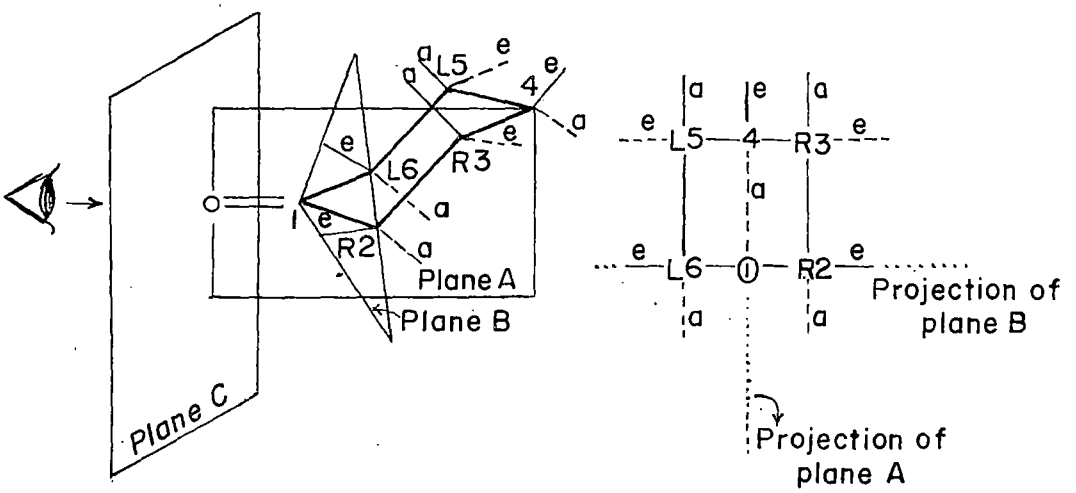
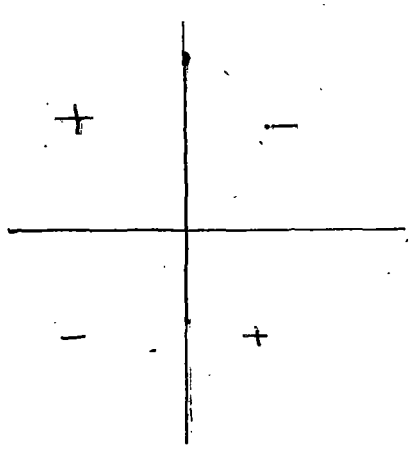

Plane C
Plane A
Plane B
L5
L6
R3
R2
4
1
Projection of plane B
Projection of plane A

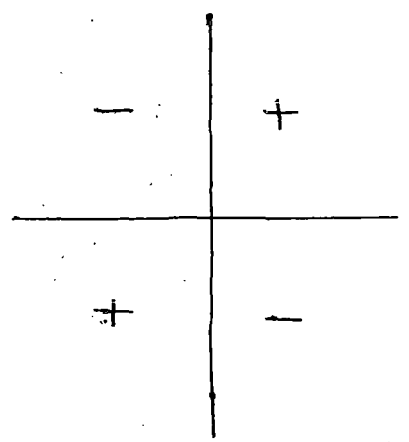
Fig-6

left, called L-6). The equatorially oriented substituents attached to these carbon-atoms C-2 and C-6 lie nearly in the nodal plane B. Thus, the planes A and B correspond to the nodal planes xz and yz of the orbitals mentioned in Fig. 6. The planes A and B provide four Octants, the back Octants (Fig. 6). A third plane C, perpendicular to plane A and dissecting the oxygen-carbon atom (C-1) bond produces four additional Octants, called front Octants. It should be pointed out that the exact nature and position of plane C are still uncertain. The four back Octants defined by planes A and B are the most important ones for practical purposes (Fig. 7).

The Octant rule states that substituents lying in planes A and B make no contribution to the Cotton effect associated with the carbonyl. This includes the equatorial substituents on carbon atoms C-2 and C-6 provided that they are exactly in the plane and both substituents on carbon atom C-4.



Back Octants



Front Octants

Fig. 7

The atoms or groups of atoms situated in an axial configuration on C-2 (lower right Octant) as well as axial and equatorial substituents on C-5 (upper left Octant) make a positive contribution to the Cotton effect. And the substituents located in an axial configuration on carbon C-6 (lower left Octant) as well as the axial and equatorial substituents on carbon C-3 produce a negative Cotton effect.

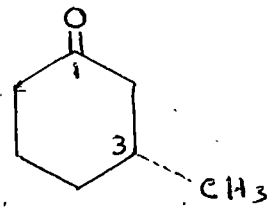
Extensive studies have shown that alkyl group of all kinds and halogen atoms (except fluorine) in a given Octant make contributions of the same sign to the Cotton effect[18,19,20]. The signs of the rear Octant are shown in Fig. 7. A given substituent produces the largest effect when it is in an axial position α to the carbonyl group of a cyclohexanone in a chair conformation.

Section E: Applications of the Octant Rule:

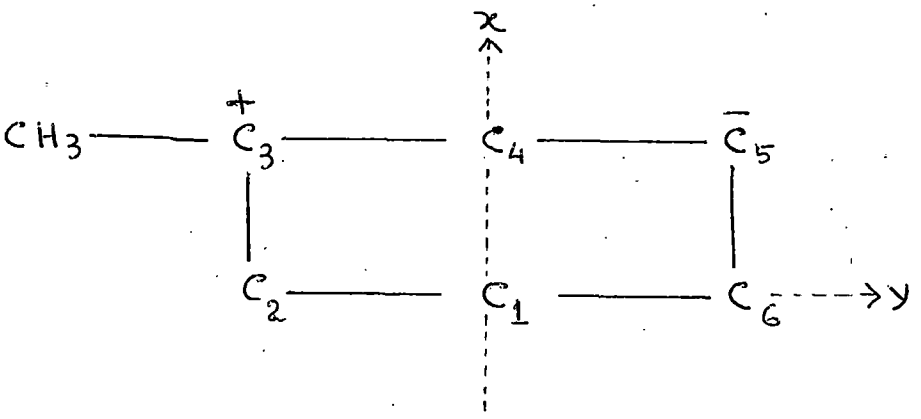
The main utility of the Octant rule is as follows: If the absolute configuration[21] of a ketone is known, its conformation can be determined. Conversely, if the conformation of the compound is established, its correct absolute configuration can be assigned[22-28].

The study of the Cotton effect associated with (+) 3-methyl cyclohexanone 1 clearly illustrates the Octant Rule. The carbon atoms C-2, C-4 and C-6 lie in nodal planes and thus make no contribution to the Cotton effect. The contributions due to C-3

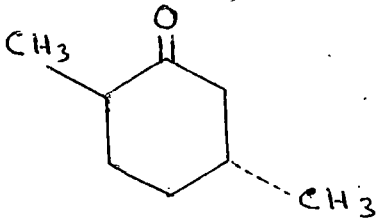
and C-5 cancel each other, being equal and opposite.



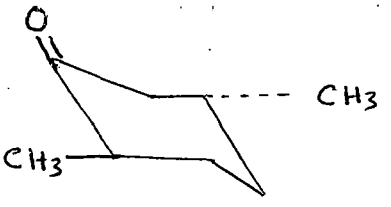
1



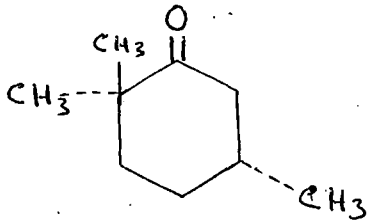
However, the methyl groups on C-3, being on a positive Octant, is alone responsible for the positive Cotton effect of this substance. The molecular amplitude of the O.R.D. curve of (+) 3-methyl-Cyclohexanone is a =+25.



2



2a



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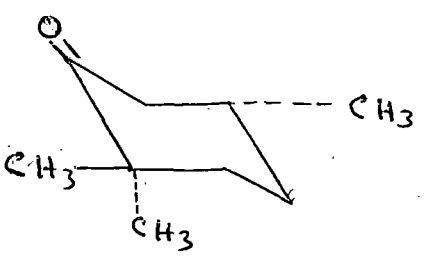
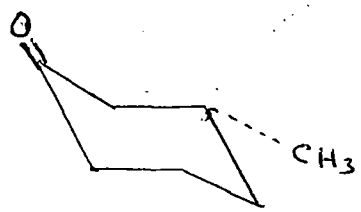


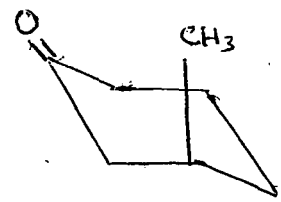
3a

In (+)-trans-3,6-dimethyl cyclohexanone 2 the equatorial methyl at C-6 2a lies in plane B and makes no contribution to the Cotton effect. Hence the compound shows the same positive Cotton effect as 1 (a = +28). But in compound (+) 3,6,6-trimethyl cyclohexanone 3 one axial methyl group is introduced, which being in a positive Octant enhances the positive Cotton effect (a = +81).

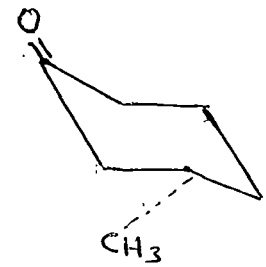
(+) 3-methyl cyclohexanone (1) can exist in two inter convertible conformations represented by 1a and 1b. Since the ketone showed positive Cotton effect; the conformation 1a represents the correct stereochemistry[29].



1a



1b

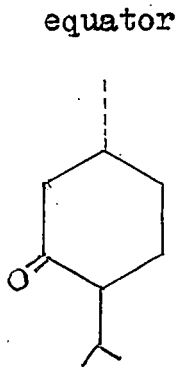


1c

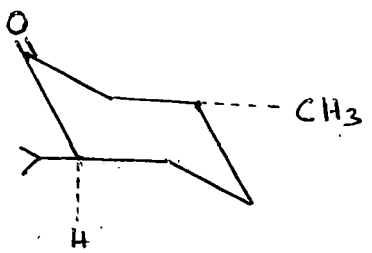
The Cotton effect should be negative for conformation 1b and 1c also would be expected to show a -ive Cotton effect, thus confirming indirectly the absolute configuration 1a assigned to (+) 3-methyl cyclohexanone.

Absolute Configuration of monoterpenes (-)-menthone 4 and (+) isomenthone 5:

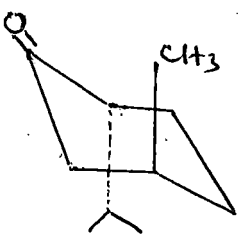
(-) menthone 4 shows a weak positive Cotton effect curve[22,23,28,30] whose amplitude is similar to that of trans, 3,6-dimethyl cyclohexanone 2. This can best be explained by the preferred conformation 4a in which both the alkyl groups are equatorially oriented (as in 2a).



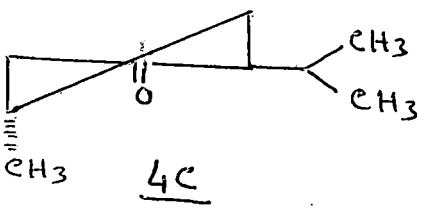
4c



4a

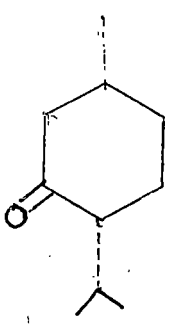


4b



4c

Isomerisation of (-) menthone 4a leads to (+)-isomenthone 5, for which two chair conformations 5a and 5b can be drawn. Since the Octant rule predicts a positive Cotton effect for conformation 5a and negative Cotton effect for 5b, the latter could be excluded.



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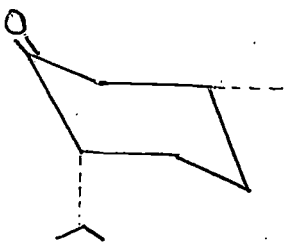
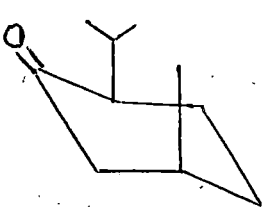
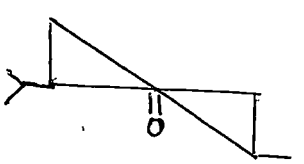


5a



5b

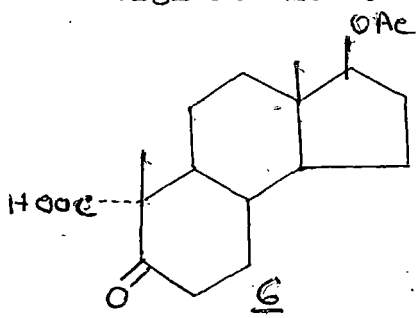


5c

Quantitative study[24] of the Cotton effect associated with (+)-isomenthone 5 indicates that there are contributions from conformers other than 5a. Although the twist form 5c would be expected to show a strongly positive Cotton effect[25], its participation together with conformer 5a can be excluded a priori[24].

Quantitative studies of the Cotton effect associated with α-equatorial alkyl cyclohexanones have indicated that in such cases an equatorial methyl[26], isopropyl[24] and t-butyl[27] group does not lie exactly in the nodal plane. In these cases, the equatorial alkyl substituent makes a positive contribution to the Cotton effect when situated on the right side next to the carbonyl.

The c.d of axial alkylated compounds 6 to 11 has also been measured. It was observed[31] in agreement with the Octant rule that an axial methyl group in α-position with respect to the carbonyl group has a major influence on the intensity and sometimes the sign of the Cotton effect.



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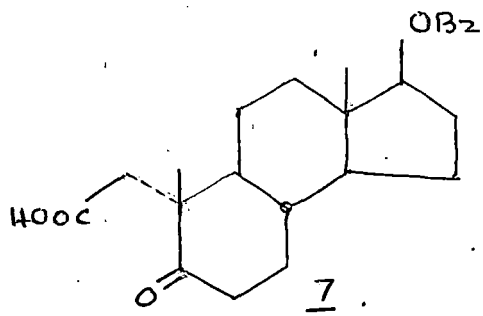

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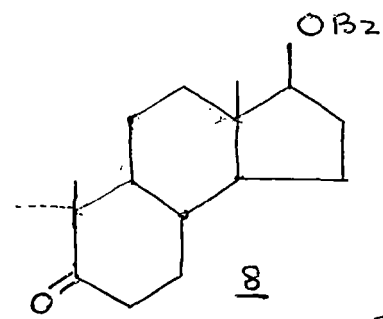

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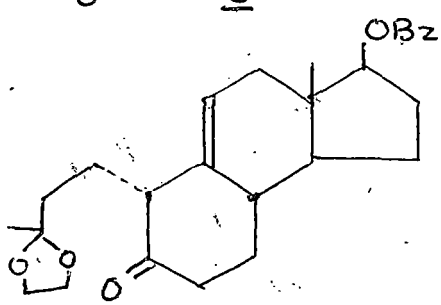

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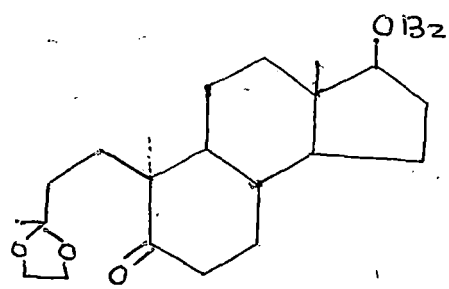

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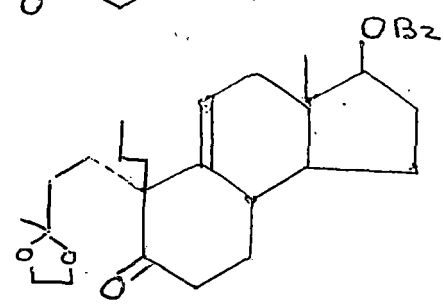
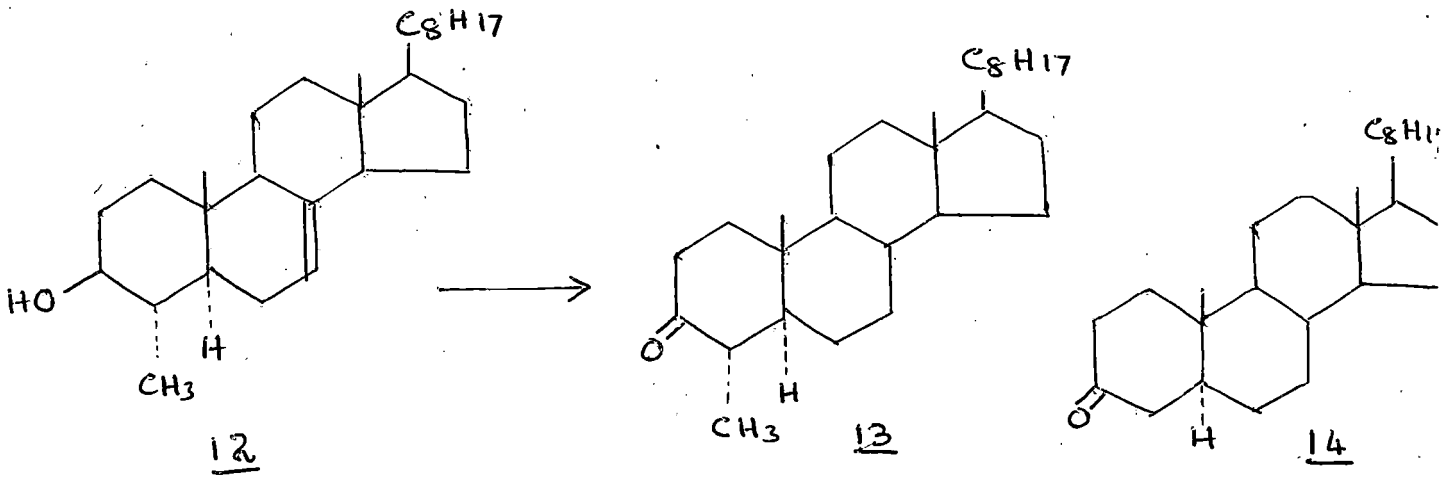


11

For example, in cases of acids 6 and 7 it has been pointed out[31] that, as expected, the introduction of an axial methyl group at C-10 has a positive effect on the c.d. maximum. However, the positive increment is higher than would be expected for a methyl group. In order to release the newly introduced 1,3-interactions between the 10β-methyl and the 6β, 8β and 11β-hydrogen atoms, the cyclohexanone ring probably partially adopts a boat or twist conformation.

Lophenol: The presence of adjacent alkyl groups was also shown[32] to have a strong inhibitory effect upon (hemi) ketal formation and advantage of this was taken in the structure elucidation[33] of lophenol 12, a novel type of biogenetically important plant sterol.

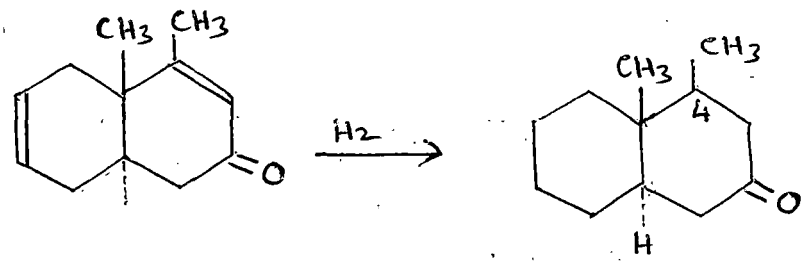


Reduction of the 6-7 double bond followed by oxidation gave lophanone 13, whose o.r.d. curve was similar to that of cholestanone 14, except that there was noted only a slight reduction in amplitude upon the addition of hydrochloric acid, in contrast to the marked

one (64%) suffered by cholestan-3-one 14 . This observation strongly suggested the presence of a methyl group adjacent to the oxygen function and this helped greatly in the identification of lophanone 13 as 4 α-methyl-cholestan-3-one.

Determination of stereochemistry of a Methyl group:

In connection with their work[34] in the eremophilone series Djerassi and coworkers, carried out the optical rotatory dispersion curve of the ketone 16 obtained by hydrogenation of 15 in methanol and noted that the curve underwent a marked reduction in



15 16

amplitude upon addition of hydrochloric acid, which would only be compatible with an equatorial orientation of the C-4 methyl group. The axial isomer would almost certainly have inhibited the formation of the (hemi) ketal.

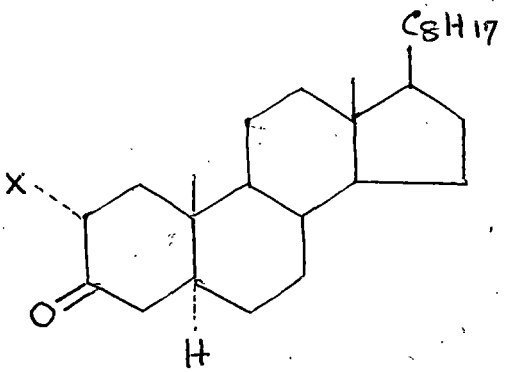
Section F: α-Halo Ketones:

The results accumulated[35] with U-V spectroscopic measurements demonstrated that the maximum associated with an isolated carbonyl group in a six membered ring is subjected to a hypsochromic

shift of about 5 mμ by an adjacent equatorial bromine atom (i.e. to shorter wave length) but an axial bromine produces a bathochromic shift (i.e. to a longer wavelength) of 28 mμ. It is to be expected that corresponding shifts should be observed in the rotatory dispersion peaks (or troughs) of α-halo cyclohexanones as compared with those of halogen free precursors[36,37].

Equatorial halogen:

It will be observed from Table I that by comparison with cholestan-3-one 17 introduction of equatorial F(18), Cl(19), or Br(20) produces a slight bathochromic shift of practically the same amplitude (2 to 3 mμ) whereas an iodine atom causes a somewhat larger change (8 mμ)



17, X = H
18, X = F
19, X = Cl
20, X = Br
21, X = I.

Table I[37]

Substance	R.D. peak mμ	Mol. rot. MeOH	U.V. max for MeOH mμ
Cholestan-3-one 17	307	3710°	286 +
2α-Fluoro 18	309	2650°	280
2α-Chloro 19	310	3130°	289 +
2α-bromo 20	310	3190°	282 +
2α-Iodo 21	315	4400°	258

..+.. in ethanol

From the above table (Table I) it will be seen that with the exception of 21 (X = I), the range of wavelength shifts is comparable with that observed in the corresponding U-V spectra (hypsochromic shift of 4-7 mμ). Thus a slight bathochromic shift (+3 mμ) is observed in going from 17 to 20 (2α-bromo). A second characteristic feature associated with the equatorial halogen atom is that the rotatory dispersion amplitude of the parent ketone is not affected to a marked extent.

Axial Halogens:

Rotatory dispersion measurements of α-axial halogen substituted cyclohexanones exhibited a move to a higher wavelength[36] of 20+5 mμ(Table II), and the r.d. amplitude is greatly increased.

Axial Iodine caused a shift to a higher wavelength of 32 mμ.

Table II[36]

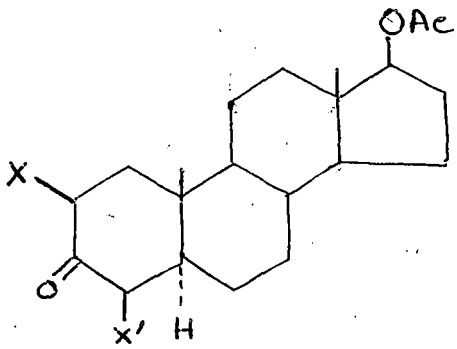
Substance	R.D. first extremum	Wavelength shifts of axial halogen			
		Iodine	Br	Cl	F
Cholestan-2-one	310				
3α-iodo	342	+32			
Friedelin	315				
2α-bromo	335		+20		
4α-bromo	335		+20		
Cholestan-3-one	307				
2 β-chloro	327,			+20	
11-keto progesterone					
3,20-bisketal	321.5				
9α-fluoro	340.0				+18.5

Section G: The Axial Halo Ketone Rule:[38] Its applications in Organic Chemistry:

The sign of the Cotton effect associated with a given cyclohexanone is not altered by introduction of an equatorial bromine atom on either side of the keto group. On the other hand,

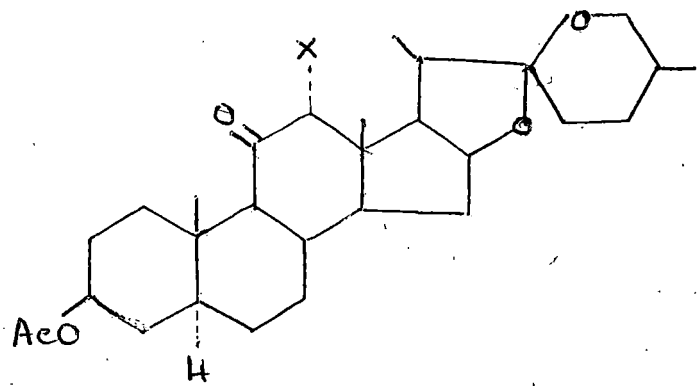
this does not apply when the halogen atom exhibits an axial orientation, because in that event a reversal in sign may occur. The effect of an axial halogen atom is highly dependent on the site of the substitution.

Thus 2β-bromo androstanε-17β-ol-3-one acetate 22 shows a strongly positive and 4β-bromo androstan-17β-ol-3-one-acetate 23 a strongly negative Cotton effect. Numerous other examples of this phenomenon have been recorded in the literature[39] and this also applies to axial chlorine. Thus 5α-spirostan-3β-ol-11-one-acetate 24 has a positive and its 12α-chloro derivative 25, a negative Cotton effect. It should be noted, however, that axial fluorine behaves anomalously and that the generalisations stated below applies to axial bromine, chlorine and probably also iodine, but not to fluorine.



22, X = Brγ, X' = H

23, X' = Brγ, X = H



24, X = H

25, X = Cl

The "axial halo ketone rule" states the matter as follows: (1) Introduction of equatorial halogen in either adjacent position of a keto group in a cyclohexanone does not alter the sign of the Cotton effect of the halogen free ketone.(2) The effect of introducing an axial chlorine, or bromine (and probably Iodine) atom next to the keto group of a ketone may affect the sign of the Cotton effect of the parent ketone.

The Cotton effect of the α-halocyclohexanone may be predicted by viewing along the O = C axis in a model so placed that the carbonyl group occupies the head of the chair (or boat) closest to the observer. If the halogen is now on the left of the line of view(Fig. 8)the compound will exhibit a negative Cotton effect (a) but if it is on the right, a positive Cotton effect will be observed (Table III).

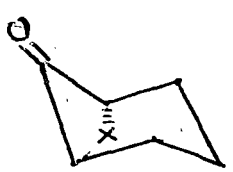
Table III

Axial α-halo Ketone	Cotton Effect	
	Halogen free Ketone	α-Halo Ketone
3 α-Bromo androstan-2-one-17 β-ol propionate	+	+
7 α-Bromo cholestane-3 β,5 α-diol-6 one-3 acetate	-	+
7 α-Bromo cholestane-3 β,5 α,[illegible]-diol-6one 3,5-diacetate	-	+

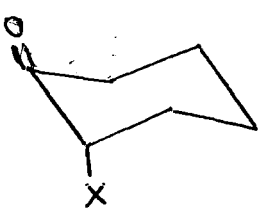
Contd..

Table III (Contd.)

Axial α-halo Ketone	Cotton Effect	
	Halogen free Ketone	α-Halo Ketone
6β-Bromo cholestan-3β-ol-7-one-acetate	-	+
9α-Bromo ergostan-3β-ol-11-one-acetate	+	+
12α-Bromo ergostan-3β-ol-11-one acetate	+	-
12α-Chloro-11-keto tigogenin acetate	+	-
12α, 23-Dibromo-11-Keto tigogenin acetate	+	-
Methyl 11β-bromo-3α-acetoxy-12 Keto cholanate	+	-
2α-Bromo friedelin	-	-
4α-Bromo friedelin	-	+



(a) Negative Cotton Effect



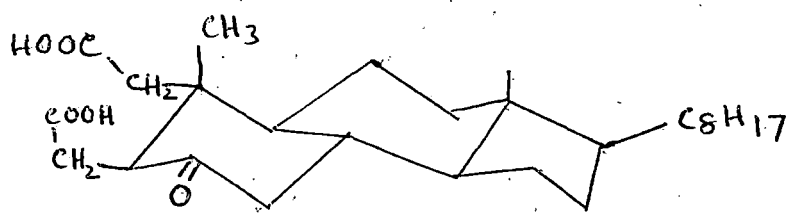
(b) Positive Cotton Effect.

Fig. 8

The rule may be used in four ways:

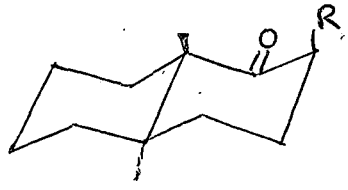
1. If there is doubt whether an α-halogen is equatorial or axial, reversal of the Cotton effect upon halogenation proves location of the halogen. The converse is obviously not true. The halogen could be axial without reversing the Cotton effect, but even in this case it should be readily distinguishable from equatorial halogen through the bathochromic shift of the extrema and the increased amplitude[39].

2. If the configuration and conformation of the parent ketone are known and the α-halogen is axial (demonstrated as above (1) or by infra-red or U-V measurements) its location may be deduced from the sign of the Cotton effect of the haloketone. For example 2,3 -seco cholestan-6-one-2-3-dioic acid 26 upon bromination gives an axial α-bromo derevative which, a priori, could be the 5-or the 7-bromide. Since the Cotton effect for this bromide is negative, the haloketone rule implies that it is the 5-isomer.

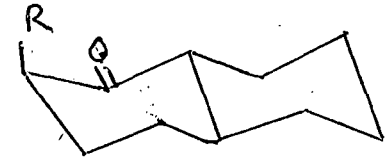


26

3. If the conformation of the ring system is fixed and the location of the axial α-halogen is known, the absolute configuration of the α-haloketone (and thus the parent ketone) may be deduced. An example is provided by (-) trans-1-decalone. This ketone may, a priori, have either configuration 27 or 28. Now bromination of trans-1-decalone gives, among other products, the



OR



27 R = H 28

29 R = Br 30

axial 2-bromo ketone. That the product is not the equatorial 2-bromo ketone may be deduced from I.R. as well as rotatory dispersion data as shown above; that it is not a 9-bromoketone follows from the fact that its reduction returns pure trans-1-decalone whereas the 9-bromo ketones-which are also formed in the bromination -upon reduction give the expected mixture of cis and trans-decalone[40]. When (-)-trans-1-decalone is brominated the product is (+)-2β-bromo -trans-1-decalone 29 or 30 which has a strong positive Cotton effect. According to the haloketone rule, this means that the dextrorotatory bromoketone has configuration 29 rather than 30. Therefore, (-) trans-1-decalone must have configuration 27 - thus providing an independent corroboration of the configurational assignment.

4. If the α-haloketone is axial and the position of the halogen and the configuration of the molecule are known, the conformation of the ring may be deduced. For example, chlorination of (+)-3-methyl cyclohexanone (absolute configuration as in Fig. 9) gives a mixture from which a pure crystalline isomer was isolated and shown, by chemical means, to be 2-chloro-5-methyl-cyclohexanone[41]. Two configurational isomers are possible, and each may exist in one or the other of two conformations as in Fig. 9 . The spectral properties of the crystalline chloroketone indicate that in Octane solution it has axial halogen and the Cotton effect in Octane is negative; this is compatible with configuration A but not with B. Hence the chloroketone is the trans-isomer. When the rotatory dispersion curve is measured in methanol, the Cotton effect is found to have become positive. This means that the trans isomer has changed from the diaxial conformation A to the diequatorial conformation A', presumably because in methanol the dipole repulsion between the carbonyl group and the adjacent equatorial halogen is not so serious as in Octane with its much lower dielectric constant.

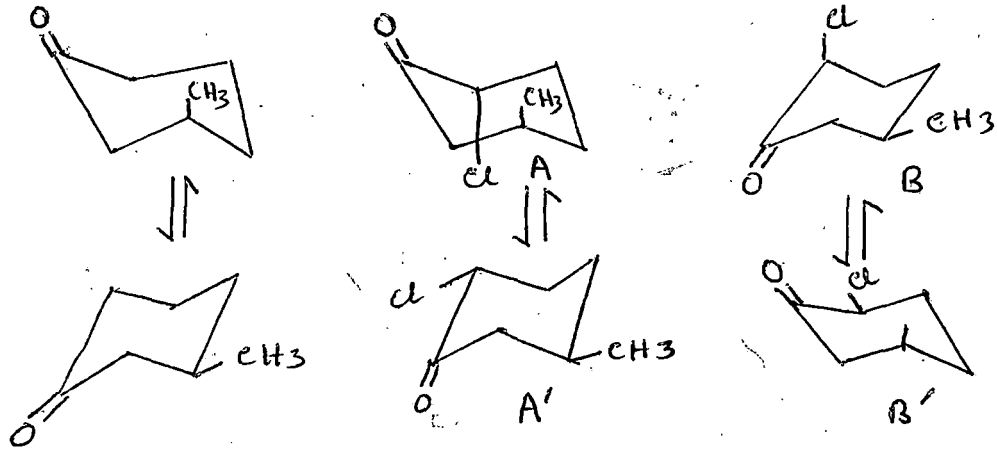
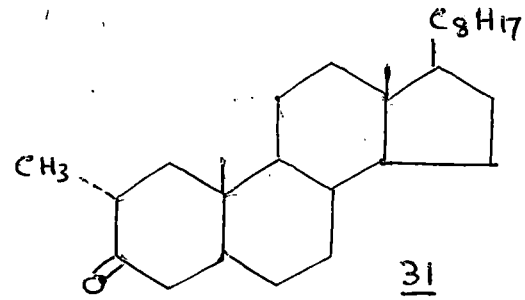


Fig. 9

Bromination of 2α-methyl cholestan-3-one: Boat Conformation of 2α-Bromo-2β-methyl cholestan-3-one.

Bromination of 2α-methyl cholestan-3-one 31 at the 2-position gave a 2-bromo-2-methyl-cholestan-3-one. I.R and U-V measurements[42] indicate that the bromine in this compound is axial and thus it appeared at first that the bromination product was - 2β-bromo-2α-methyl cholestan-3-one whose formation would have involved the usual axial attack of bromine in a kinetically controlled step. However, when the rotatory dispersion curve of the bromo ketone was recorded, it was found[43] that instead of the expected strong positive Cotton effect it shows a negative one. 2β-bromo-2α-methyl-3 keto steroids have been prepared[43] and they do indeed, show the expected strong positive Cotton effect. It, therefore, appears that the above discussed bromination product is 2α-bromo-2β-methyl cholestan-3-one and that its anomalous Cotton effect is due to the existence of the ring A in the boat form.



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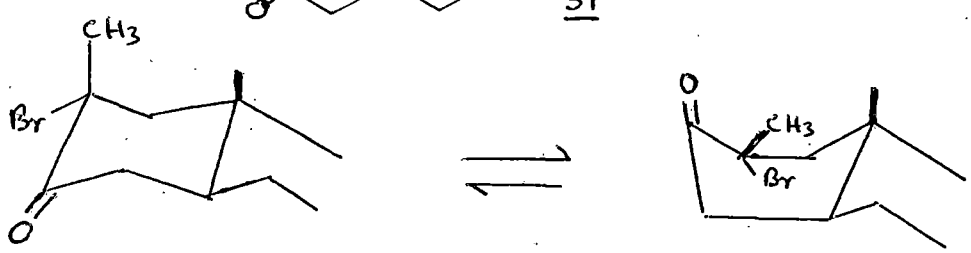


2α-bromo-2β-methyl-Cholestan-3-one

In the chair form the bromine is equatorial and the Cotton effect should be unchanged from that of the parent ketone, this is not the case here.

Section H ; Effect of α-OH and α-acetoxy ketones: Anti Octant effect:

Information on the conformation of a ketone can generally be obtained from the sign, amplitude and position of its Cotton effect. With α-hydroxy ketones and α-acetoxy ketones, however, the situation is complicated by the fact that their circular dichroism (c.d), optical rotatory dispersion (o.r.d) spectra do not always follow the well known shift rules for U-V $n-\pi^*$ absorption bands of α-substituted ketones[35]. Thus it is known that the position of the $n - \pi^*$ bands in ultraviolet spectra is dependent on the nature and conformation of the α-substituent, and an axial hydroxyl group shows a bathochromic shift stronger than that of an axial acetoxy group, while on equatorial hydroxyl group shows a hypsochromic shift stronger than that of an equatorial acetoxy group[44]. Although most o.r.d and c.d. curves follow the same rule, exceptions[45] are known in steroids in α-ketal grouping in ring C (Table IV). This also appears to be true in some B-ring α-ketals[46].

Table IV

No.	Substance	Rotations dispersions, in Dioxan Solution	$\Delta\lambda e$	$\Delta\lambda a$
1.	11-keto-tigogenin	325 mμ	-	-
2.	12-α-hydroxy-11-keto-tigogenin	337.5 mμ		+12.5
3.	3-O-Acetyl-12α-acetoxy-11-keto tigogenin	340 mμ		+15
4.	12β-hydroxy-11-keto-tigogenin hydrate	315 mμ	-10	-10
5.	3-O-Acetyl-12β-acetoxy-11-keto-tigogenin	322.5 mμ	-2.5	
6.	3-O-Acetyl-hecogenin	312.5 mμ		
7.	3-O-Acetyl-11α-acetoxy-hecogenin	310 mμ	-2.5	
8.	3-O-Acetyl-23α-brom-11β-hydroxy-hecogenin	332.5 mμ		+20
9.	3-O-Acetyl-11β-acetoxy-23α-brom-hecogenin	335 mμ		+22.5

The deduction from the Octant rule that an equatorial OR group will have little influence on the Cotton effect, whereas an axial group will show a definite contribution applies in most cases[47] and leads

Table V α-Ketols and their acetates.

Position of Keto group	Series	α-Substituent OR	amplitude a	Contribution to O.R. Δa
3	5α-Cholestane	none	+58	
	2α-oAe(eq.)	2α-OAe(eq)	+66	+8
		2β-OAe(ax)	+166	+58
3	4,4'-dimethyl	none	-12	
	-5α-Cholestane	2α-OAe(eq)	+38	+50
		2β-OAe(ax)	+124	+136
4	5α-Cholestane	none	-95	-
		5α-OH(ax)	-45	+50
6	5α-Cholestane	none	-78	-
		5α-OH(ax)	-110	-32
		5α-OAe	-89	-11
11	5α-Spirostan	none	+43D	-
		12β-OH(eq)	+44D	+1 ½
		12β-OAe(eq)	+42D	-1 ½
		12α-OH(ax)	+42D	-1
		12α-OAe	+88D	+45
11	5β-Cholanate 12	none	-	-
		12β-OH(eq)	+14	
		12β-OAe(eq)	+28	
		12α-OAe(ax)	+73	
11	5α-Ergostane	none	+12	
		12α-OAe	+85	+73
	5α-Ergostane	none	+19	
		9α-OH(ax)	+68	+49
11	5β-Etianate	none	+10	
		12α-OH	+40	+30
		12α-OAe	+44	+34
		12β-OAe	+171D	+71
12	5α-Spirostan	none	-	
		11α-OAe(eq)	411D	
		11β-OH(ax)	+77D	
		11β-OAe(ax)	+162D	
13	5β-Cholanate	none	+10	
		11α-OH(eq)	+17	+7
		11β-OH(ax)	+39	+29

Table V (Contd)

Summary for α-Ketols

C = O	C-OH	Octant behaviour	
3(5α)	2	✓	Normal
4	5α	✓	
6	5α	✓	
11	9		
11	12	X	abnormal
12	11	X	

The above table showed that for the steriod α-ketols it is generally true that (a) eq. (OH) or acytoxy substituent have little influence on the Cotton effect.

(b) axial (OH) or acetoxy substituents in ring A and B make large contribution to the Cotton effects of the same sign as chlorine or bromine in the same pattern.

(c) axial (OH) or acetoxy substituents in ring C makes large contribution to the Cotton effect of opposite sign to those given by chlorine or bromine in the same portion.

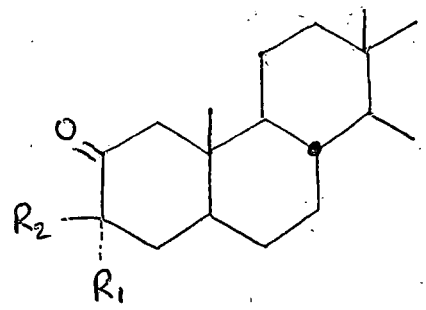
to the correct prediction of the Cotton effect of 4 β-acetoxy-cholestan-3-one[48]. but not of 3 α-acetoxy cholestanone[48] or a 12 α-acetoxy-11-ketone or a 5 α-acetoxy-6-ketone, although no conformational change is to be expected. As shown in Table VI,

Table VI

O.R.D

Compound	Peak	Trough	Molecular amplitude a
5α-Cholestan-2-one	$[\phi]_{310}$ +6290°	$[\phi]_{267}$ -5820°	+121
3α-OH-5α-Cholestan-2-one-acetate	$[\phi]_{317}$ +2540°	$[\phi]_{257}$ -2330°	+ 49
3β-OH-5α-Cholestan-2-one-acetate	$[\phi]_{305}$ +6740°	$[\phi]_{270}$ -5320°	+120°
5α-Cholestan-3-one	$[\phi]_{307}$ +3700°	$[\phi]_{267}$ -2940°	+ 55°
2α-OH-5α-Cholestan-3-one-acetate	$[\phi]_{305}$ +3450°	$[\phi]_{265}$ -2790°	+62
2β-OH-5α-Cholestan-3-one-acetate	$[\phi]_{290}$ +1820°	$[\phi]_{250}$ +6201°	+121
4α-OH-5α-Cholestan-3-one-acetate	$[\phi]_{300}$ +1850°	$[\phi]_{260}$ -2410°	+ 43
4β-OH-5α-Cholestan-3-one-acetate	$[\phi]_{320}$ +730	$[\phi]_{270}$ -970°	+17

3β-hydroxy-5α-cholestan-2-one-acetate 32c shows the same amplitude as 5α-cholestan-2-one in agreement with the Octant rule, since the equatorial acetoxy function lies in a nodal plane. The

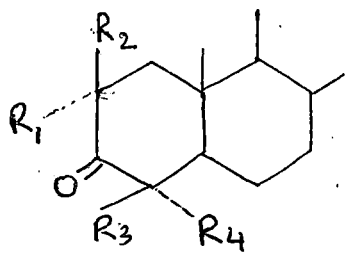


32a, $R_1 = R_2 = H$

32b, $R_1 = Ac$, $R_2 = H$

32c, $R_1 = H$, $R_2 = Ac$

molecular amplitude of the 3α-acetoxy derivative 32b is considerably reduced when compared with the parent unsubstituted 5α-cholestan-2-one 32a. Since a 3α-axial substituent in ring A in the chair form would be expected to enhance the Cotton effect, it is suggested that this ring is distorted in 32b in order to release the non-bonded interactions between the 3α-substituent and the 1α- and 5α-axial hydrogen atoms. However, in a recent NMR study of axial and equatorial alcohols, it was indicated[48] that the coupling constant of the 3-equatorial proton in 32b is in perfect agreement with a normal chair conformation for Ring A. The unexpected o.r.d curve of 2β-acetoxy-cholestan-3-one 33b[48], on the other hand, has been taken as evidence for a twist conformation of ring A.



33a, $R_1 = R_2 = R_3 = R_4 = H$

33b, $R_1 = R_3 = R_4 = H$, $R_2 = Ac$

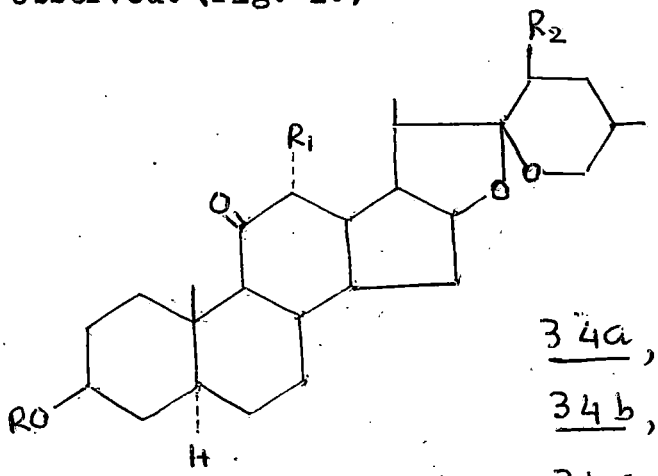
33c, $R_1 = Ac$, $R_2 = R_3 = R_4 = H$

33d, $R_1 = R_2 = R_3 = H$, $R_4 = Ac$

33e, $R_1 = R_2 = R_4 = H$, $R_3 = Ac$

The 4β-acetoxy derivative 33e is also distorted in order to release 1,3-diaxial interactions between the 4β-acetoxy and the 10β-methyl groupings. The molecular amplitude of the 4α-isomer 33d, is somewhat decreased when compared with 33a. It has been suggested that this may be due to reorientation of the acetoxy group in 33d in order to avoid interactions with the 6α hydrogen atom. The acetoxy function is no longer in the nodal plane and makes a mild negative contribution to the Cotton effect. Djerassi and coworkers[49a and 49b] have shown that fluorine atoms gave contributions of opposite sign to alkyl (or chlorine or bromine). Fig 10. 11-keto tigogenin 34a is characterised by a weak positive single Cotton effect curve associated with 11-keto steroids and introduction of an axial bromine or chlorine atom at C-12 as 34b and 34c results in the anticipated increased amplitude and bathochromic shift as well as in an inversion of the sign of the Cotton effect. The latter observation is in accord with the empirical rule 49a already discussed. However, introduction of a fluorine

atom at C-12 as in 12α-fluoro-11-keto tigogenin acetate 34d exhibits the same wavelength shifts as does the corresponding 12α-chloro analog 34c but the Cotton effect is of opposite sign. This grossly different behavior of axial fluorine is also observed in the case of 9α-fluoro-11-keto-progesterone-3-20-bisethylene ketal 35 which showed a negative Cotton effect in contrast to the 9α-bromo compound 36 where a positive single Cotton effect was observed. (Fig. 10)

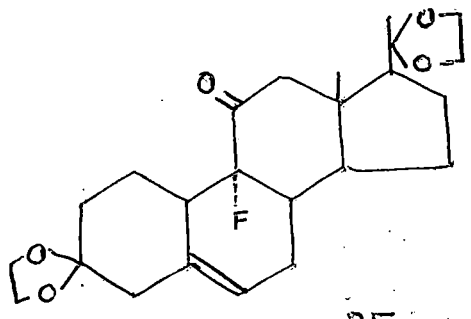


34a, R = R1 = R2 = H

34b, R = Ac, R1 = R2 = Br

34c R = Ac, R1 = Cl, R2 = H

34d R = Ac, R1 = F, R2 = H



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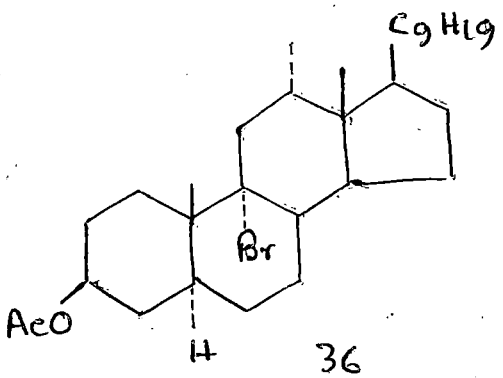

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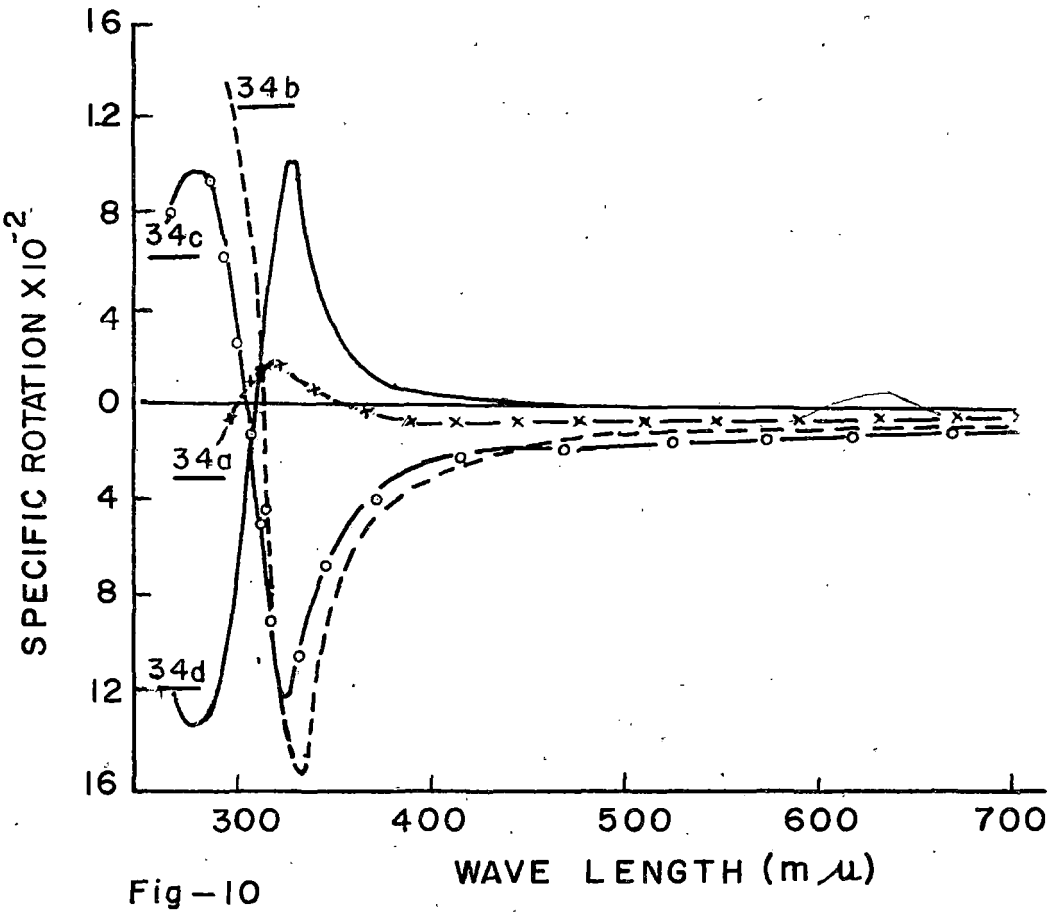
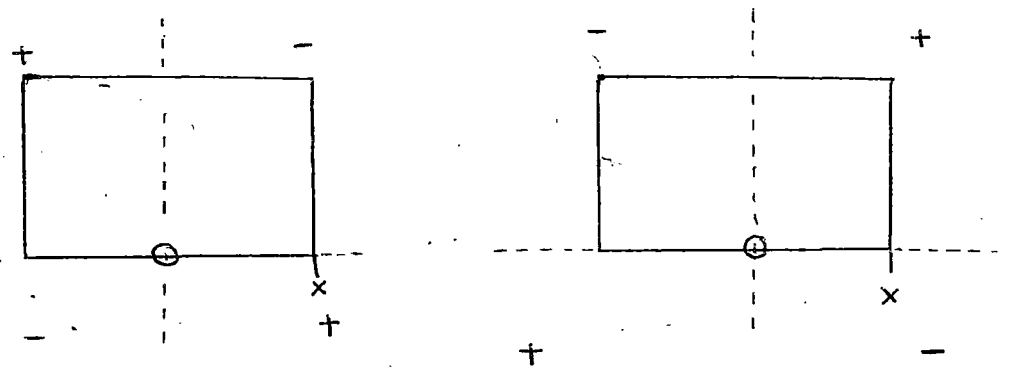

16
12
8
4
0
4
8
12
16
34b
34c
34a
34d
SPECIFIC ROTATION X10^{-2}
300
400
500
600
700
WAVE LENGTH (mμ)

Fig – 10

This behaviour has been termed "Anti-Octant behaviour". (Fig. 11).



Normal Octant Sign (for rear Octants)

Anti-Octant Signs (for rear Octants)

..... nodal planes

Fig. 11

Recently, Enslin and his colleagues[50] carried out c.d. studies on ring A hydroxy and acetoxy-ketones and some of the results show anti-Octant effects. (Table VII[50]) (next page)

Table VII

Compound	CD in Methanol			CD in Hexane		
	$\Delta\epsilon$ (nm)	Shift[a](nm) of ketone $n\rightarrow\pi^*$ band	$\Delta\Delta_{max}$ contribution[b]	$\Delta\epsilon_{max}$ (nm)	Shift[a](nm) of ketone $n\rightarrow\pi^*$ band	$\Delta\Delta_{max}$ contribution[b]
3β-OAc-5α-cholestan-2-one[e]	+3.2(289) -1.1(210[c])		0	+2.25(300) +2.35(292) -1.0(210[c])		0
3α-OAc-5α-cholestan-2-one[e]	+1.45(300) -1.0(215[c])		A0	+1.0(314) +1.05(305) -1.0(215[c])		A0
5α-cholestan-4-one	-2.1(291)			-1.0(315) -1.6(304) -1,6(296)		
3β-OH-5α-cholestan-4-one	-1.95(289) +0.35(215[c])	2^-	+0.15	-2.25(286) +0.5(215[c])	14^-	-0.65
3β-OAc-5α-cholestan4-one	-2.1(291) +1.25(210[c])	0	0.0	-1.5(299) -1.6(291) +1.2(210[c])	5^-	0.0
5-OH-5-αcholestan-4-one	-0.85(302)	11^+	+1.25,0	-1.1(306)	6^+	+0.5,0

Table VII (Contd.)

Compound	CD in Methanol			CD in Hexane		
	$\Delta\epsilon$ (nm)	Shift[a] (nm) of ketone $n \rightarrow \pi^*$ band	$\Delta\Delta$ max contribution[b]	$\Delta\epsilon$ (nm) max	Shift[a] (nm) of ketone $n \rightarrow \pi^*$ band	$\Delta\Delta$ max contribution[b]
5-OAc-5α-Cholestan-4-one	-2.9(294) +2.4(214)	3^+	-0.8,AO	-2.3(299) +1.2(222)	1^-	-0.7,AO
5β-cholestan-4-one	0.20(284)			+0.25(307) +0.25(297)		
5-OH-5β-cholestan-4-one	-0.9(306)	22^+	-1.1,0	-0.25(326sh) -0.30(316)	19^+	-0.55,0
5-OAc-5β-cholestan-4-one	+1.05(297) -1.2(220)	13^+	+0.85,AO	+1.2(300) -1.05(220)	2^+	+0.95,AO
5α-cholestan-6-one	-1.60(292)			-0.7(315) -1.2(305) -1.1(296)		
5-OH-5α-Cholestan-6-one	-2.55(303)	11^+	-0.95,0	-1.45(308)	8^+	-0.25,0
5-OAc-5α-cholestan-6-one	-1.9(293) -0.8(220)	1^+	-0.3,0	-1.2(302sh) -1.35(296) -0.55(222)	1^-	-0.15,0
5β-cholestan-6-one	-4.2(294)			-3.2(308) -3.4(299)		
5-OH-5β-cholestan-6-one	-5.1(288) +2.45(215[c])	6^-	-0.9	-4.4(296) +2.9(215[c])	17^-	-1.2

Table VII (Contd.)

Compound	CD in Methanol			CD in Hexane		
	$\Delta\epsilon$(nm)	Shift[a](nm) of ketone $n \rightarrow \pi^*$ band	$\Delta\Delta_{max}$ contribution[b]	$\Delta\epsilon$(nm) max	Shift[a](nm) of ketone $n \rightarrow \pi^*$ band	$\Delta\Delta_{max}$ contribution[b]
5-OAc-5 β-cholestan-6-one	-3.7(293) +0.95(220)	1⁻	+0.5	-2.6(300) -2.75(293) +1.15(220)	7⁻	+0.65
4,4,14α-trimethyl-19(10$\rightarrow$9β) abeo-10α-pregn-5-en-2,11,20-trione	+6.45(297)					
4,4,14α-trimethyl-19(10$\rightarrow$9β) abeo-10α-pregn-5-en-3, 11,20-trione	+6.65(295) +12.0(214)					
Anhydro-22-deoxocucurbitacin D	+4.15(302) +16.0(216)					

a bathochromic + hypsochromic-.
b "O" Octant contribution, "AO" anti-octant contribution.
c end absorption.
e The results on these compounds should be compared with an ORD amplitude of a = +121 for the parent ketone in dioxan solution.

The results of three α-ketals with axial OH-group viz. the 5α-hydroxy-4-one, the 5β-hydroxy-4-one and the 5α-hydroxy-6-one show that in each case the α-substituent makes a significant contribution of the predicted sign.

Anomalous results were obtained for two α-ketals with equatorial OH groups. In hexane solution, where strong intra-molecular H-bonding is expected, the spectra of 3β-hydroxy-5α-cholestan-4-one and the 5β-hydroxy-6-one revealed an unmistakable contribution due to the equatorial substituent. This effect in methanol, where the polar nature of the solvent would be expected to weaken intra-molecular hydrogen bonds was no longer significant in the 3β-hydroxy-4-one but was still apparent in the 5β-hydroxy-6-one. The influence of strong intra-molecular H-bonding was evident as strong end absorption at 210-15 nm in the C.D. spectra of these compounds, particularly in hexane solution. Significant changes were observed in the corresponding OAc-derivatives. The axial OAc-group of the 5α and 5β-acetoxy-4-ones and the 3α-acetoxy-2-ones make a strong contribution to the ketone Cotton effect, but of a sign opposite to that predicted by the Octant rule -i.e. (anti-Octant contribution). The sign of the Cotton effect in the 5α-acetoxy-6-one accords with prediction, but the magnitude of the effect is strongly reduced by comparison with that of the corresponding OH-compound. The effect of this anti-Octant behavior of axial OAc-group is demonstrated clearly in the case of 5-hydroxy-5β-cholestan-4-one where acetylation of the tertiary OH-group leads to reversal of the sign of the

Fig 12

ketone n-π effect. The C.D. spectra of 3β- acetoxy-5α-cholestan-2-one and 3β-acetoxy 5α-cholestan-4-one and the data already reported in the literature (Table V, Table VI) and Table VIII) for

Table VIII

c.d and O.R.D data for related derivatives of 5α-cholestane; c.d. curves measured for solutions in diexan (D) or ethanol(E); O.R.D curves measured for solutions in methanol-dioxan (2:1)

	C.D			O.R.D	
	Solvent	λ (mμ)	Δε	λ(mμ)	a
3 β-hydroxy-4-one	D	286	-1.91	303/263	-101
	E	287	-2.32		
3β-acetoxy-4-one	D	290-295	-1.82	305/265	-105
	E	289	-2.08	222	+59a
4 α-hydroxy-3-one	D	290	+1.32	298/258	+62
	E	283	+1.12		
4 α-acetoxy-3-one	D	287	+0.95	298/259	+39
		227	+0.44	226	-5[a]
	E	284	+0.83		
		227	+0.64		

various A-ring equatorial α-ketal acetates are in agreement with the Octant rule since these OAc groups contribute insignificantly to Cotton effects of the corresponding parent ketones. The result

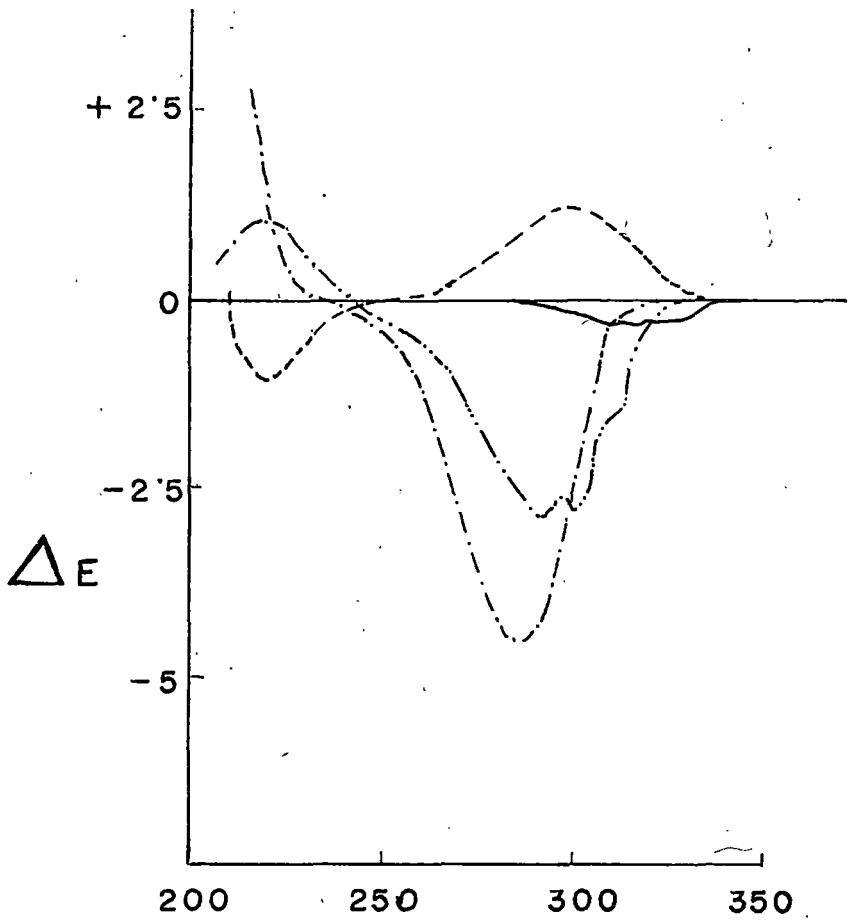


Fig-12 CD spectra in haxene solution

5-hydroxy-5β-cholestan-4-one

5-acetoxy-5β-cholestan-4-one

5-hydroxy-5β-cholestan-6-one

5-acetoxy-5β-cholestan- -one

reported by Enslin and his coworkers[50] on the 5β-acetoxy-6-one is however, an important exception since this equatorial OAc-group makes a demonstrable positive contribution in both hexane and methanol (Table VII).

Enslin and his colleague studied the space-filling models (Courtauld Atomic Models) of the above α-ketals and their acetates and made the following observations (1) free rotation of the OH-groups of all the α-ketals is possible, but in axial cases, 1,3-diaxial interactions suggest some conformational preference- which is strengthened by the known (albeit weak) OH——πCH bond[51]. (2) In compounds with an equatorial OH-group, the strong OH····n-OH bond[51] should result in a conformational preference. (3) In axial OAc compounds there is complete restriction of rotation about the -C-O- bond of the OAc group. In the equatorial OAc- compounds such rotation is also severely contained, but not to the same extent as axial cases. However, one notable exception is 5-acetoxy-5β-cholestan-6-one, where rotation about the C-O bond of the equatorial (relative to the 6-ketone)α-substituent is completely frozen since this function is at a cis-ring junction and is therefore axial relative to the A-ring. Significantly, this is also the one-α-ketal acetate in which an equatorial OAc-group makes a remarkable contribution to the ketone $n-\pi^*$ Cotton effect.

(4) Due to the severe rotational barrier imposed upon OAc-groups in these compounds, two discrete conformers appear to be possible, in one in which the OAc-carbonyl group is oriented toward the ketone

and in the other, away. It is therefore feasible that in the former the proximity of the two optically active functions may lead to coupling of their respective n-π^* transitions. It is also possible that a more important factor - that is perturbation of the ketone n-π^* transition by the lone pairs on the "ether oxygen" of the adjacent OAc group and the spatial orientation of these lone pairs relative to the ketone may be decisive in determining anomalous behaviour.

(5) Since the models demonstrate that conformational preferences of the α-substituents exist, and further that the constrained orientation of the C-O-oxygen lone pairs in each α-ketol acetate differs from that preferred in each α'-ketol, this difference may well influence the amplitude and sign of the ketone n-π^* transition. Consequently, any factor which alters the lone pair orientation in a sterically restricted environment will result in an observable effect upon this transition whether the α-substituent is equatorial or axial.

Snatzke and Veithen recently[52] studied the C.D. curves of 5-amino-5α'-cholestan-6-one and its N-acetyl derivative and they observed a large difference in amplitude between the C.D. maxima of 5-amino-5α-cholestan-6-one and its N-acetyl derivative. Enslin and his colleagues suggest that here also, a difference in orientation of the nitrogen lone pairs of the two compounds relative to the CO-group could play a role.

Recently, Bartlett and his co-workers[53] measured the o.r.d and c.d. of a series of hydroxy and acetoxy derivatives of (+) bornan-2-one [(+) Camphor 37 $R^1 = R^2 = H$] and (-) bornan-3-one [(-)-epi-camphor 38; $R^1 = R^2 = H$] which are summarised in Table IX. These data also present a clear picture of apparent anti-Octant effect by oxygen containing substituents.

Table IX

C.D. of bornanone derivatives: Summary of $\Delta\epsilon$ values and differences ($\Delta\Delta\epsilon$). Solvent, Hexane. Principal c.d. maxima only are shown. Signs of $\Delta\Delta\epsilon$ are "Anti-Octant" in every case.

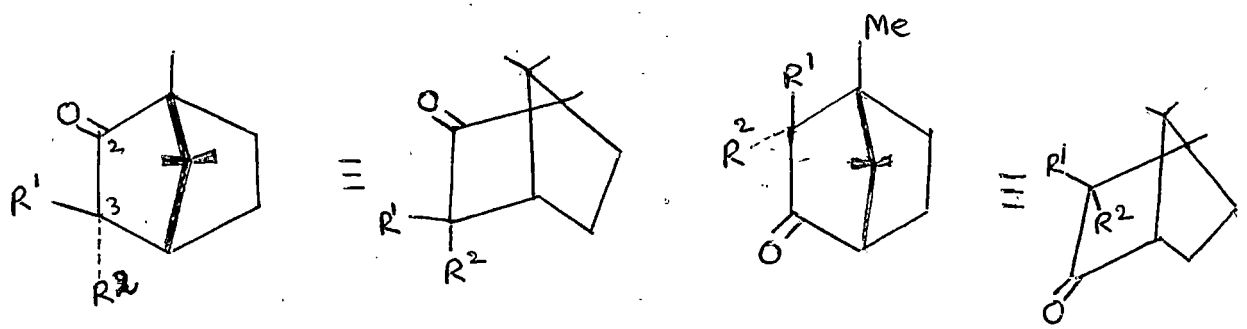
Hydroxy groups	λ/nm	$\Delta\lambda$/nm	$\Delta\epsilon$	$\Delta\Delta\epsilon$
Bornan-2-one	303		+1.50	
endo-3-OH	320[a]	+17	+0.31[a]	-1.19
exo-3-OH	304	+1	+1.85	+0.35
Bornan-3-one	306		-1.46	
endo-2-OH	317[b]	+11	-0.76	+0.70
exo-2-OH	304	-2	-2.07	-0.61
Acetoxy groups				
Bornan-2-one	303		+1.50	
endo-3-OAc	325[c]	+22	+0.36[e]	-1.14

Table IX (Contd.)

Hydroxy groups	λ/nm	$\Delta\lambda$/nm	$\Delta\epsilon$	$\Delta\Delta\epsilon$
Acetoxy groups				
exo-3-OAc	304	+1	+2.18	+0.68
Bornan-3-one	306		-1.46	
endo-2-OAc	327[d]	+21	-0.23[d]	+1.23
exo-2-OAc	308	+2	-2.34	-0.88

Additional maxima of opposite sign at shorter wavelength.

	$\Delta\epsilon$	λ/nm		$\Delta\epsilon$	λ/nm
a	-0.20	288	c	-0.09	283
b	+0.16	283	d	+0.35	292



37 38

(+)-Bornan-2-one
[(+)-Camphor]

(-) Bornan-3-one
[(-) epi-Camphor]

All the C.D. substituent differences ($\Delta\Delta\epsilon$) values for solutions in hexane in Table IX above are of "Anti-Octant" sign. The endo-isomer in each pair produces the larger effect, although the magnitudes of the differences vary. The C.d data for solutions in dioxan and methanol (Table X) show a similar trend, except that endo-2-hydroxy bornan-3-one shows a small "Octant" substituent effect in methanol.

According to the authors, all the hydroxy ketones exhibited very little or no fine structure in their c.d. curves even in hexane, which may be the result of intramolecular hydrogen-bonding between the hydroxy and ketone groups in non-polar solvent. Joris and Schleyer[51] have found evidence for moderately strong hydrogen bonding in the I.R. spectra of some related hydroxy ketones in carbontetrachloride. In the case of exo-hydroxy ketones 37 (R^1 = OH, R^2 = H) and 38 (R^1 = OH, R^2 = H) there is no appreciable bathochromic solvent shift between methanol and hexane, in contrast the parent ketones, however, show a pronounced shift on changing the solvent.

The acetoxy-ketones are in many ways analogous to the hydroxy compounds, but with the unusual features exaggerated.

The authors stated that "there is no certainty that the conformation of the acetoxy-group itself is fixed, although the preferred conformation of many other acetoxy-compounds[54] appears to be

Table X

Cotton effects of camphor and epi-camphor derivatives

Temperature, 22-24°

Solvents: M, methanol; D, dioxan; H, hexane; polarities as measured by E_T values are respectively 55.5, 36.0 and 30.9. $a/\Delta\epsilon$ should be 40.28 in the theoretical case of a perfect Gaussian c.d. curve. The experimental values are given to the nearest whole number.

Fine structure: 0, none; W, weak; M, medium; S, strong. The partial band of largest $\Delta\epsilon_{max}$ is shown in brackets.

* $[\Phi] \times 10^{-2}$ for single extremum.

Compound	Solvent	U.v. absorption λ_{max}/nm; (ϵ_{max} in parentheses)	C.d. λ/nm	$\Delta\epsilon$	O.r.d. λ/nm of extrema	a	$a/\Delta\epsilon$	Fine structure (c.d.)
Bornan-2-one	M	290(30)	295	+1.39	314/276	+58	42	0
(+)-Camphor	D		309sh	+1.04				
			299	+1.45	315/275	+63	43	W
			290sh	+1.20				
	H	292(24)	314sh	+1.01				
			303	+1.50	320/277	+63	42	MW
			294sh	+1.34				
endo-3-Hydroxybornan-2-one	M		314	+0.71	330/294	+36	43	0
	D		324	+0.61				
			314	+0.53	323/300	+31	49	W
			320	+0.31	331/303	+19	52	0
	H		288	-0.20	303/273	-9	42	
exo-3-Hydroxybornan-2-one	M	303(34)	307	+1.64	328/287	+67	41	0
	D		311	+1.70	333/287	+69	41	0
	H	301(31)	304	+1.85	329/283	+86	46	0

Table X (Contd.)

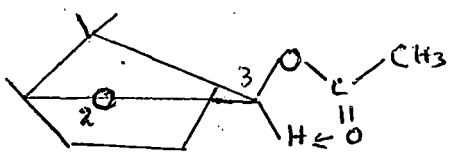
Compound	Solvent	U.v.absorption λ_{max}/nm; (ϵ_{max} in parentheses)	C.d. λ/nm	$\Delta\epsilon$	O.r.d. λ/nm of extrema	a	$a/\Delta\epsilon$	Fine structure (c.d.)
endo-3-Acetoxy-bornan-2-one	M		333	-0.03	330*	-2*pk		
			323	+0.06				W
			292	-0.425	306/270	-19	45	
	D		323	+0.365	330/306	+16	44	M
			312	+0.300				
			288	-0.155	306/270	-11	37	
	H	302(45)	325	+0.36	331/308	+17	47	MS
			313	+0.34				
			304sh	+0.10	303/263	-8		
			283	-0.09				
exo-3-Acetoxy-bornan-2-one	M		305	2.15	325/285	+95	44	O
	D		307	2.23	327/285	+103	46	O
	H	290(71)	304	2.18	328/285	+93	43	O
Bornan-3-one (-)-epi-Camphor	M		299sh	-1.58		-62	38	W
			295	-1.65	310/271			
	D		312sh	-1.04				
			303	-1.52	316/276	-64	42	W
			294sh	-1.36				
	H	295(27)	317sh	+0.93	[illegible]			
			306	-1.46	320/276	-62	42	M
			296sh	-1.34				

Contd.

Table X (Contd)

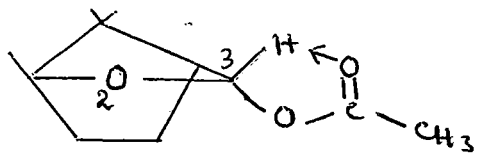
Compound	Solvent	U.v. absorption λ_{max}/nm; (ϵ_{max} in parentheses)	C.d. λ/nm	$\Delta\epsilon$	O.r.d. λ/nm of extrema	a	$a/\Delta\epsilon$	Fine structure (c.d.)
endo-2-Hydroxy-bornan-3-one	M		313	-1.78	329/289	-79	44	0
	D		329sh	-1.16				
			316	-1.34	331/297	-60	45	0
	H	300(19)	317	-0.76	330/297	-37	49	0
			283	+0.16				
exo-2-Hydroxy-bornan-3-one	M	303(37)	307	-1.64	325/281	-64	39	0
	D		311	-1.80	330/285	-72	40	0
	H	302(26)	304	-2.07	323/278	-82	40	0
2ndo-2-Acetoxy-bornan-3-one	M		333	+0.02	333	+7*		
			325	-0.05				
			293	+0.55	310/272	+19	35	0
	D		325	-0.25	331/308	-16	64	
			315sh	-0.09				W
			291	+0.38	308/273	+17	45	
	H	304(54)	327	-0.23	332/311	-14	61	W
			316sh	-0.08				
			292	+0.35	311/269	+15	43	
exo-2-Acetoxy-bornan-3-one	M		303	-2.40	326/280	-122	51	0
	D		308	-2.57	330/285	-115	45	0
	H	304(48)	308	-2.34	332/284	-105	45	0

of the type found by Mathieson[55] for secondary acetoxy groups, in which the carbonyl is eclipsed with secondary hydrogen (as in projection 39a and 39b).



39a

3β-exo



39b

3α-endo

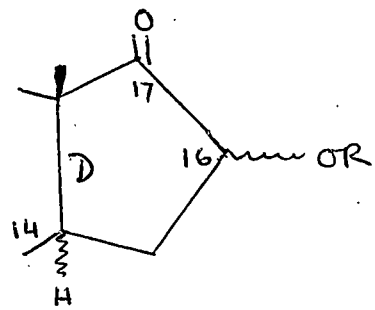
This should not be taken for granted in the present series of compounds because dipole- dipole interaction between the ketone and the ester carbonyl group may significantly alter the situation.[9]

The endo-acetoxy ketones (137, R^1 = H, R^2 = OAc) and (38, R^1 = H, R^2 = OAc) exhibit complex c.d. curves, possibly owing to solvation. The exo-compounds (acetoxy) show simpler curves than the endo-epimers. They do not show c.d. maxima of opposite sign (Table X).

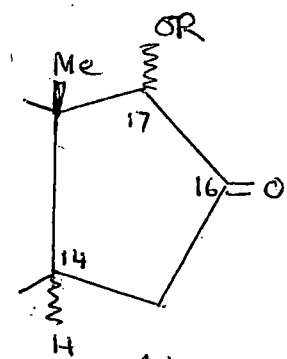
It will be observed that the four compounds (indicated by foot notes in Table IX) which show double c.d. maxima of opposite signs are the endo-3-hydroxy-2-one and the endo-2-hydroxy-3-one (in hexane only) and the corresponding endo-acetoxy ketones in all these solvents; in all cases this additional maximum (Ca 290 nm) shows an "Anti-Octant" effect with respect to the parent compound's maximum at 303 nm.

The authors have discussed in the paper the possible explanations for this effect. According to them, since the bornanones have relatively rigid structures conformational equilibria in the carbon framework can probably be excluded. Although a moderate degree of flexibility in substituted bornanes and related compounds has recently been demonstrated from X-ray studies[56], the authors suggest that the possible distortions are unlikely to alter significantly the Octant dispositions of structural features in these compounds and should not afford two conformationally distinct species. Further, they stated that although there is the possibility of two or more conformers about the C-OH and C-OAc bonds- such conformer are not likely to afford C.d. maxima of opposite sign. They, however suggest that there is the possibility that the "double humped" curves represent permitted and forbidden branches of the same transition.

Fishman and coworker[57] studied the O.r.d. of steroid ring-D Ketol-acetates (40, 41) which also present another example of "Anti-Octant" effect by oxygen-containing substituents (Table XI).The



40



41

anti-Octant A a values for OAc found in the bornanone series in Methanol (Ca ±60) agree well with values (Table X) recorded for the steroidal acetate 40 and 41.

Since, halogeno-substituents α-to the carbonyl in ring D of steroids show normal Octant effects on the o.r.d. and c.d. curves, it may be presumed that they cause no major distortion of Ring D. In the present case, also it is reasonable to assume that "Anti-Octant" behaviour of acetoxy group in ring D arises due to some cause other than conformational change.

Table XI

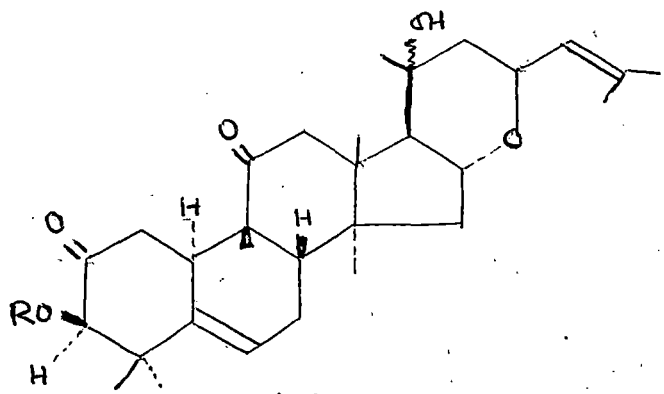
O.R.D. data for Steroid ring D Ketol acetates[56]

Solvent-dioxan.

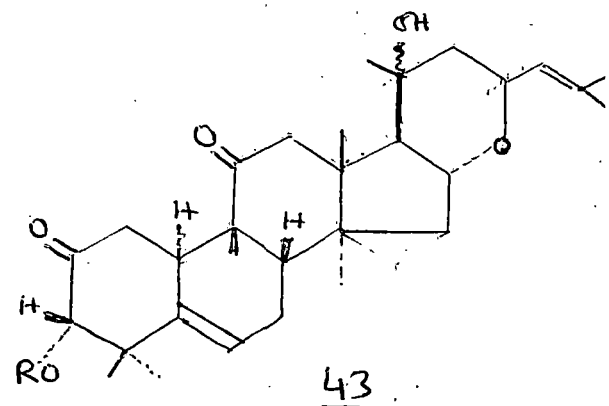
Ketone or acetoxy-ketone	a	Δa for OAc
14 α-Configuration		
17-CO	+132	
17-CO; 16α-OAc	+176	+44
17 CO; 16 β-OAc	+85	-47
16 CO;	-264	
16 CO; 17α-OAc	-313	-49
16-CO; 17 β-OAc	-202	+62
14 β -Configuration		
17 CO;	+35	
17 CO; 16α-OAc	+51	+16
17 CO; 16β-OAc	-57	-92
16 CO	+110	
16 CO; 17α-OAc	+ 96	-14
16 CO; 17β-OAc	+194	+84

C.D. measurements and Stereochemistry of the epimeric 3-hydroxy-2-Ketones viz. anhydro-22-deoxo isocucurbitacins D and anhydro-22-deoxo-3-epi-isocucurbitacin D:

In 1967, G.Snatzke and his co-workers[58] deduced the stereochemistry of the epimeric-3-hydroxy-2-ketones- the isomers (42; 43, R = H) on the basis of c.d. and other physical data.



42



43

The isomers 42 (R = H) and 43 (R = H) exhibited U.V maxima of 288-289 mm. The corresponding acetates (42; R = Ac) and (43 R = Ac) had U.V. maxima at 291 mm, while the I.R. peak due to the 2-ketone group appeared at 1736-37 cm^{-1}. These results indicated an equatorial conformation of the 3-hydroxyl or 3-acetoxy group in these compounds. The bathochromic shift (2 to 6 mm) of the c.d.

maxima on acetylation of the isomers (42, R = H) and (43, R = H), $\Delta\epsilon$ max remaining unaltered (Table XII and Fig. 13)also supported this conclusion. On the basis of the U.V. shift rules the fact that the C.d. maxima of the isomer (43; R = H) and its acetate (43; R = Ac) appear at a higher wavelength than those of the isomer (42; R = H) and its acetate (42; R = Ac) could have been considered as evidence for an axial orientation of the OR group in (43, R = H or Ac). Similarly from the N.M.R spectral data, the fact that proton at position 3 resonates at a higher field (43, R = Ac) at τ 5.05 than in 42 (R = Ac) at τ 4.92, could have been considered as evidence for an equatorial hydrogen (axial OAc group) in isomer 43 (R = OAc).

Table XII

Circular dichroism of ring A-Keto Chromophores

Compound	Position of maxima in mμ, $\Delta\epsilon$ in parentheses[58]
(42, R =H)	275sh (-3.02), 285(-3.46), 304sh(-1.15)
	315 sh (-0.12), 318 (0), 325 (+0.37)
(42 R = Ac)	279sh (-2.31), 291(-3.00), 298sh (-2.75)
(43, R = H)	250 (+0.13), 270(0), 295(-0.52), 304(-0.40)
(43, R = Ac)	267 (+0.08, 273(0), 297 (-0.48), 305(-0.40)

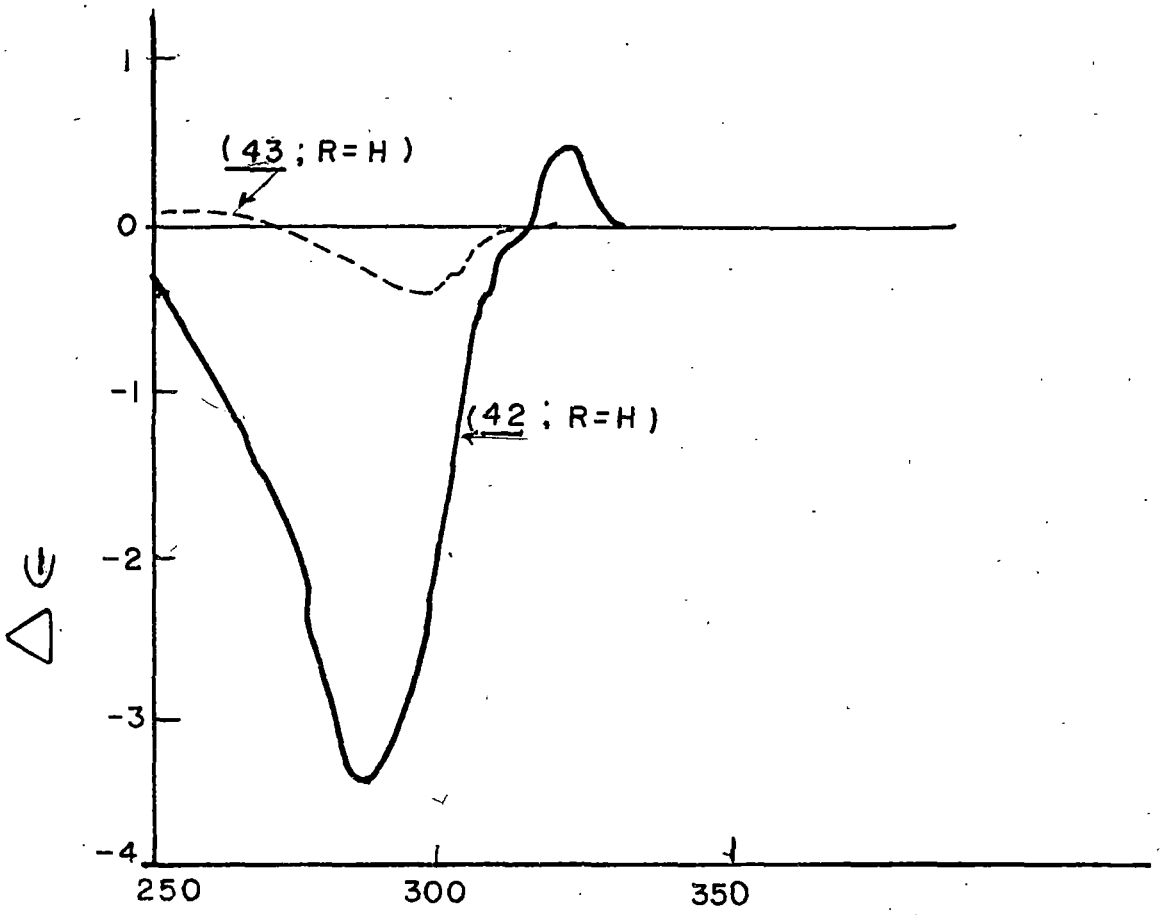


Fig. 13. Cotton effects of ring A →chromophore in 3-hydroxy-2-ketones (42 R=H) and (43 R=H)

These conclusions are, however, valid only if it is assumed that ring A is in the chair conformation in both isomers. In compounds with ring A in twist conformation e.g. in 2β-acetoxy cholestan-3-one[48] and 2β - bromo lanost-8-en-3-one[59] the Cotton effects show a hypsochromic shift compared to the 2α-isomers in the chair conformation. Furthermore, in the n.m.r. spectra of 2α-and 2β-acetoxy cholestan-3-one it has been found that the proton at position 2 resonates at lower field when it is axial on a twist ring (τ 4.88 in 2β-isomer) than when it is axial on a chair ring (τ 4.93 in 2α-isomer)[48]. Thus an equatorial conformation of the acetoxy group in both isomers (42, R = Ac) and (43, R = Ac) would require that ring A is in the twist conformation in the first case and in the chair conformation in the second.

The c.d. maxima of the isomers (42, R = H) and (43, R = H) differ in their amplitudes considerably (Table XII) indicating that the ring A in these two compounds has different conformations. From an Octant projection of the 2-ketone in the all chair form; one can predict a moderate negative Cotton effect Fig. 14; the C and D rings are somewhat mobile relative to ring A as a result of the Δ^5- double bond.

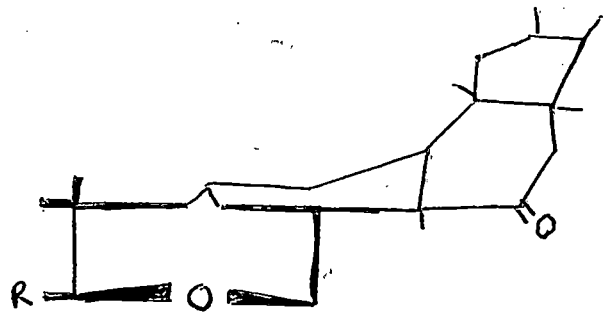


Fig. 14 Octant projection of a 2-oxo-cucurbitacin (R = OH or OAc) Ring A in chair conformation.

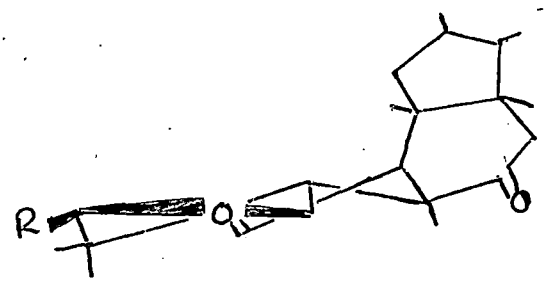


Fig. 15 Octant projection of a 2-oxo cucurbitacin (R = OH or OAc). Ring A in twist conformation.

In the twist form (Figure 15), according to known rules[25,47] a strongly negative Cotton effect can be predicted since the 2-ketone is one of the points of the twist. On the basis of above, the authors assigned isomer (43, R = H) a structure with ring A in the chair form and isomer (42, R = H), a structure with ring A in the twist form.

From considerations of (a) different interactions (b) energy differences between the chair form and twist from (c) geometry for hydrogen bonding, the authors deduced that ring A will prefer a conformation in which the hydroxyl group is in the equatorial position. For R = Ac in the 3β-isomer (42) in the all chair conformation there is a destablising repulsion between this axial group and the 1β-hydrogen. In the twist form, a 3α-axial acetoxy group suffers a flag-pole-flag-pole interaction with the 10α-hydrogen. From the above considerations they assigned OR group in compound (43, R = H or ØAc) as 3α-configuration (ring A chair) and compound (42, R = H or Ac) as 3β-configuration. It may be noted that in the two isomers, the double bond is in the nodal plane (x, z) of the CO-group and is therefore not expected to exert any direct influence upon the chromophore.

CHAPTER-II

Autoxidation Studies on β -amyrone: Investigation on the stereochemistry of 2 acetoxy-3-keto β -amyrin and 3-acetoxy 2 keto β -amyrin.

Section A: Introduction

A number of 2,3 isomeric diols of triterpenoids have been isolated from natural sources. A new method for the preparation of 2,3-diols of triterpenoids, from the diosphenol obtained by the autoxidation of a 3-keto triterpenoid has been described by Misra and Khastgir[62]. They[62] have shown that three out of the four isomeric 2,3-diols of Δ^{12}- oleanene can be successfully synthesised employing the diosphenol 45 as the starting material. The scheme of their procedure has been depicted in Chart 1 . The generality of the method has now been demonstrated by the synthesis of the similar 2,3-diols in the oleanonic acid series and Moretanone (Isohopanone) series (the details have been described in Part II Section B and C Pages 129, 160 of this thesis).

During their studies on the autoxidation of β -amyrone 44 Khastgir and colleagues[62] prepared 2-acetoxy-3-keto-β-amyrone and 3-acetoxy-2-keto-β-amyrin and assigned the structure and stereochemistry 47 and 49 respectively to these compounds on the basis of

NMR spectral evidences only. From the discussions which follow it will be evident that the n.m.r. spectral data alone were not sufficient to assign the stereochemistry of the compounds 47 and 49. We therefore undertook o.r.d. study of these compounds with the hope that this would provide a second criterion in addition to n.m.r. spectral evidence in establishing the stereochemistry of the compounds. The results of our o.r.d. and other physical studies are described in the following lines.

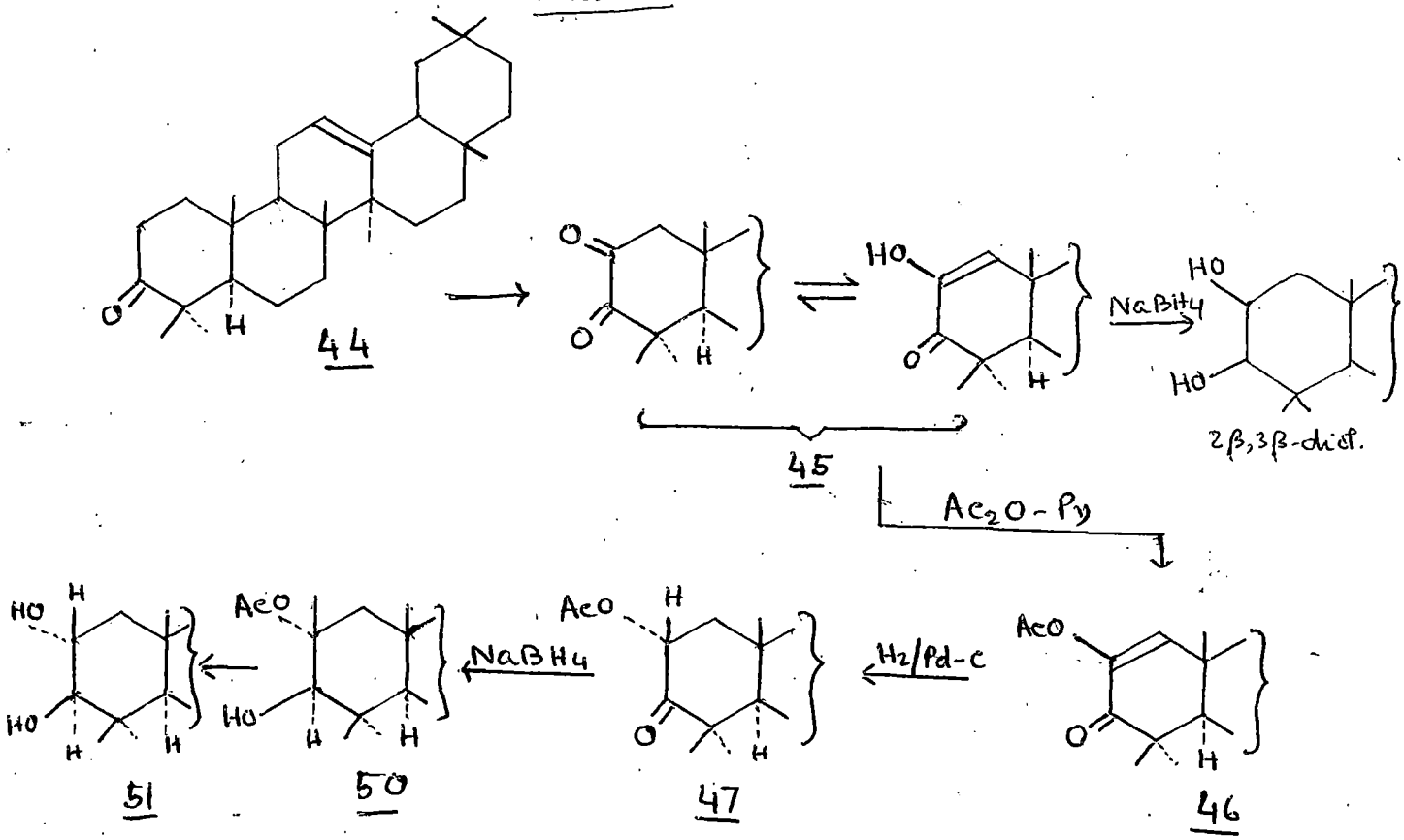
Section B: Autoxidation Studies on β-amyrone:

The starting material β-amyrone 44 was obtained by the acid isomerisation of Taraxerone[60] isolated from the bark of Sapium baccatum Roxb[61] (see experimental page 66). Oxidation of β-amyrone[62] 44 by passing oxygen through a suspension of it in tert. butanol containing K-tert butoxide gave a compound m.p. 200-202°, $(\alpha)_D$ 124.37°. The material showed positive ferric chloride coloration and two spots on a chromatoplate indicating that it was a mixture of diketone and diosphenol 45. Its U.V spectrum exhibited a maximum at 270 mμ (ϵ ,7932) and its I.R. spectrum in nujol showed peaks at 1100, 1650, 2960 and 3570 cm^{-1}. Acetylation of 45 with acetic anhydride and pyridine gave the acetate 46 (TLC homogenous), m.p. 172-73°, $(\alpha)_D$ 107.69°. The acetate showed a U.V absorption maximum at 236 mμ(ϵ, 9915) and its I.R. spectrum in nujol exhibited peaks at 1205, 1685, 1720, 2950 cm^{-1}. These spectral data are

in good agreement with the structure 45 and 46 for the diosphenol and its acetate respectively.

Diosphenol acetate 46 on hydrogenation in the presence of 10% Palladium-on-charcoal catalyst gave a solid product 47, m.p. 158-60°, $(\alpha)_D$ 108·57°, I.R. in KBr 1225, 1238, 1730 and 1750 cm^{-1}. The NMR of this compound (Fig. 16) showed a multiplet at 5.62 ppm for the proton at C-2[63], but no signals were detected in the region 4.95 ppm region characteristic for protons α to a keto group ($-CO.CH_2$) as was observed in the case of compound 49. As the X part of an ABX pattern with a width of 20 H_Z (sum of J) the proton must be axial with an axial-axial and axial-equatorial coupling with ring A in the chair conformation[48]. The NMR data is thus in accord

Chart-I



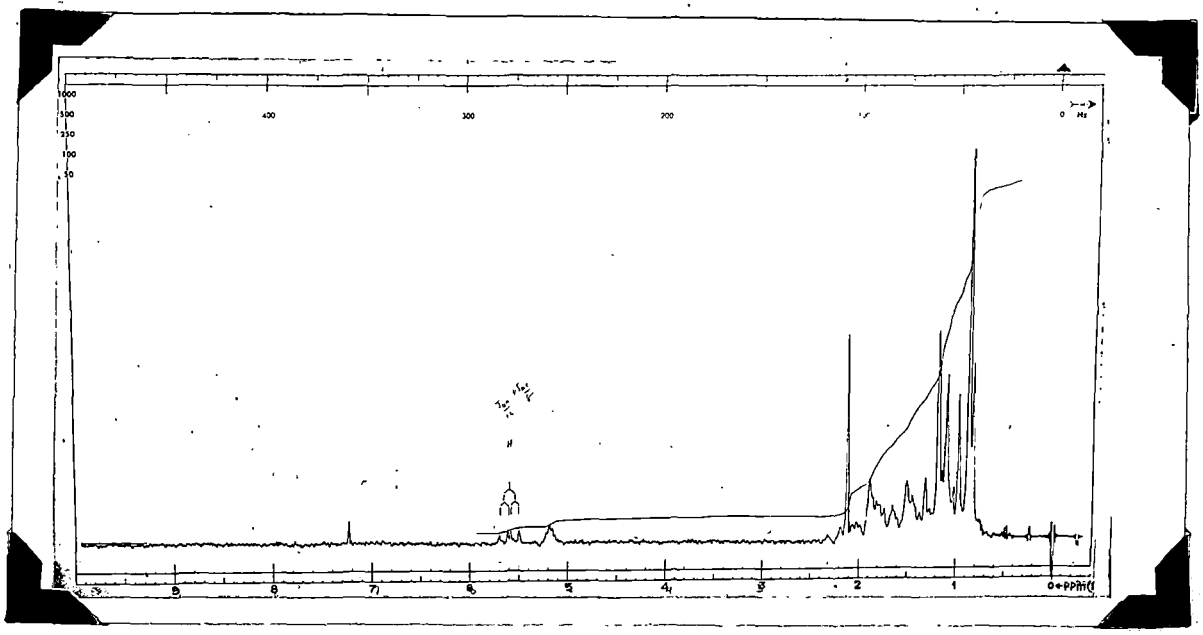
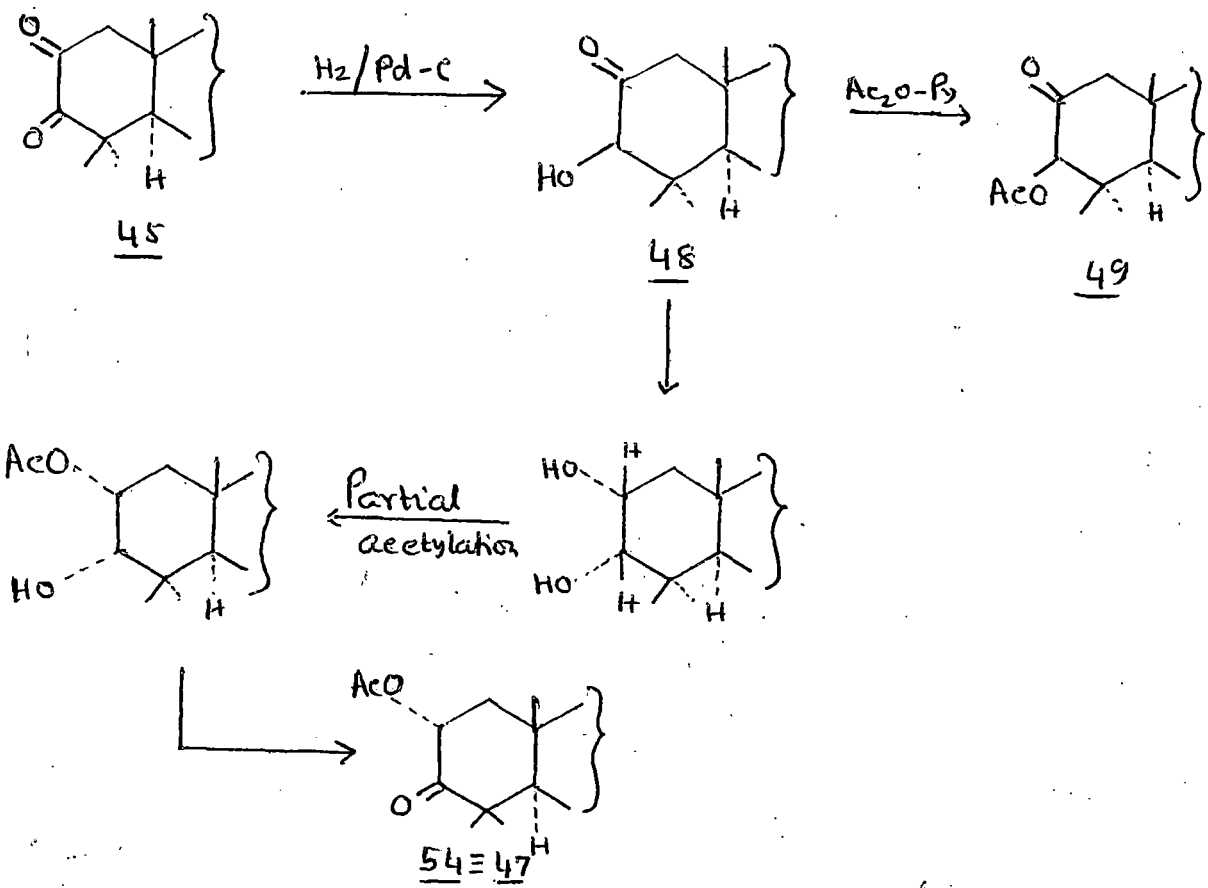


Fig. 16 NMR spectrum of 2α-acetoxy-β-amyrone 47



45 48 49

54 ≡ 47

with the formulation of this product as the 2α-acetoxy-3-ketone 47 — the acetoxy group at C-2 being equatorial.

O.R.D. studies on 44 and 47 : Anti-Octant effect of the acetoxy group:

It occurred to us that the NMR signals exhibited by 47 as described above could have been considered as evidence for an 2β-axial orientation of the 2-acetoxy group in the boat conformation of ring A as well as for 2α-equatorial acetoxy group with

the chair conformation of Ring A. We have already described the o.r.d. and c.d. studies of Bull and Enslin and Klyne et al (page 34,42 of this thesis) from which it appears that in many cases, though not in all, the effect of an acetoxy group to the carbonyl chromophore is anti-Octant i.e. the contribution to the carbonyl $n-\pi^*$ Cotton effect is opposite to that of an alkyl group in the same position. Since o.r.d. studies are expected to throw more light on the stereochemistry of 47, we carried out o.r.d. measurements on the parent ketone 44 (Fig. 17) and 47 (Fig. 18). If we consider our parent ketone 44, the conformation of ring A is most likely to be a flattened chair to relieve the diaxial interaction between the 10β-methyl and 4β-methyl group and this leads to a positive Cotton effect[64]. The same conformation is possible for the 2α-acetoxy derivative 47 but as a consequence of the flattening of ring A, the 2α(equatorial) acetoxy group does not lie in the nodal plane of the carbonyl group but protrudes into the back upper right Octant. An alkyl group in that situation would make a negative contribution to the Cotton effect but the acetoxy group shows an "anti-Octant" effect and makes a positive contribution. Therefore, as the o.r.d. studies indicates (Fig. 18), if the compound 47 has the 2α-acetoxy configuration, we would expect it to have a more positive Cotton effect than the parent ketone. The alternative 2β-acetoxy configuration with the boat conformation of ring A would lead to a small negative Cotton effect. In the ORD curve of the

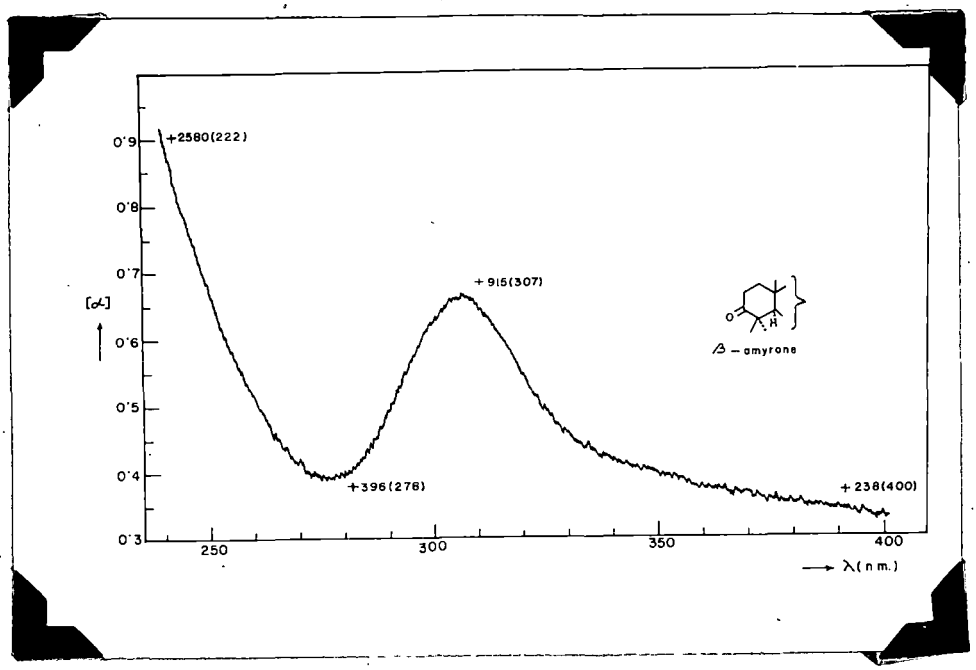


Fig. 17 ORD curve of β -amyrone 44

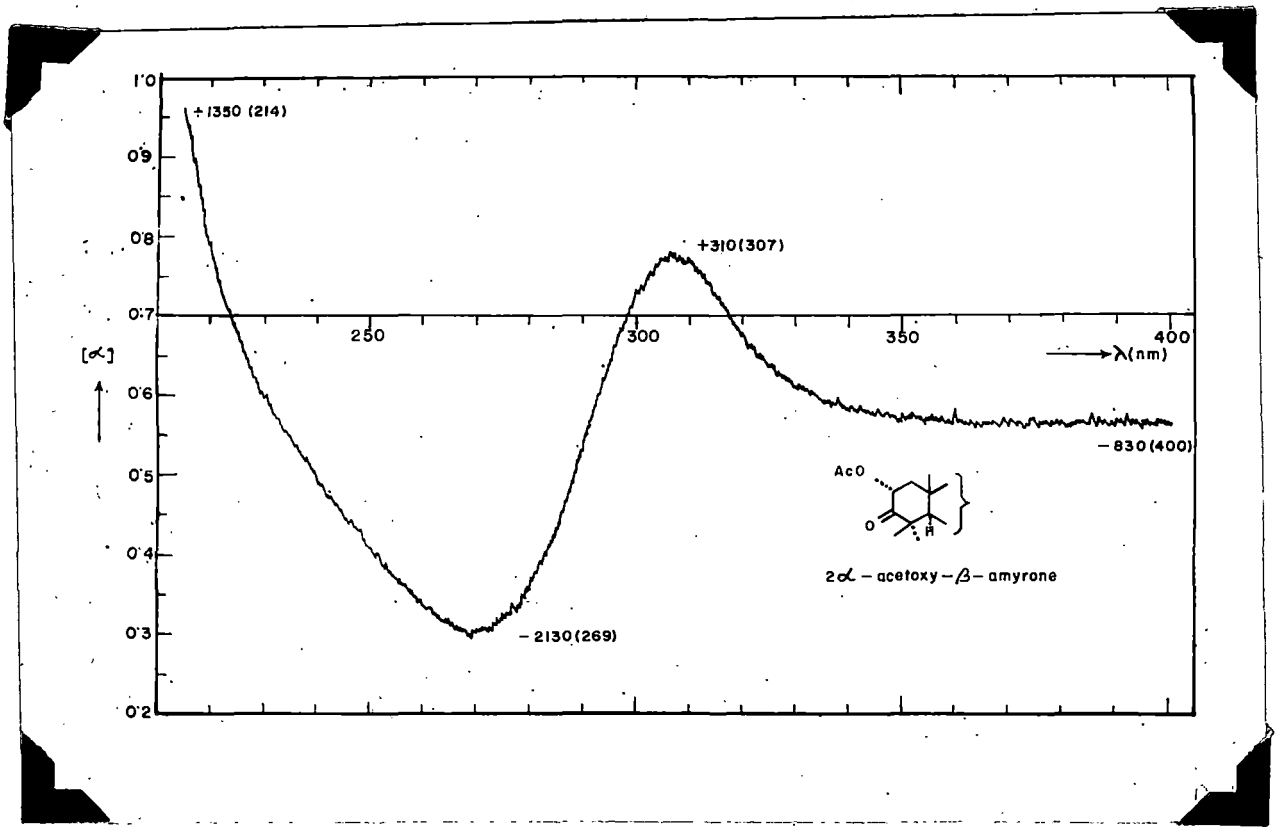


Fig. 18 ORD curve of 2α-acetoxy β -amyrone 47

compound 47 the amplitude [redacted] is greater than the amplitude in the corresponding parent ketone. Therefore, from O.R.D. results coupled with n.m.r. spectral data it appears most likely that compound 47 has the 2α-equatorial acetoxy configuration.

O.r.d. study on 2-keto-3-acetoxy -β-amyrin 49:

Hydrogenation[62] of 45 in presence of 10% Palladium-on-charcoal catalyst gave a solid 48, m.p. 180°-82°, $(\alpha)_D$ 101.70° λ_{max} 270 mμ (ϵ = 43), ν^{nujol}_{max} 1720 and 3500 cm^{-1} (~~Fig.~~ → NMR spectrum (Fig. 19) of 48 showed a signal for an olefinic proton (C-12H) at 5.09 ppm as a multiplet. A broadened multiplet appeared at 3.89 ppm which collapsed to a doublet (J = 1.5 Hz) and also a hydroxyl proton (-OH) signal at 3.43 ppm which disappeared upon treatment with D_2O . This information along with the presence of AB doublets at 2.48 ppm (d, J = 12.5 Hz) and 2.08 (d,d, J = 12.5, 1.5 Hz) suggested structure 48 for the hydrogenated product. The small coupling (1.5 Hz) of C-3H is consistent with a long range coupling to one of the protons at C-1. Acetylation[62] of the latter gave the acetate 49, m.p. 276-78°, $(\alpha)_D$ +127.08°, UV λ_{max} 276 mμ (ϵ , 81), IR ν^{KBr}_{max} 1725 and 1740 cm^{-1}.

The NMR spectrum of 49 (Fig. 20) has the olefinic proton signal at 5.18 ppm (broad multiplet) and the doublet (C-3H) shifted to 4.95 ppm (d, J = 1.5 Hz) consistent with acetylation of the C-3

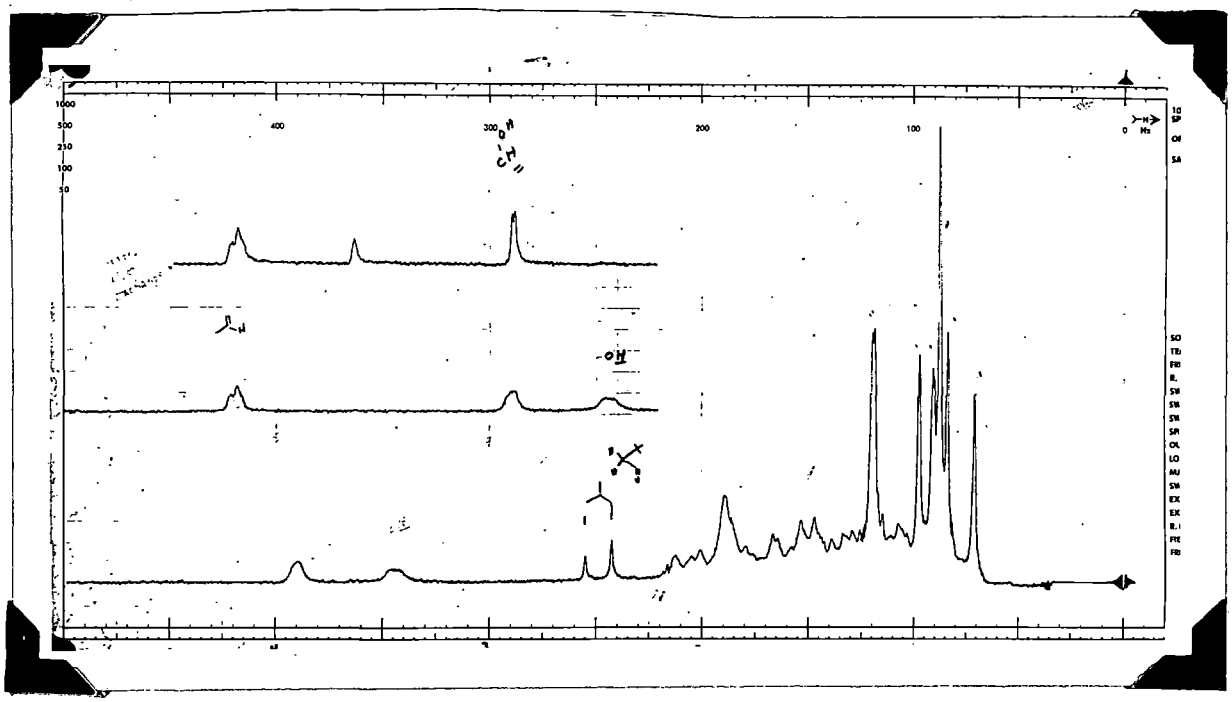


Fig. 19 NMR spectrum of 2 keto-β-amyrin 48

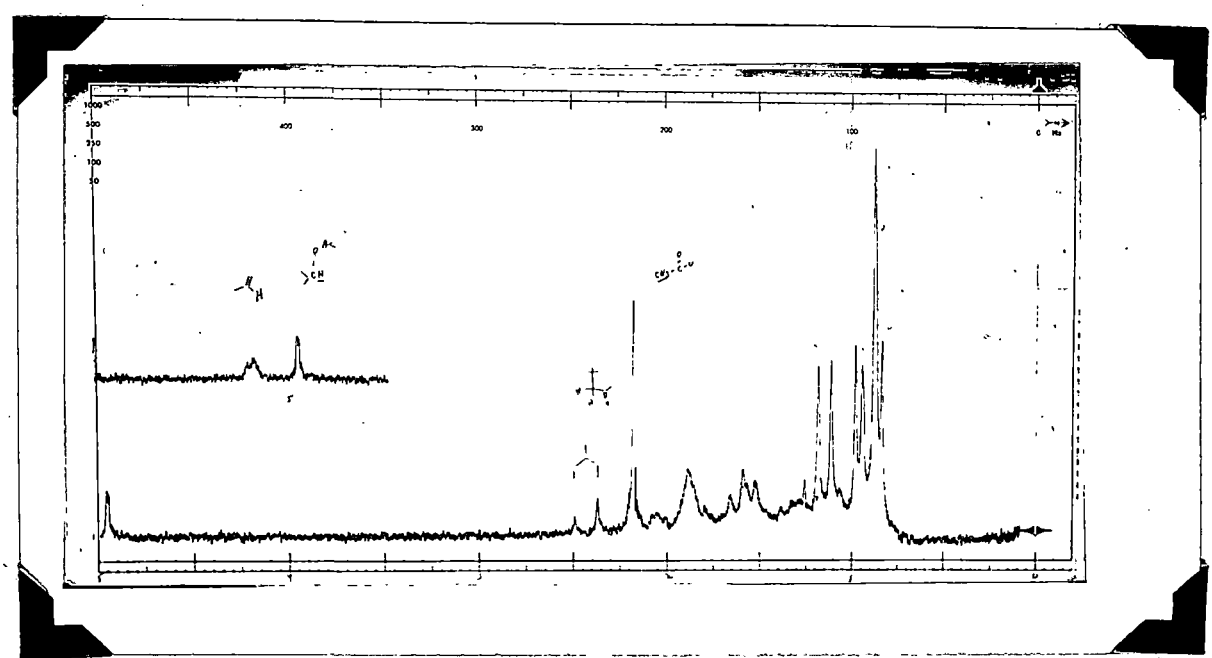


Fig. 20 NMR spectrum of 2 keto-β-amyrin acetate 49

hydroxyl group. Half of the AB pattern appeared at 2.42 ppm (d, J = 12.5 Hz), the other half was obscured by other signals but was estimated to be near 2.15 ppm. During the process of hydrogenation it is presumed that the attack of the hydrogen atom took place from the less hindered side (1, 4-addition) with the formation of 2-keto β-amyrin 48.

O.r.d. measurement of 49 was carried out and is shown in Fig. 21. The compound 49 with chair conformation of ring A and a 3β-equatorial acetoxy group would be expected to exhibit a positive Cotton effect. This is in accordance with the o.r.d. experimental results. Thus the stereochemistry as shown in 49 is consistent with the o.r.d. and n.m.r. spectral evidences.

The stereochemistry of 2-acetoxy group in 47: Chemical evidences:

In order to adduce further chemical evidences for the stereochemistry of 2-acetoxy group Misra and Khastgir[62] carried out the reduction of 47 with sodium borohydride buffered at pH 8 (to reduce isomerisation)[63] and obtained the reduced product 50, m.p. 246°-48°, ν_{max}^{nujol} 3430 (hydroxyl), 1720, 1245 cm^{-1} (acetate). The compound 50 on hydrolysis gave a solid m.p. 202-4°, $(\alpha)_D$ 60.0°. The latter has been assigned structure 51 as it has been found to be identical with an authentic sample of Δ^{12}-oleanene 2α, 3β-

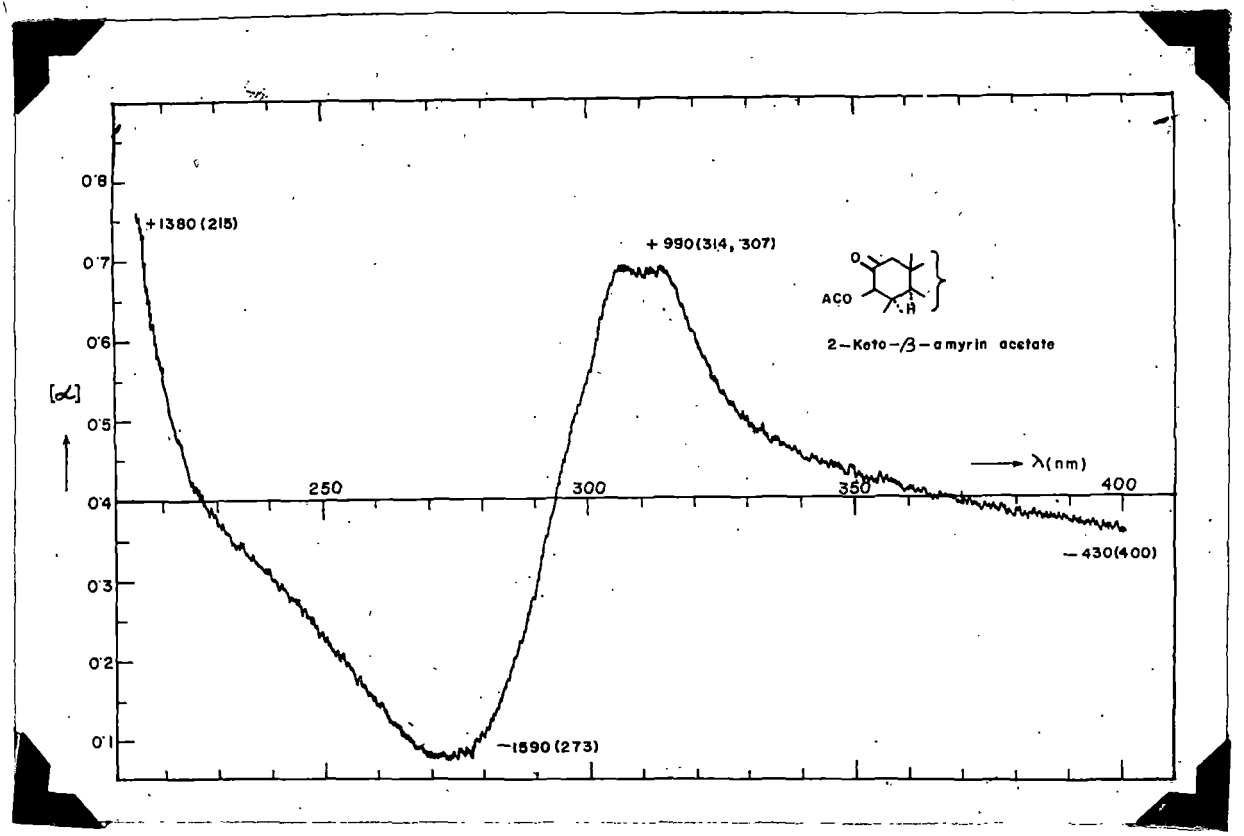


Fig. 21 ORD curve of 2 keto-β-amyrin acetate 49

diol (m.m.p) prepared by Sengupta et al[65] from crategolic acid. The NMR of 51 (Fig. 22) showed signals for C-2H and C-3H at 3.67 (t, d, 10, 10.5 Hz) and 3.00 ppm (d, 10 Hz) respectively in addition to the olefinic proton signal at 5.18 ppm. The splitting of C-2H is consistent with its having large axial-axial coupling to C-3H_a and C-1H_a and a smaller axial-equatorial coupling to C-1 H_e. The above evidences clearly indicate the α-equatorial orientation of the acetoxy group in 47 provided we argue that in the sodium borohydride reduction no isomerisation had taken place.

Further it has also been reported by Misra and Khastgir[62] that Meerwein Pondorff reduction of 2-keto-β-amyrin 48 afforded a solid m.p. 278-80° $(\alpha)_D$ 71.28°, ν_{max}^{nujol} 3420, 2960, 1450, 1040 and 835 cm^{-1} and has been assigned by them the 2α, 3α-diol structure 52 on the basis of its NMR spectra. The NMR of 52 (Fig. 23) has a doublet at 3.41 ppm (after D_2O exchange) with splitting of 3 Hz, indicative of either equatorial-equatorial or axial-equatorial coupling. The signal for C-2H appeared as a double triplet at 3.98 ppm with splittings of 12.5, 3 and 3 Hz, establishing it as an axial proton with one axial-axial and two axial-equatorial splittings.

In order to provide further chemical evidence for the stereochemistry of the 2-acetoxy group in 47 we prepared the monoacetate 53 by the partial acetylation of 52 with a calculated amount of acetic anhydride (1.1 mole) in pyridine for 12 hours at

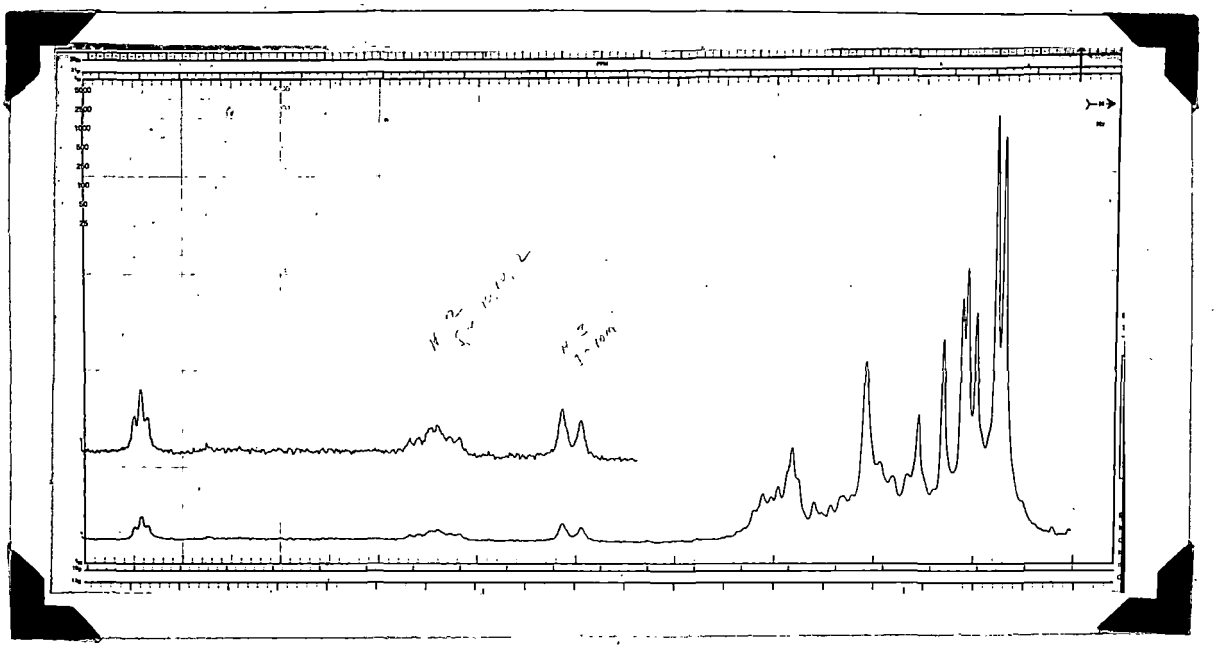


Fig. 22 NMR spectrum of 2α , 3β -diol 51

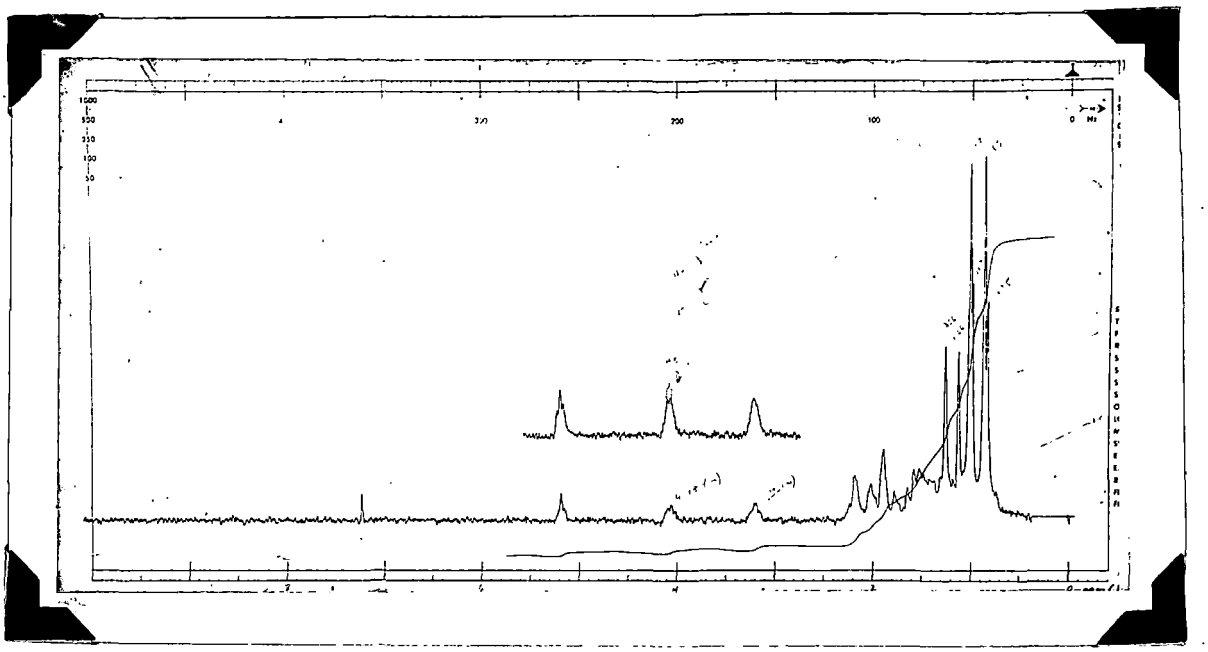


Fig. 23 NMR spectrum of 2α , 3α diol 52

0° C. The monoacetate 53 had m.p. 195°-7°, $(\alpha)_D$ 89.53°, IR ν_{max} I.R. ν_{max}^{nujol} 3450 (OH), 1750(OAc) and 1720 (ketonic carbonyl), 1280 cm^{-1} (Fig. 24) . Oxidation of the latter compound by CrO_3-Pyridine method[66] gave a crystalline solid 54 (TLC homogenous) m.p. 158-9° $(\alpha)_D^{108.5^{\circ}}$ which was indistinguishable from compound 47, (mixed melting point determination and Co-TLC) thus providing a further complimentary evidence for α-equatorial orientation of the acetoxy group in 47.

O.R.D. studies, NMR spectral observations and chemical evidences lead us to conclude without any ambiguity that the 2-acetoxy group in 47 has the α-equatorial configuration with ring A in the chair conformation.

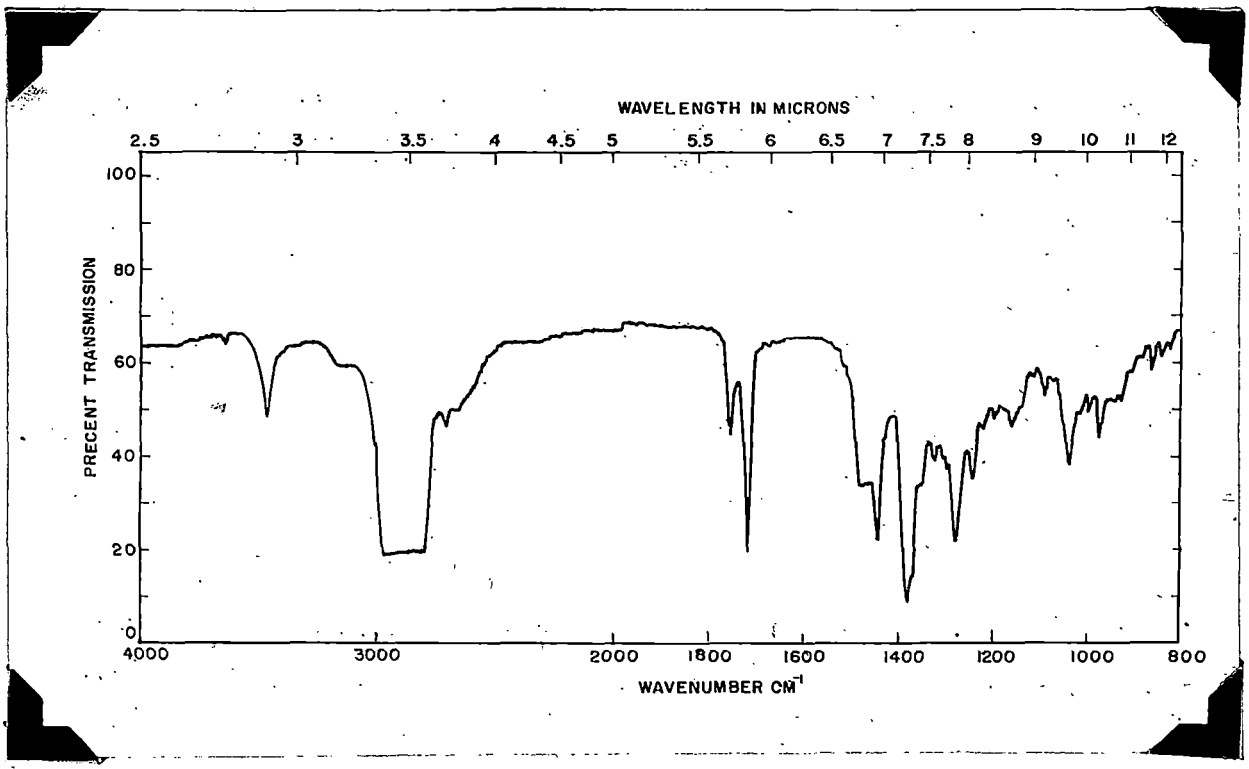


Fig. 24 IR spectrum of 2α-acetoxy-β-amyrin 53

CHAPTER -III

EXPERIMENTAL

Melting points are uncorrected. Petroleum ether used throughout the investigation had b.p. 60-80°. All optical rotations were determined in chloroform solution unless otherwise stated. NMR spectra were determined on Varian A-60 and HA-100 spectrometers using chloroform - d solution containing tetramethyl silane as reference. IR spectra were recorded in a Perkin Elmer 337 and 221 spectrophotometer. U.V. absorption spectra were taken in a Zeiss VSU-1 spectrophotometer in 95% ethanol solution unless otherwise stated. Thin layer chromatography was done on chromatoplate of Silica gel G (E.Merck) and the spots were developed with sulphuric acid-acetic anhydride (9:1) mixture.

Isolation of taraxerone[61]:

Dried and powdered Stembark of Sapium baccatum Roxb (2 Kg) was extracted with benzene in a soxhlet apparatus for twenty hours. On cooling the benzene extract a yellow insoluble solid precipitated out which was collected by filtration and was kept aside (latter identified as 3,3'-di-o-methyl ellagic acid[66]). From the clear filtrate benzene was distilled off and the residual gummy solid (30 gm) was taken up in ether (2 lit.). A cloudy precipitate which remained in the ether extract was separated out by filtration. The clear ether solution was washed with 10% sodium hydroxide solution (4 x 200 ml)

and then washed with cold water till the washings were neutral and dried over anhydrous sodium sulphate. The solvent was evaporated, when the neutral material (10.6 gm) was obtained as a yellow gummy solid which after chromatography and crystallisation from chloroform-methanol gave shining crystal (1.3 gm) m.p. 238-40° $(\alpha)_D$ +10.8°. Its melting point was not depressed when mixed with an authentic sample of taraxerone. It also showed identical IR throughout the region when compared with that of an authentic specimen of taraxerone.

Other compounds isolated were 1-hexacosanol, taraxerol and another solid of m.p. 210-15°.

Isomerisation of taraxerone[60]: Preparation of β-amyrone 44

To a suspension of taraxerone (600 mg) in glacial acetic acid (140 ml) maintained at 90° was added conc. hydrochloric acid (4 ml) and the reaction mixture was heated on a water bath for twenty minutes during which the solid dissolved in the solvent. The reaction mixture was then cooled and diluted with water. A solid came out which was taken up in ether. The ether layer was washed with water till neutral. On removing the solvent a solid came out (520 mg). The solid on crystallisation from chloroform and methanol mixture afforded fine crystals m.p. 174-6°, $(\alpha)_D$ +105.6° which was found to be identical with an authentic sample of β-amyrone 44 (m.m.p. and rotation).

Autoxidation of β-amyrone 44 : Isolation of Diosphenol 45

β-amyrone (2 gm) suspended in potassium tertiary butoxide in tertiary butanol (prepared from 6 gm of potassium and 160 ml of tertiary butanol) was shaken in a stream of oxygen for three hours. The reaction mixture was diluted with water and then 6N hydrochloric acid was added till the solution was acidic. It was then extracted with chloroform (150 ml), washed with water till neutral and the combined extract was dried (Na_2SO_4). On removal of the solvent under reduced pressure, a yellowish gummy foam was obtained (1.8 gm). The latter on crystallisation from acetone and methanol gave colorless crystals of the diosphenol 45, (1.2 gm), m.p. 200-2°, $(\alpha)_D$ +124.27°. It gave a positive ferric chloride test for diosphenol. TLC of the compound 45 showed two spots on chromatoplate (using benzene as solvent), an upper spot R_f = 0.77 of slightly weaker intensity than the lower spot R_f = 0.75. These were assumed to be due to the tautomeric mixture of the diketone and the diosphenol 45.

Found: C, 79.50, H, 9.83%

Calculated for $C_{30}H_{46}O_2$ $\frac{1}{2}CH_3OH$ C, 79.15; H, 10.62%

UV : λ_{max} 270 mμ(ε , 5104)

IR : ν^{nujol}_{max} 3570, 2960, 1650, 1100cm^{-1}.

Preparation of Diosphenol acetate 46 : Acetylation of Diosphenol 45

Diosphenol 45 (500 mg) was acetylated by treatment with acetic anhydride (10 ml) and pyridine (10 ml) overnight at room temperature. After working up in the usual manner the crude acetate (460 mg) was obtained. This was then chromatographed over a column of alumina (20 gm) deactivated with 0.8 ml of aqueous acetic acid.

Table I

Chromatography of the above solid (460 mg)

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1-2	Oil (trace)
Petroleum ether: benzene (9:1)	3-4	Nil
Petroleum ether: benzene (4:2)	5-9	Solid m.p. 165-7° (320 mg)

Further elution with more polar solvents did not afford any solid material.

The solids (320 mg) from fractions 5-9 (table I) were collected which after crystallisation from a mixture of chloroform and methanol afforded crystals m.p. 172-3°, $(\alpha)_D$ 107.69°. It showed a single round spot on a chromatoplate.

Found: C, 80.17; H, 9.78%.

Calculated for $C_{32}H_{48}O_3$: C, 80.00; H, 10.00%

UV: λ_{max} 236 mμ, (ϵ, 9915)

IR: ν_{max}^{Nujol} 2950, 1720, 1685, 1205 cm^{-1}.

Hydrogenation of diosphenol acetate 46 : Preparation of 2α-acetoxy β-amyrone 47:

To diosphenol acetate 46 (200 mg) dissolved in absolute ethanol was added 10% palladium-on-charcoal catalyst (50 mg) and the mixture was shaken in an atmosphere of hydrogen till the absorption of hydrogen ceased (absorption of one mole equivalent of hydrogen within one hour). The solution was filtered and after removing the solvent from the filtrate an oily residue (200 mg) was obtained which was chromatographed over silica gel (20 gm).

Table II

Chromatography of the above oily residue (200 mg)

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1-3	Oil (trace)
Petroleum ether: benzene (9:1)	4-8	Solid m.p. 155-7° (120 mg)

On elution with more polar solvents did not afford any crystalline solid.

The solids from fractions 4-8 (table II) were combined and after crystallisation from chloroform and methanol mixture afforded a crystalline solid m.p. 158-60°, $(\alpha)_D$ +108.57° and was found homogenous on a chromatoplate (R_f 0.38 in benzene).

Found: C, 79.18; H, 10.28%

Calculated for $C_{32}H_{50}O_3$: C, 79.66; H, 10.44%

IR: ν^{KBr}_{max} 1730, 1238, 1225 cm^{-1}.

NMR (100 Me/S): Peaks at 2.12 (3H, -O-COC<u>H</u>$_3$), 5.19 (1 H multiplet, vinyl proton), 5.5, 5.595, 5.6, 5.7 (1H, -Co-C<u>H</u>(OAc)-CH_2) ppm (Fig.16)

Hydrogenation[62] of Diosphenol 45 : Preparation of 2 Keto-β-amyrin 48:

Diosphenol 45 (500 mg) dissolved in a mixture of absolute ethyl alcohol (20 ml) and ethyl acetate (150 ml) was stirred in presence of 10% palladium-on-charcoal catalyst (50 mg) in an atmosphere of hydrogen till the absorption ceased. The catalyst was removed by filtration and the solvent was distilled off under reduced pressure from the filtrate. A solid residue (460 mg) was obtained which after crystallisation from chloroform-methanol furnished a solid of m.p. 180-182°, $(\alpha)_D$ 101.70°. The solid did not give ferric chloride coloration and showed one single spot on chromatoplate (R_f 0.44 in benzene).

Found: C, 82.29; H, 10.94%

Calculated for $C_{30}H_{48}O_2$: C, 81.81; H, 10.90%

UV: λ_{max} 270 mμ (ε, 43).

IR : ν^{nujol}_{max} 3500 cm^{-1} (hydroxyl), 1720 cm^{-1}(carbonyl).

NMR (100 Mc/S): Peaks at 3.88 (1H, H-C-OH), 3.44 (-C-OH), 2.42, 2.55 (2H, CO-CH_2), 5.18 (multiplet, vinyl proton) ppm.

Fig. 19.

Acetylation of 2 Keto-β-amyrin 48 : Preparation of 2-keto-β-amyrin acetate 49

The hydroxy ketone 48 (200 mg) was treated with acetic anhydride (5 ml) and pyridine (5 ml) and kept overnight at room temperature. Next day a crystalline solid separated out from the

solvent mixture, which was collected by filtration. The latter after crystallisation from chloroform-methanol mixture gave crystals m.p. 276-8° $(\alpha)_D$ 127.08°. The filtrate on dilution with ice cold water precipitated from above a solid which after usual working up and crystallisation from chloroform methanol mixture afforded crystals m.p. 276-8° and was found to be identical with the above acetate. The solid 49 showed a single spot on chromatoplate (R_f 0.35 in benzene).

Found: C, 79.56; H, 10.05%

Calculated for $C_{32}H_{50}O_3$: C, 79.66; H, 10.37%

UV: λ_{max} 276 mμ (ε, 81)

IR: ν_{max}^{KBr} 1725 and 1740 cm^{-1}

NMR (100 Me/S): Peaks at 4.95 (1H, H-C-OCOCH$_3$), 2.49, 2.37 (2H, -CO-CH$_2$), 2.16(3H, -OCOCH$_3$), 5.2 (multiplet 1H, vinyl proton)ppm (Fig. 20).

Sodium borohydride reduction of 2α-acetoxy-β-amyrone 47: Preparation of Δ^{12}-Oleanene 2α-acetoxy-β-amyrin 50:

To 2α-acetoxy-β-amyrone (300 mg) dissolved in dry dioxan (25 ml) was added, with cooling a slurry of sodium borohydride (300 mg) prepared in a NH_4Cl-NH_4OH buffer (P_H = 8, 4 ml). The mixture was stirred at room temperature for two hours. A portion of the solvent was removed by distillation, cooled and acidified with dilute hydrochloric acid and then extracted with ether. The ethereal

layer was washed with water till neutral and dried (Na_2SO_4). Removal of ether gave a solid residue (250 mg) which was chromatographed over a column of alumina (30 gm, deactivated with 1.2 ml of 10% aqueous acetic acid) developed with petroleum ether. The residue was dissolved in benzene, poured on the column and was eluted with the following solvents.

Table III

Chromatography of the above solid 250 mg

Eluent	Fractions 50 ml each	Residue on evaporation.
Petroleum ether	1-4	Oil small amount (12 mg)
Petroleum ether: benzene (4:1)	5-7	Nil
Petroleum ether: benzene (3:2)	8-14	Solid (210 mg) m.p. 240-5°

Further elution with more polar solvents did not afford any solid material.

The solid from fractions 8-14 (Table III) were collected and crystallised from chloroform-methanol mixture. After two crystallisation pure 2α-acetoxy-β-amyrin 50 m.p. 246-8° was obtained.

Hydrolysis of Δ^{12}-Oleanene-2α-acetoxy-β-amyrin 50 : Preparation of Δ^{12} Oleanene 2α, 3β-diol 51:

To the Δ^{12} oleanene 2α-acetoxy-β-amyrin 50 (200 mg) in dioxan (40 ml) was added 10% sodium hydroxide solution (10 ml) and the mixture was heated under reflux for three hours. The reaction mixture was then cooled, diluted with water and extracted with ether. The ethereal layer after washing with water till neutral was dried (Na_2SO_4). The solvent was removed and a solid (190 mg) m.p. 196-8° was obtained. After crystallisation from methanol it afforded pure 2α, 3β diol 51, m.p. 202-4° $(\alpha)_D$ +60.00°. This was found to be identical with an authentic sample of 2α, 3β dihydroxy - Δ^{12}-oleanene.

Found: C, 81.84; H, 11.62%

Calculated for $C_{30}H_{50}O_2$: C, 81.44; H, 11.31%

UV: No absorption in the range 220-300 mμ.

I.R. spectra: ν_{max} 3360, 2970, 1425, 1375, 1350, 1050, 1030 cm^{-1}

NMR spectra (100 Mc/S): Peaks at 2.94, 3.14 (1H, H-C_3-OH), 3.74 (quartet of a doublet, H-C_2-OH), 5.18 (multiplet 1 H, vinyl proton) ppm Fig. 22.

Preparation of Δ^{12}-Oleanene 2α, 3α-diol 52 : Meerwein Pondorff reduction of 2 keto-β-amyrin 48:

A mixture of 2 keto-β amyrin 48 (500 mg), Al-isopropoxide (650 mg) in dry isopropanol (12.5 ml) was distilled slowly with the addition of isopropanol to maintain constant volume. After 5 hours the distillate no longer contained acetone and the solution was concentrated to a small volume. The reaction mixture was diluted with water followed by 10% sulphuric acid solution (20 ml) and then extracted with ether. The product obtained after removal of ether was dissolved in benzene (6 ml) and poured on a column of alumina (25 gm deactivated with 1 ml of 10% aqueous acetic acid) developed with petroleum ether. The following solvents were used for elution.

Table IV

Chromatography of the above product (450 mg)

Eluent	Fractions 50 ml each	Residue
Petroleum ether	1-3	Nil
Petroleum ether: benzene (3:1)	4-6	Nil
Petroleum ether: benzene (1:1)	7-9	Nil
Petroleum ether: benzene (1:3)	10-12	Nil
Benzene	13-15	Nil

Contd...

Table IV (Contd)

Eluent	Fractions 50 ml each	Residue
Benzene: ether (4:1)	16-21	Solid (400 mg) m.p. 276-8°
Benzene: ether (4:1)	22-25	Solid (48 mg) m.p. 196-8°

Further elution with more polar solvents did not give any solid material.

The solid from fraction 16-21 (Table IV) were combined which after crystallisation from chloroform-methanol afforded the crystalline 2α, 3α-diol 52 (350 mg), m.p. 278-80°, $(\alpha)_D$ 71.28°.

Found: C, 81.40; H, 11.08%

Calculated for $C_{30}H_{50}O_2$: C, 81.44; H, 11.31%

U.V. : No absorption in the region 220-300 mμ

IR spectra: ν_{max}^{nujol} 3420, 2960, 1450, 1040, 835 cm^{-1}

NMR (100 Mc/S): Peaks at 3.2 (broad 1H, $\underline{H}$-C_3-OH), 4.06, 4.1 (doublet 1H, $\underline{H}$-C_2-OH), 5.2 (multiplet 1H, vinyl) ppm. (Fig. 23)

The solids from fractions 22-25 (table IV) after crystallisation from methanol afforded a crystalline solid (28 mg) m.p. 200-2° identical with 2α, 3β-diol 51 described on page 75.

Partial acetylation of Δ^{12}-Oleanene-2α, 3α-diol 52 : Preparation of 2α-acetoxy -3α-hydroxy β-amyrin 53:

200 mg of the 2α, 3α-diol 52 was treated with pyridine (12 ml) and acetic anhydride (0.5 ml, 1.1 mole) and the reaction mixture was allowed to stand at 0°C for 12 hours. The reaction mixture was then poured on ice-water and extracted with ether. The ethereal layer was washed with water till neutral and dried (Na_2SO_4). After removal of ether a solid (150 mg) was obtained. The solid was dissolved in benzene (2 ml) and poured on a column of alumina (12 gm, deactivated with 0.5 ml of 10% aqueous acetic acid) developed with petroleum ether. The following solvents were used for elution.

Table V

Eluent	Fractions 50 ml each	Residue on evaporation
1. Petroleum ether	1-3	Oil (trace)
2. Petroleum ether: Benzene (4:1)	4-8	Solid (120 mg) m.p. 193-4°

Elution with more polar solvent did not give any solid material.

The solids from fractions 4-8 (table V) were combined which after crystallisation from methanol afforded crystalline mono-acetate 53 m.p. 195-7°, $(\alpha)_D$ 89.53°.

This compound showed a single spot on a chromatoplate (R_f 0.409) in benzene.

Found: C, 79.40; H, 10.61%

Calculated for $C_{32}H_{52}O_3$: C, 79.29; H, 10.81%

I.R. : ν_{max}^{nujol} 3450 (-OH), 1750 ($-OCOCH_3$) 1720(ketonic carbonyl), 1280 cm^{-1} (Fig. 24).

Oxidation[67] of 2α-acetoxy-3α-hydroxy β-amyrin 53 : Preparation of 2α-acetoxyβ-amyrone 54:

40 mg of chromium trioxide was added to a solution of pyridine (5 ml) in methylene chloride (35 ml) and the mixture stirred for 15 minutes. 200 mg of 2α-acetoxy-3α-hydroxy β-amyrin dissolved in methylene chloride (10 ml) was added to the stirred solution and the stirring was continued for another 15 minutes. The solution was then decanted and taken in ether. The ethereal layer was washed with 5% sodium hydroxide (5 ml portions twice) and then with water till neutral and dried over Na_2SO_4. On removal of ether a solid was obtained which on crystallisation from chloroform-methanol gave crystalline ~~the~~ solid 54 m.p. 158-9° $(\alpha)_D$ 108.57° which was indistinguisable from 47 described in page 71 (no depression of m.m.p. with 47).

It gave a single spot on a chromatoplate (R_f 0.38 in benzene) even Co TLC with 47 gave the same R_f value.

Found: C, 79.28; H, 10.30%

Calculated for $C_{32}H_{50}O_3$: C, 79.66; H, 10.37%

I.R. : ν_{max}^{KBr} 1730, 1238, 1225 cm^{-1} .

R E F E R E N C E S

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PART - II

STUDIES ON AUTOXIDATION: SYNTHESIS OF ISOMERIC 2, 3-DIOLS OF ISOHOPANE (MORETANE) AND METHYL OLEAN-12-EN-28-OATES.

PART - II

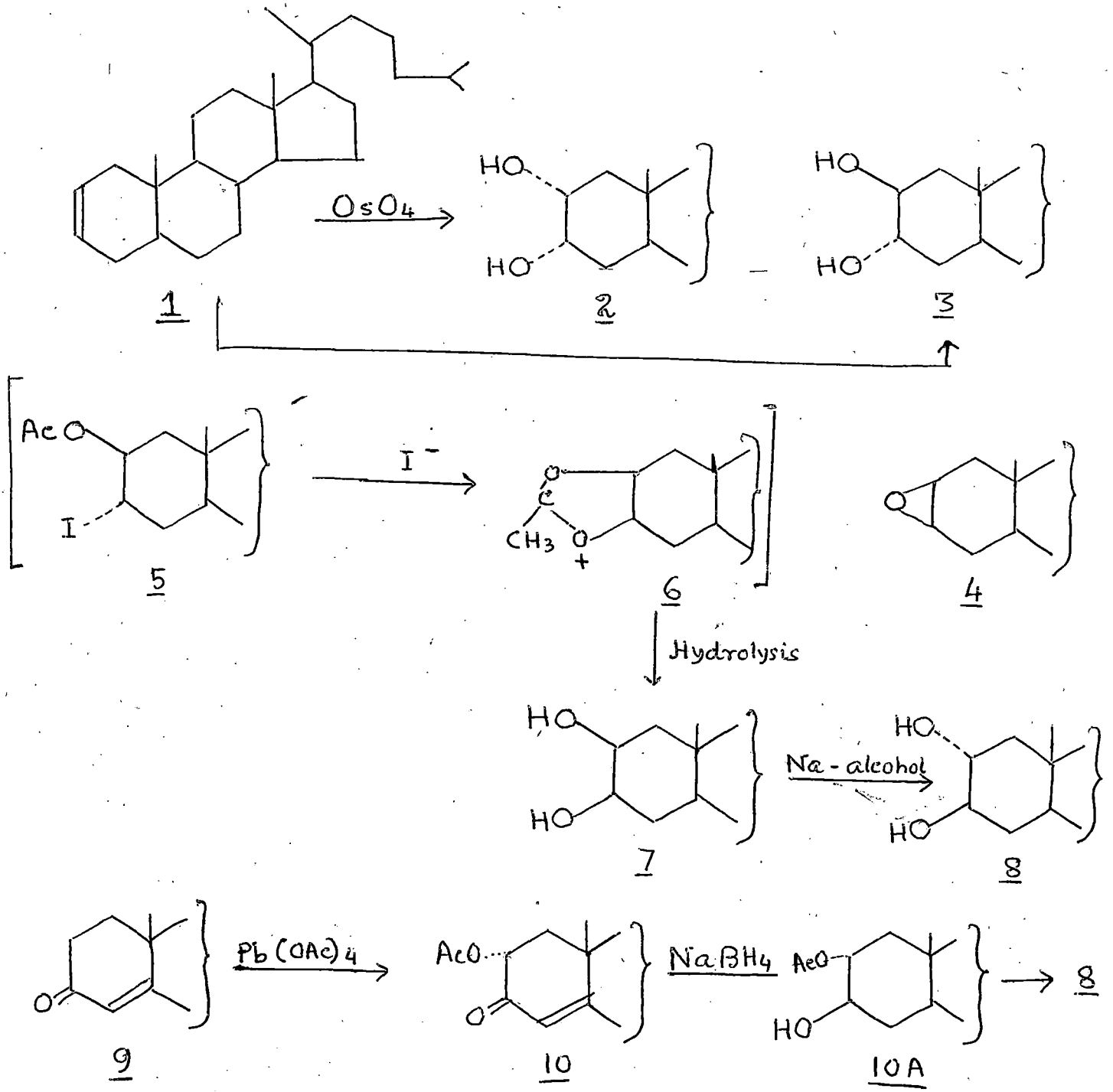
CHAPTER - I

A short review of synthesis of isomeric 2,3-diols of triterpenoids:

Section A: Introduction

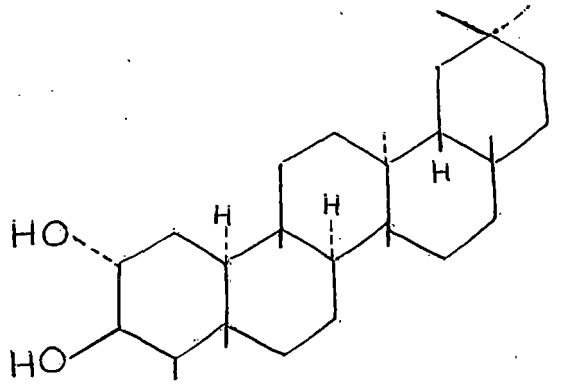
In the course of elucidation of the configurations of sapogenins containing a 2,3-diol system, methods were developed for production of the four possible isomers in this series. The four cholestane 2, 3-diols have also been prepared by the methods developed in this connection[1,2]. Δ^2-Cholestane 1 on reaction with osmium tetroxide gives the 2α, 3α-diol 2 whereas peracetic acid oxidation and subsequent hydrolysis affords 2β, 3α-diol[1-3] 3. 2α, 3α-or 2β, 3β-oxido-cholestanes 4 on diaxial opening also gives the same diol 3. The 2β, 3β diol 7 has also been prepared[1,2] according to the procedure of Winstein and Buckles[4] by treatment of Δ^2-cholestene 1 with silver acetate, iodine and moist acetic acid. The reaction probably involves formation of a cyclic 2α, 3β-iodonium ion which on acetolysis with inversion at C-2 gives 5. Expulsion of iodide ion with inversion at C-3 forms a resonant oxonium-carbonium ion 6 which leads to a mono-acetate which on hydrolysis gives 7. As the diol 7 contains one axial substituent at C-2, it is epimerized by treatment with sodium in ethanol at 180° to the diequatorial 2α, 3β-diol 8. Diol 8 was also obtained from cholestenone 9 which reacts with lead-

tetracetate to give in about 10% yield a product 10 having 2α-acetoxy group[5,6]. Reduction of 10 with sodium borohydride followed by hydrogenation gives the 2α, 3β-diol 8.

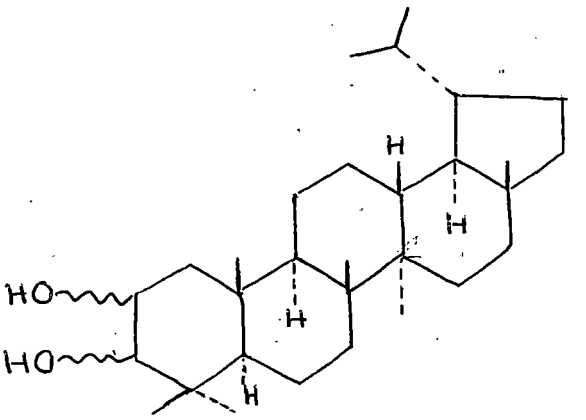


Section B: Synthesis of 2,3-diols of lupane and friedelane series.

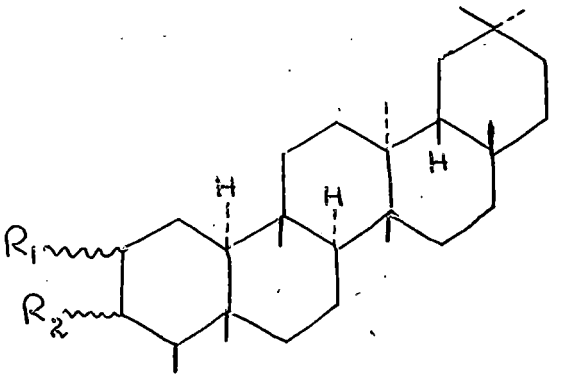
McGinnis[7] et al have reported the preparation of all the four possible stereoisomeric lupane 2,3-diols 12. Samson et al[8] have synthesised 2α, 3α-13A; 2β, 3β-13B; 2α, 3β-13C friedelane diols and shown that the last named isomer is identical with natural pachysandiol A[9] 11.



11



12 (A, B, C, D)



13A $R_1 = R_2 = \alpha$-OH

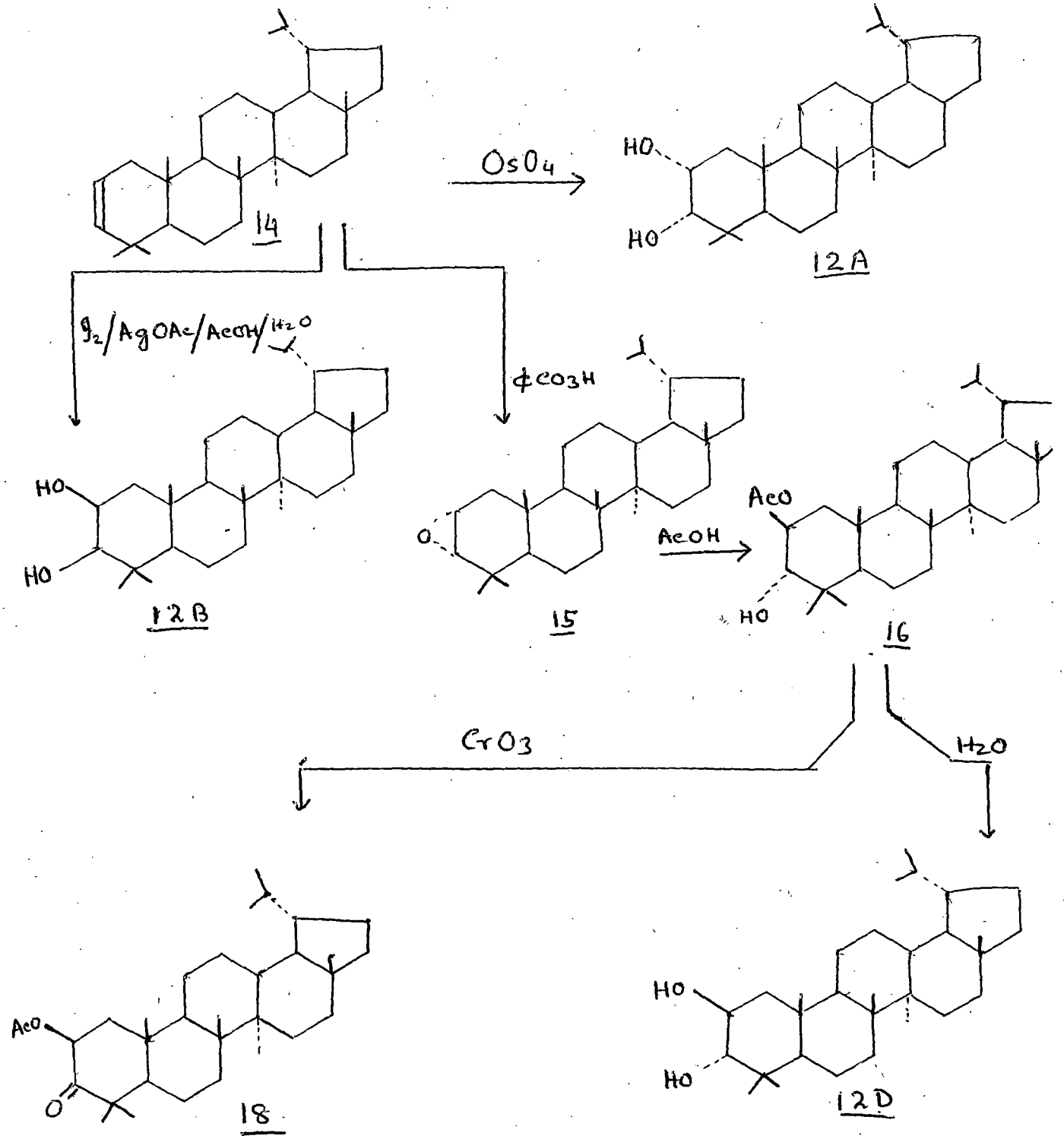
13B $R_1 = R_2 = \beta$-OH

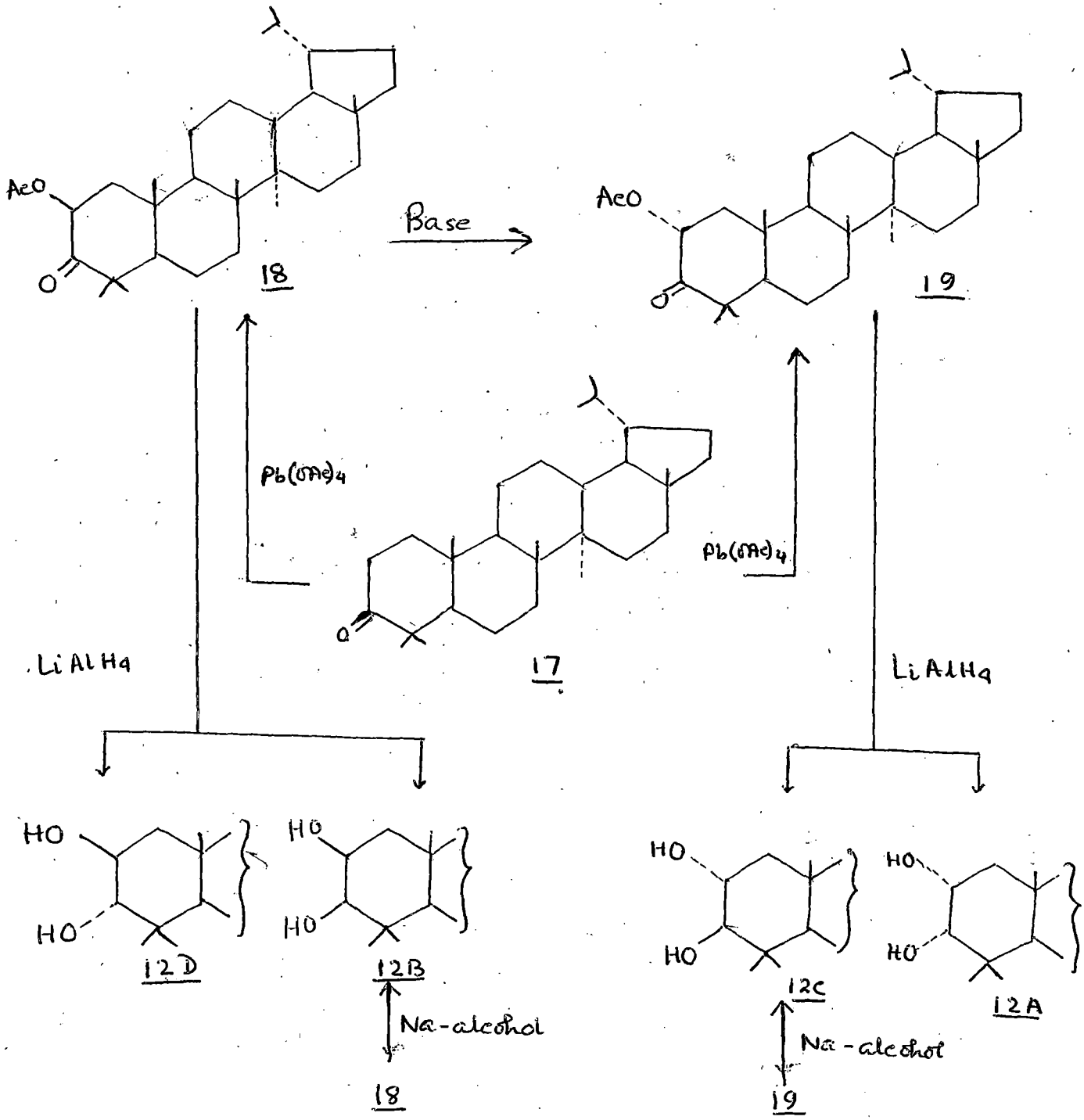
13C $R_1 = \alpha$-OH ; $R_2 = \beta$-OH

Synthesis of Lupane-2, 3-diols[7] 12 (Chart I)

Lup-2-ene 14 was converted to lupane-2α,3α-diol 12A, lupane-2β, 3β-diol 12B and lupane 2α, 3α-epoxides 15 by the action of osmium tetroxide, iodine-silveracetate-acetic acid and perbenzoic acid respectively. The epoxide 15 was opened with acetic acid to give 2β-acetoxy-lupane-3α-ol 16 which on mild alkaline hydrolysis yielded the diaxial trans diol, lupane 2β, 3α-diol 12D. In order to increase the poor yield of the 2β, 3β-diol 12B obtained by the above procedure and also to prepare the remaining isomer: lupane 2α, 3β-diol 12C, lupane-3-one 17 was treated with lead tetracetate to give 2β-acetoxy-lupane-3-one 18 and 2α-acetoxy-lupane-3-one 19 as the major and minor products respectively. The 2β-acetoxy isomer 18 could also be prepared by the chromic acid oxidation of 2β-acetoxy-lupane-3α-ol 16. This acetoxy ketone 18 upon equilibration with base furnished the 2α-acetoxy-3-ketone 19 which was then reduced with lithium aluminium hydride mainly to lupane-2α, 3α diol 12A and with sodium and isopropanol to lupane-2α, 3β-diol 12C. The 2β-acetoxy-3-ketone 18 was similarly reduced with lithium aluminium hydride to give the 2β, 3α-diol 12D as the main product and with sodium and isopropanol to the 2β, 3β-diol 12B.

CHART I

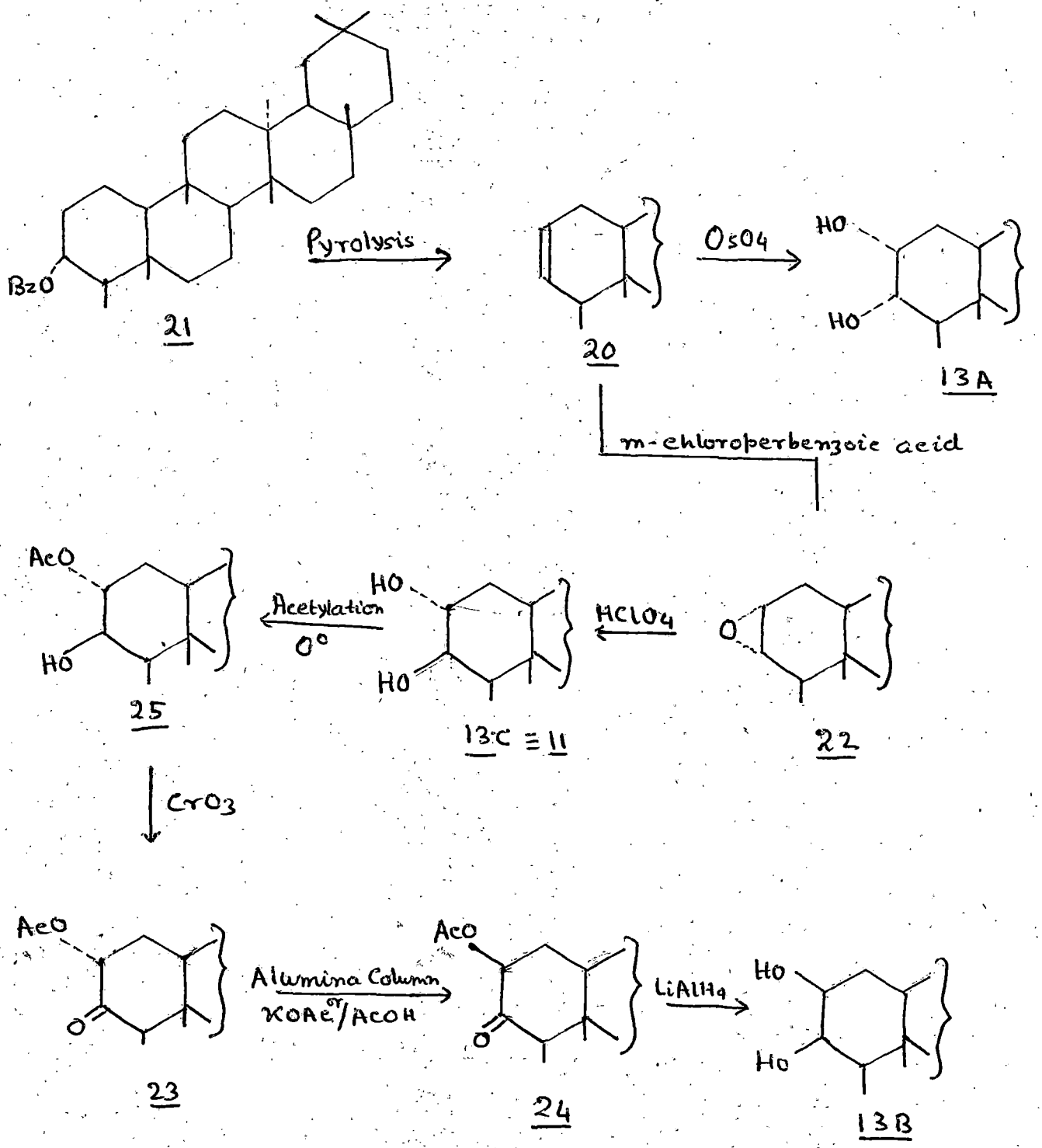

OsO4
HO
HO
12A
14
I2/AgOAc/AcOH/H2O
HO
HO
12B
MCO3H
O
15
AcOH
AcO
HO
16
CrO3
H2O
AcO
O
18
HO
HO
12D


AcO
O
18
Base
AcO
O
19
Pb(OAc)4
O
17
Pb(OAc)4
LiAlH4
LiAlH4
HO
HO
12D
HO
HO
12B
Na-alcohol
18
HO
HO
12C
HO
HO
12A
Na-alcohol
19

Synthesis of Friedelane-2α, 3α 13A; 2β, 3β -13B; 2α, 3β -13C-diols[8]:(Chart II)

Friedel-2-ene 20, obtained by the ~~hydrolysis~~ pyrolysis of friedelanol benzoate 21 was converted to friedelane-2α, 3α-diol 13A by the action of osmium tetroxide and a 2, 3-epoxide 22 by the action of m-chloroperbenzoic acid. The 2,3-epoxide 22 was opened with perchloric acid to yield friedelane 2α, 3β -diol 13C identical with naturally occuring pachy-sandiol A[9] 11. Kikuchi and Toyoda[9] has suggested that cerin acetate was 2α-acetoxy friedelan-3-one 23, contrary to its previous formulation as 2β-acetoxy-friedelane-3-one 24 on the following grounds: Paschysandiol A-2-monoacetate 25 obtained by the acetylation of Pachysandiol A 11 at 0°, could be oxidised to cerin acetate 23 with chromic acid. The cerin acetate so obtained, on prolonged absorption on alumina, was isomerized to another 2-acetoxy-3-ketone 24 which must hence be the more stable 2β-(e, equatorial)-acetoxy isomer. Consequently the original cerin acetate must be less stable 2α-(axial) acetoxy isomer 23. Samson et al[8] isomerized cerin acetate 23 with potassium acetate in acetic acid and reduced the resulting 2β -acetoxy-friedelan-3-one 24 with lithium aluminium hydride and thus synthesised friedelane-2β, 3β -diol 13B . (Chart II)

CHART -II


BzO
21
Pyrolysis
20
OsO4
HO
HO
13A
m-chloroperbenzoic acid
AcO
HO
25
Acetylation
0°
HO
HO
13C ≡ 11
HClO4
O
22
CrO3
AcO
O
23
Alumina Column
KOAc/AcOH
AcO
O
24
LiAlH4
HO
HO
13B

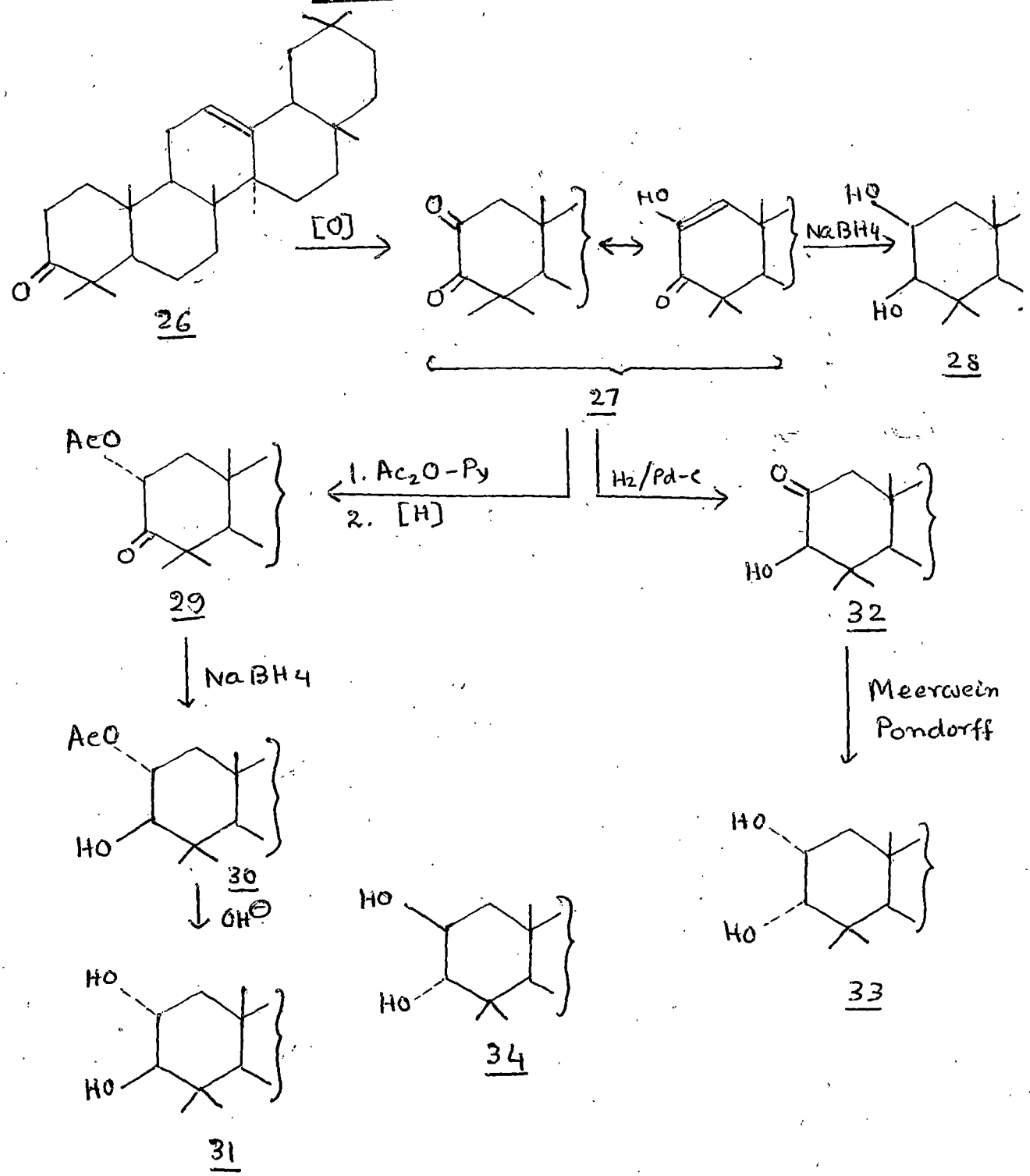
Section C: A short review of synthesis of isomeric Δ^{12}-Oleane-2,3-diols[10]:

A number of 2,3-diols of triterpenoids have been isolated from natural sources. Recently Khastgir et al[10] have synthesised three out of the four isomeric 2,3-diols (Chart IV) by using diosphenol 27 as the intermediate, from β-amyrone 26. Δ^{12}-Oleanene 2β, 3β-diol m.p. 240-42°, $(\alpha)_D$ 101.88° 28 was obtained by sodium borohydride reduction of 27. Acetylation of 27 followed by hydrogenation gave 2α-acetoxy-β-amyrone 29 which on sodium borohydride reduction at pH-8 gave 2α-acetoxy-β-amyrin 30 which on hydrolysis gave the most stable Δ^{12}-oleanene 2α, 3β-diol 31. Hydrogenation of 27 gave 32 which on Meerwein-Pondorff reduction afforded Δ^{12}-Oleanene-2α, 3α-diol, 33 having m.p. 278-81°, $(\alpha)_D$ 71.28°. The sterically most unstable 2β, 3α-diol 34 was also synthesised. The configurations assigned have been confirmed from NMR spectral evidences. The melting points and rotations of the isomeric diols, their diacetates and their acetonide derivatives have been shown in Chart III.

Chart III

	DIOL		DIACETATE		ACETONIDE DERIVATIVE	
	m.p.	$(\alpha)_D$	m.p.°	$(\alpha)_D$	m.p.	$(\alpha)_D$
2β, 3β	240-42°	101.88°	221-22°	83.63°	180-2°	102.50°
2α, 3α	278-82°	71.28°	180-82°	40.77°	199-201°	97°
2α, 3β	202-4°	60.00°	216-18°	73.42°	173-4°	-
2β, 3α	250-52°	12°	161-63°	-	-	-

Chart IV

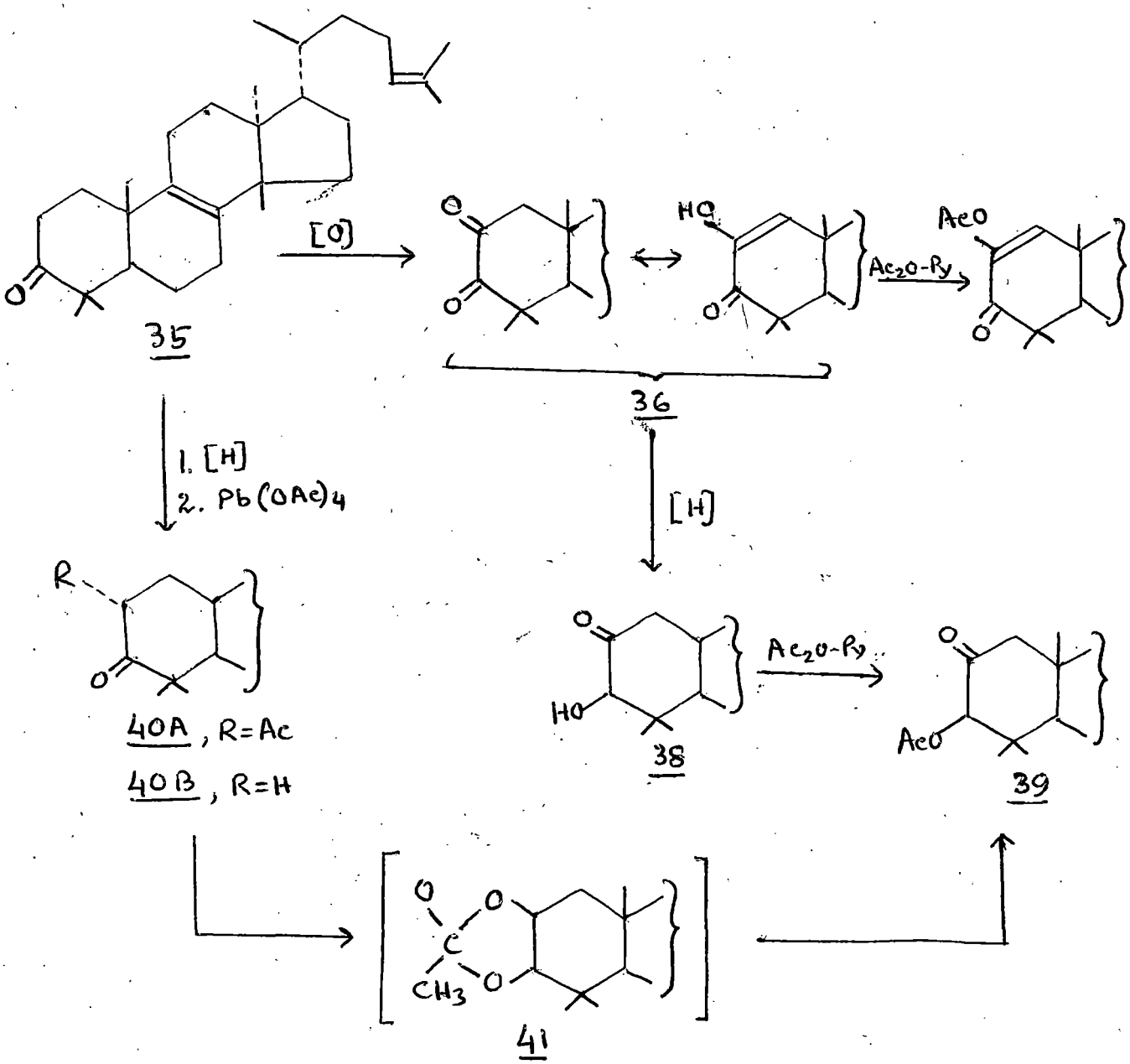

[O]
NaBH4
26
27
28
1. Ac2O-Py
2. [H]
H2/Pd-c
29
32
NaBH4
Meerwein
Pondorff
30
OH⊖
31
34
33

Section D: A short review on autoxidation and isomerization in ring A in triterpenoids :

1. Oxidation in ring A in Euphol.

Lavie and co-workers[11] studied the autoxidation of euphadiene-3-one 35 and the results of their work is summarised in the following lines. Euphadiene-3-one 35 was oxidised by shaking in oxygen in t-butanol saturated with potassium-t-butoxide[12,13]. A tautomeric mixture of diketone and the corresponding diosphenol 36 (two spots on chromatoplate) was produced by absorbing one mole of oxygen UV, λ_{max} 269 (ϵ, 7900), ν_{max} 1715, 1672, and 1653 cm^{-1}. NMR of the compound 36 showed a singlet at τ 3.60 due to vinylic proton at C-1. Acetylation gave the corresponding acetate 37, UV_{max} 236 mμ (ϵ,9000), ν_{max} 1764 cm^{-1}. NMR showed a singlet at τ 3.02 due to C-1 proton. On hydrogenation of the diosphenol 36 over palladium on charcoal (two moles of hydrogen were absorbed-one mole to reduce the side chain double bond and the second mole to reduce the enolic double bond) a non-crystallisable homogeneous solid ν_{max} 1712 cm^{-1}, NMR singlet at τ 5.95 accounting for one hydrogen and two AB type doublets centered at τ 7.69 and τ 7.35 accounting for two hydrogens, was obtained. Upon acetylation a crystalline Ketoacetate was obtained, ν_{max} 1742 and 1730 cm^{-1}, NMR singlet at τ 4.95 for one hydrogen and a broad peak at τ 7.50 accounting for two hydrogens. From the above spectral properties structures 38 and 39 were proposed for hydroxy-ketone

and ketoacetate respectively. 2α-acetoxy (equatorial) derivative

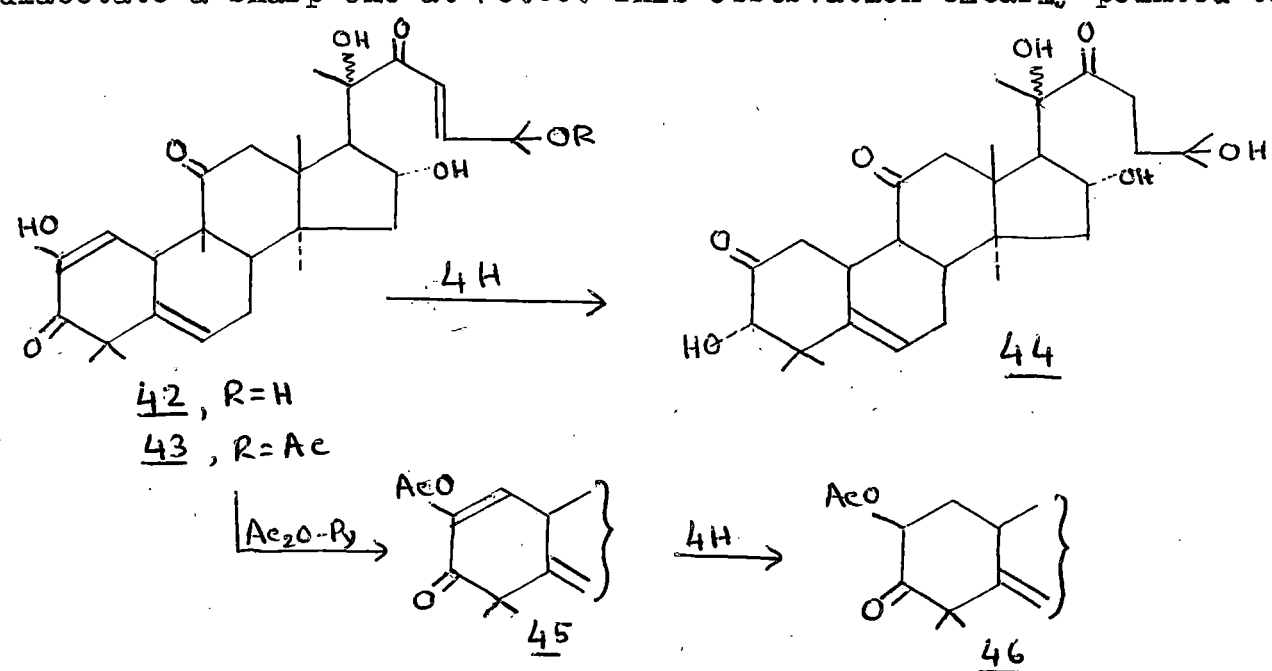


40A was prepared by the reaction of dihydroderivatives of 35 with lead tetra-acetate in acetic acid in presence of boron-trifluoride[14].

The product 40A showed IR bands at 1742 and 1730 cm^{-1} and the NMR spectra showed a quartet of lines centered at τ 4.3 (Jae = 6.5 eps and Jaa = 13.0 eps) for the C-2 proton but no signals for protons α to a keto-functions. The isomerisation of 2α-equatorial acetoxy ketone 40A into the isomer 39 was also observed and they proposed that the migration proceeded through the cyclic intermediate 41[15]. Acid hydrolysis of 40A afforded a compound which has been assigned the 2α-equatorial hydroxy 3-keto derivative 40B on the basis of its IR, ν_{max} 1718 cm^{-1}.

2. Isomerisation in ring A of the Cucurbitacins

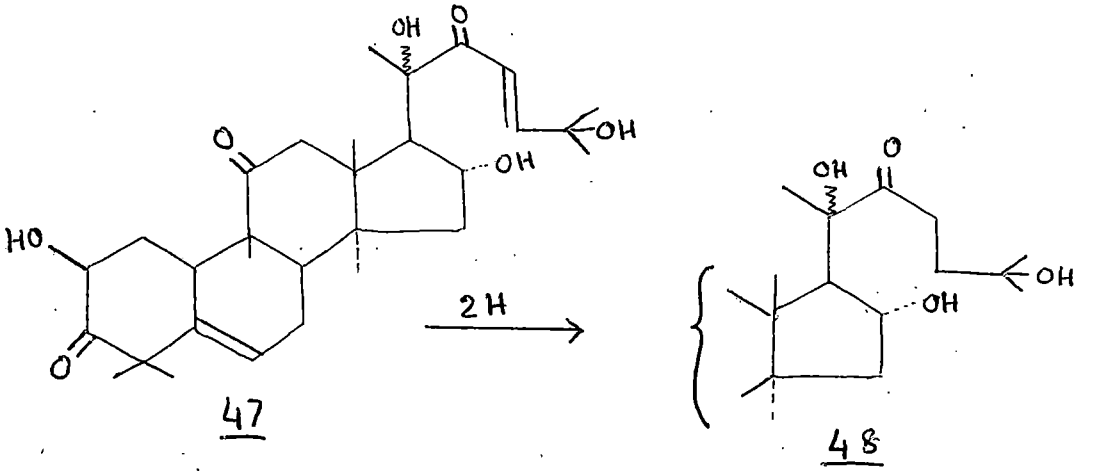
Lavie and co-workers[16,17] reported that hydrogenation of the diosphenol containing cucurbitacins namely elatericin B 42 and elaterin 43 resulted in 1,4 addition of hydrogen during the process of hydrogenation. The NMR spectrum of hydrogenated product of elatericin B was found to show a singlet at τ 6.02 and that of its diacetate a sharp one at τ 5.00. This observation clearly pointed to



the fact that the proton linked to the carbon to which the acetoxy group is also attached had no neighbouring protons and can not therefore be at C-2. The NMR spectra could be explained, if it was considered that 1, 4-addition of hydrogen to the diosphenol system took place, resulting in the conversion of Δ^1-2-hydroxy-3-keto to a 2 keto-3 hydroxy system 44.

Elatericin B diacetate 45 on hydrogenation formed the 2β-equatorial-acetoxy-3-keto derivative 46 by a normal 1,2-addition of hydrogen. This compound showed a quartet of lines related to the 2α-axial proton which is centered at τ4.4 (Jaa = 13.5 eps; Jae = 5.1 eps). The isomerisation of 2-acetoxy-3-ketoderivative 46 on a basic column of alumina as well as on an acedic column was studied. In both the cases the material recovered from the columns showed that it had remained unchanged.

The ORD curves of dihydro elatericin A 48 and tetrahydro elatericin B 46 were also interpreted. Cotton effect curves of both 2 and 3-keto derivatives were found to be positive with the amplitude



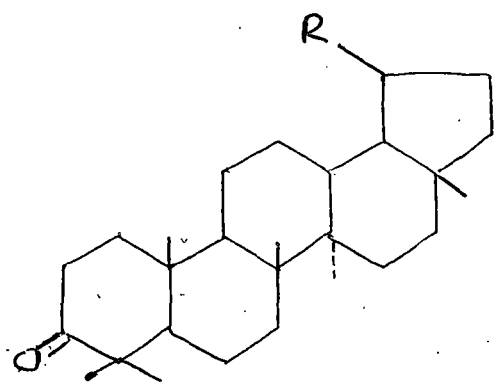
47 48

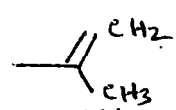
of the 2 keto-derivative being larger than that of the 3-keto form. ORD studies on 2 and 3 ketosteroids[18] and the oxomanoyl oxide series[19] also revealed the same result. The inverted stereochemistry of cucurbitacins at C-10 resulting in a mirror image of the C-10, β - analogy, should give rise to a negative Cotton effect but instead the two compounds displayed positive curves. This can be interpreted as due to the presence of two additional carbonyl chromophore, one in particular at C-11 displaying a large amplitude, which counteracts thereby the inverted rotation of the keto group in ring A as should be expected. The result is a lower positive value instead of a negative one. The peak for dihydro elatericin A 48 (3 keto) at $(\alpha)_{325}+2200^{o}$ is larger, than that of tetrahydroelatericin B 44 (2 keto) $(\alpha)_{325}+1558^{o}$. In both the cases the keto group was flanked by an equatorial (OH) substituent which is either likely to increase the Cotton effect or to render no change at all.

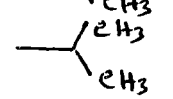
In order to obtain pure tetrahydroelatericin B 45, alkaline hydrolysis of tetrahydroelatericin B diacetate 46 was attempted but the reaction resulted in the formation of dihydroelatericin B[20] 47 λ_{max} 267 mμ (∈, 5700), positive ferric chloride coloration (characteristic of diosphenol). Tetrahydroelatericin B diacetate 48 on alkaline hydrolysis yielded the same dihydroelatericin B 47. The alkali induced autoxidation of α-hydroxy ketone in elatericin was also studied[21] and was found to occur at much slower rate.

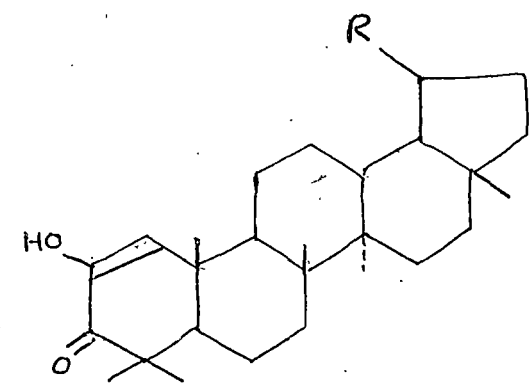
3. Oxidation in ring A in Lupeol

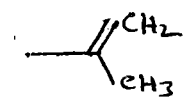
Ganguly and co-workers[22] carried out the oxidation of lupenone 49 and lupanone 50 to the corresponding diosphenols 51 and 52 respectively by passing oxygen in dry t-butyl alcohol containing potassium tertiary butoxide. Diosphenol 52 on hydrogenation afforded a non-crystalline alcohol which on acetylation yielded the keto-acetate 53. The structure 53 was assigned to the keto-acetate by examining its NMR spectra (a sharp singlet at δ, 4.95) ascribed to the C-3 proton.

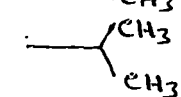


49, R= 

50, R= 



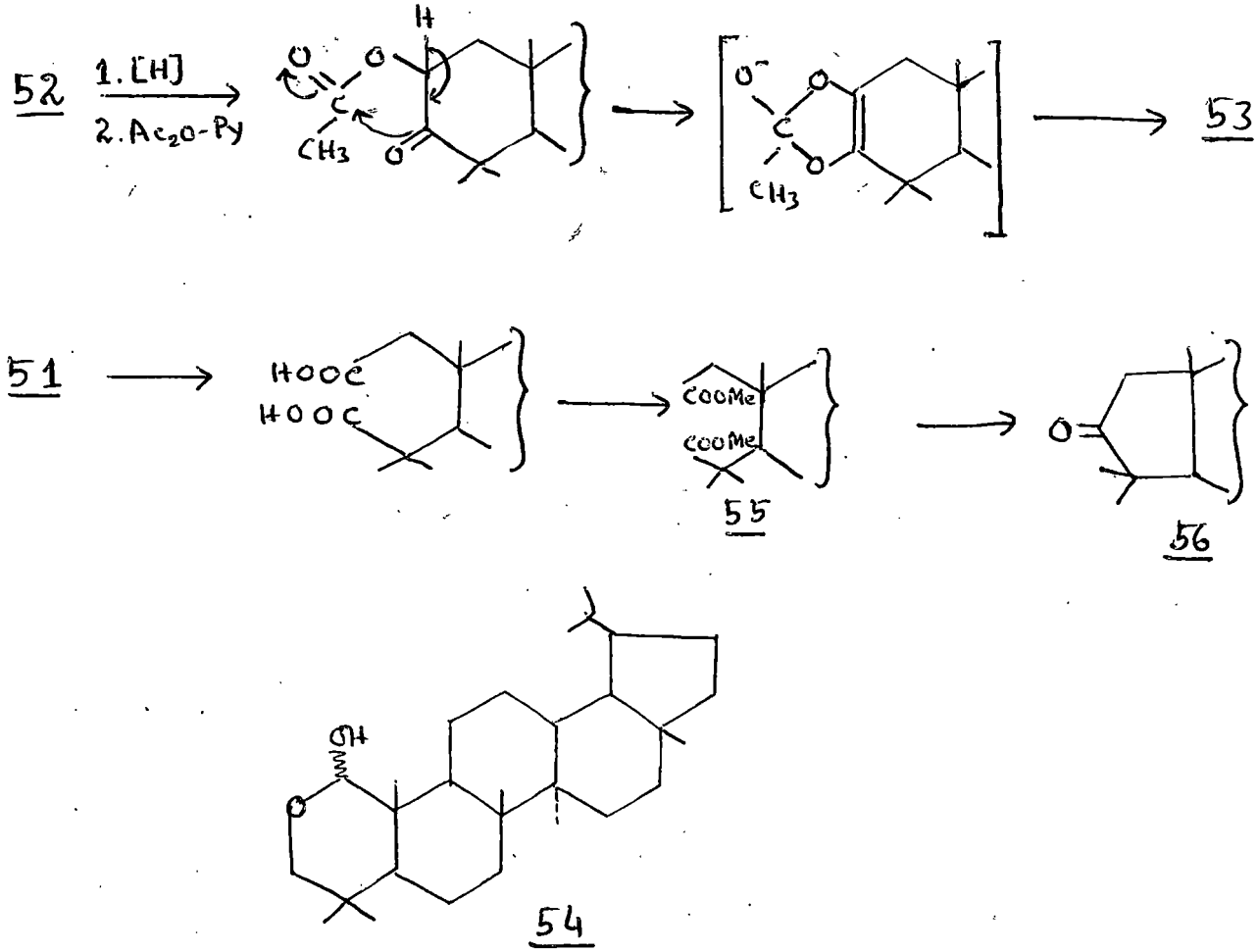
51, R= 

52, R= 

53

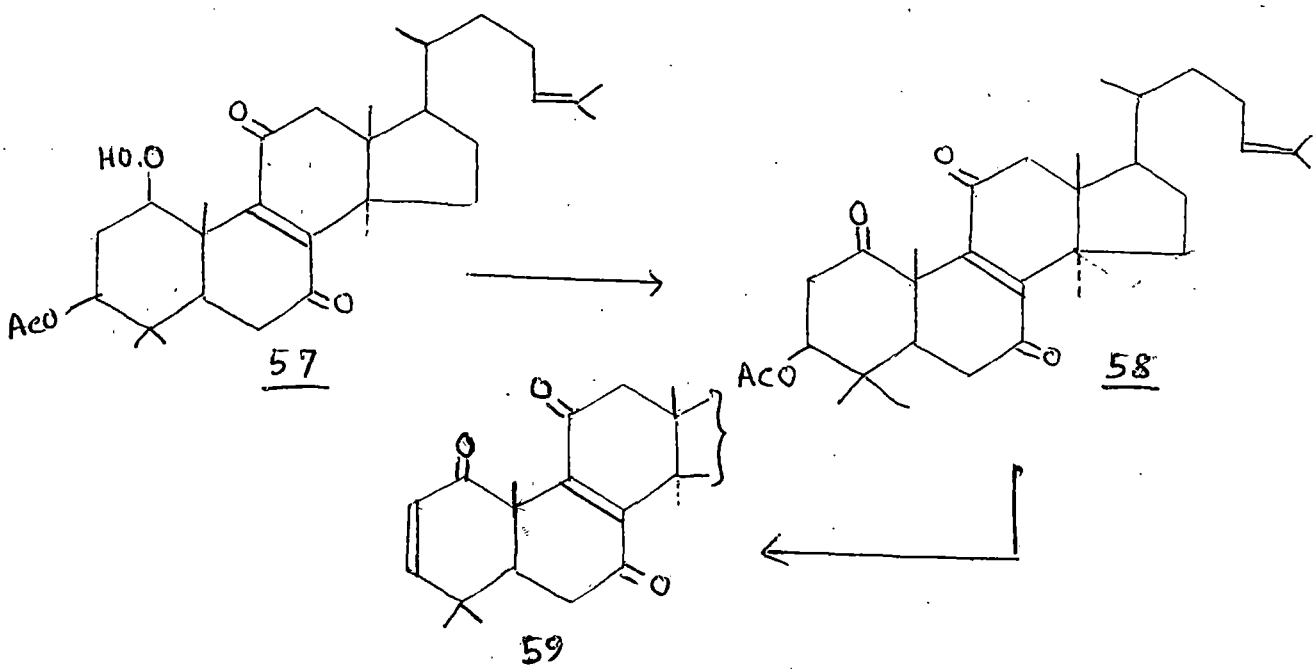
Formation of 53 from 52 was explained by the mechanism shown in Chart V. Diosphenol 52 on ozonisation gave a neutral compound $C_{29}H_{48}O_3$, whose structure was assigned as 54 on the basis of mode of formation, spectral characteristics and elemental composition. Diosphenol 51 was cleaved by alkaline hydrogen peroxide to the dicarboxylic acid $C_{30}H_{48}O_4$. The acid was converted into the dimethyl ester, 55 which on refluxing with alcoholic alkali yielded a neutral crystalline compound 56.

Chart-V



4. Autoxidation of Lanostenyl acetate

Horn and Ilse[23] stated that lanostenyl acetate in ethyl acetate was extensively converted into a mixture of 7-hydroperoxy and 7, 11-dihydroperoxy lanostenyl acetates by treatment with gaseous oxygen at 50° for 48 hours. After that Scotney and Truter[24] found that the autoxidation of lanostenyl acetate in ethyl acetate at 50° after 14 days was a mixture of at least eight peroxides (laminar chromatography). The two most plentiful peroxides were recovered and shown to be 7β-and 11β- hydroperoxy lanostenyl acetates. The structure of 7β-hydroperoxy-lanostenyl acetate was obtained by reducing it with sodium borohydride to 7β-hydroxylanostenyl acetate. The structure of 11β-hydroperoxide was proved by converting it to 11-oxo-lanostenyl acetate with ferrous ion. Furthermore, lithium aluminium hydride reduction of the 11-hydroperoxide afforded one product, which was identical with 11β-hydroxylanostenol.



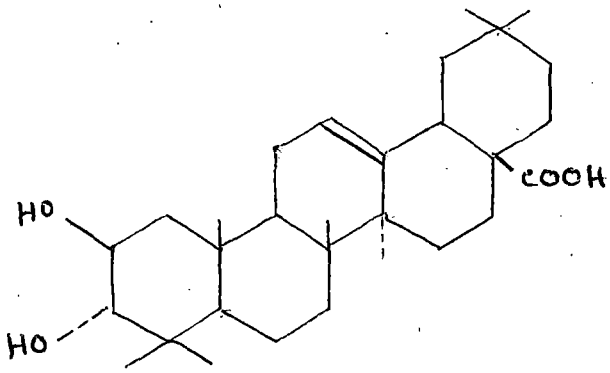
Autoxidation of 7,11-dioxolanost-8-enyl-3β-acetate in cyclohexane at 40° proceeded via 1β-hydroperoxy-7, 11-dioxolanostenyl acetate to 1, 7, 11-trioxolanost-8-enyl acetate[25]. The location of ketone at 1-position was deduced from the behaviour of the trione acetate with alkali. With alkali 1, 7, 11-trixolanost-8-enyl acetate yielded 1,7,11-trioxolanosta-2,8-diene and it had been derived from the trione acetate by elimination of the 3β-acetate group and the formation of a conjugated unsaturated grouping (57, 58, 59). That the precursor for the trione is a mono-hydroperoxide of 7,11-dioxolanostenyl acetate was established by the fact it was decomposed by ferrous ion to 1,7,11-trioxolanostenyl acetate.

In an experiment a solution of lanost-8-en-3βyl acetate in cyclohexane at 40° was oxidised by passing oxygen through it[26]. After twelve months treatment the neutral fraction was examined and was found to contain at least sixteen components. From the R_f values several components have been identified e.g. 1, 7, 11-trioxolanostenyl acetate, 1,7,11-trioxolanosta-2,8-diene. Besides these 15β-hydroxy-7-oxo, 15α-hydroxy-7-oxo, 7, 15-dioxo-and 11, 15-dioxolanostan 3β-yl acetate were also identified.

5. Oxidation of ring A in oleanolic acid.

In connection with their work to confirm the structure of bredemolic acid 60 and crategolic acid 61 Tschesche and coworkers[27,28] performed the autoxidation of ring A in methyl oleanonate 62. Methyl oleanonate was stirred in t-butanol containing

potassium metal at 25-50° with simultaneous introduction of oxygen. The reaction mixture on acidification and usual working up gave an amorphous solid for which structure 63 was proposed. The diosphenol 63 m.p. 130-35°, $(\alpha)_D$ 104 ± 4° on sodium borohydride reduction gave 2β, 3β-dihydroxy-12-en-olean-28-oate 64 which on oxidation with kilani solution gave a mixture of several compounds in which 10% of 63 was found to be present as was shown by its UV spectrum.



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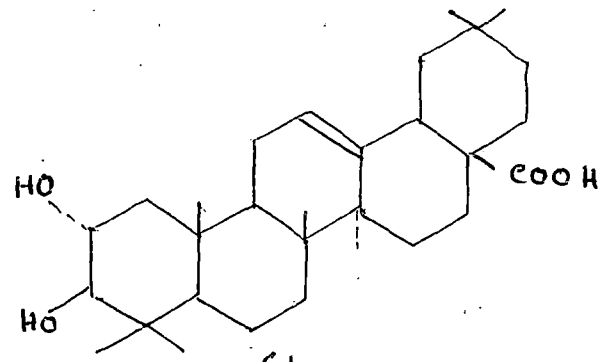

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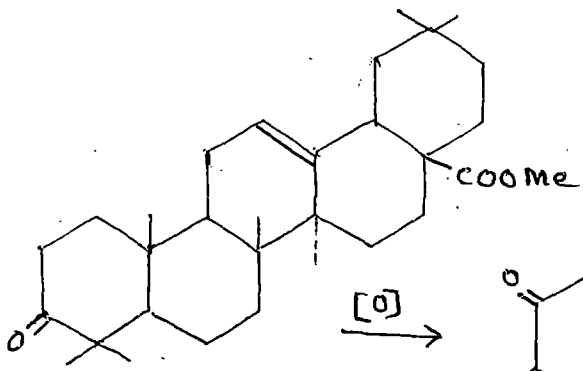
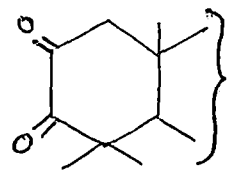


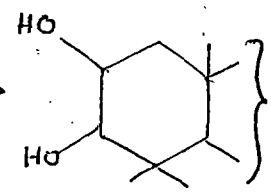
62

[O]



63

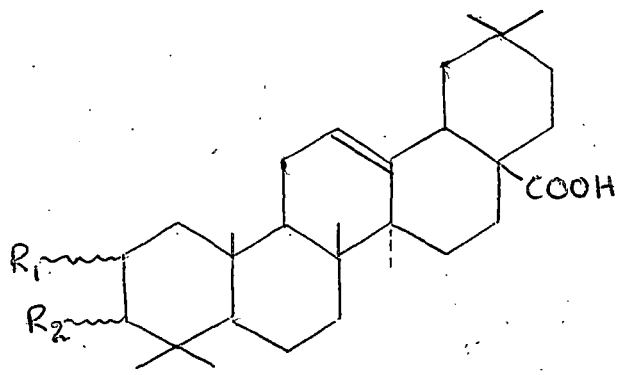
NaBH4



64

Section D: A short review on 2,3 -dihydroxy triterpene acids from natural sources:

The four stereo-isomeric 2,3-dihydroxy olean-12-en-28-oic acids are known to occur in nature viz: (1) the 2α, 3α -dihydroxyolean-12-en-28-oic acid 65A[29] (2) the 2α, 3β -dihydroxyolean-12-en-28-oic acid (crategolic/maslinic acid) 65B[30] (3) the 2β, 3β -dihydroxy olean-12-en-28 oic acid[31] 65C (4) the 2β, 3α-dihydroxy olean-12-en-28-oic acid 65D[28] (bredemolic acid).



65A $R_1 = R_2 = \alpha$-OH
65B $R_1 = \alpha$-OH, $R_2 = \beta$-OH
65C $R_1 = R_2 = \beta$-OH
65D $R_1 = \beta$-OH, $R_2 = \alpha$-OH

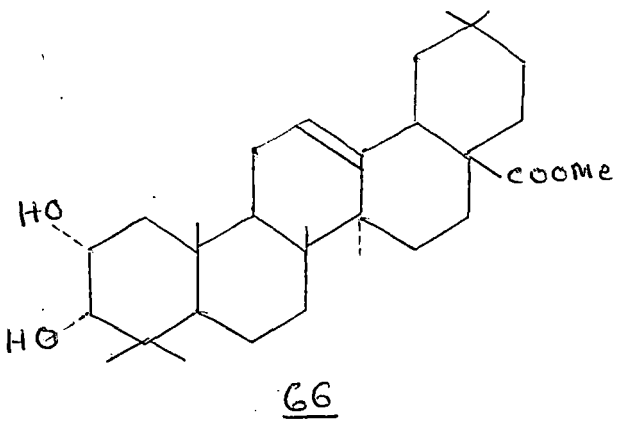
Alphitolic acid[33] in the lupeol series and 2α-hydroxy ursolic acid[34] in the ursane series are known to occur in nature.

1. The 2α, 3α-dihydroxy olean-12-en-28-oic acid:

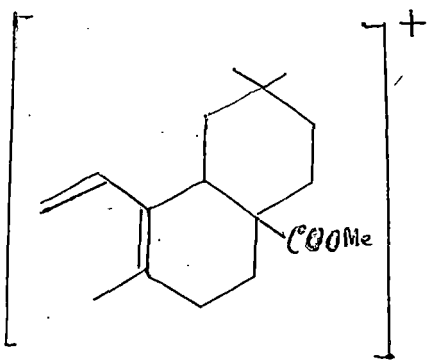
Cheung et al[29] isolated a triterpene acid 65A as its methyl ester m.p. 296-99° from shorea accuminata resin, which has been shown to be the 2α, 3α-dihydroxyolean-12-en-28 oic acid. The methyl ester 66 (ν_{max} 3340, 1725 cm^{-1}) formed a diacetate, a monoacetate and a O, O-isopropylidene derivative indicating the presence of two hydroxyl groups. All these compounds showed NMR signals due to

methyl ester group (3H, singlet δ 3.6) and a triplet for an olefinic proton (J 4Hz, δ 5.3).

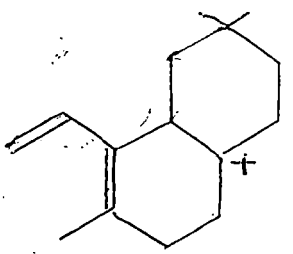
The mass spectrum of the diol methyl ester or its acetonide showed intense peaks at m/e 262, 203 corresponding to ions A and B[35]



66



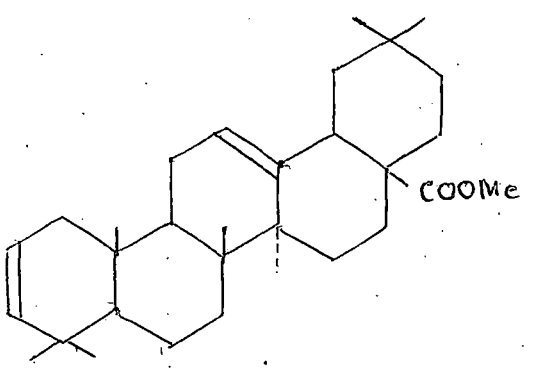
A



B

The mass fragmentation pattern established that the diol methyl ester belongs to 12-oleanene or 12-Ursene series with a 28-methoxycarbonyl group. They concluded from the NMR signal[36] of the allylic 18β-hydrogen at δ 2.8 (AB quartet), that the diol ester belonged to oleanene group. Further evidences for assignment of a 2α, 3α-configuration of the diol is supported by the following observations. In this diol, the C-3 proton showed a doublet (J 3Hz) at δ 3.35 due to vicinal coupling of equatorial and axial protons. Upon saturation (by double irradiation) of this signal, the multiplet near δ3.9 due to the proton at C-2 simplified to a four-line signal characteristics of X part of an ABX type spectrum.

The cis isomers, 2α, 3α-diol and 2β, 3β-diol were first prepared[37] by Djerassi et al[37] by osmium tetroxide oxidation of methyl oleana-2, 12-diene-28-oate 67. Cheung et al also repeated the oxidation and obtained two cis-diols and the one with higher melting point was identical to the methyl ester of m.p. 296-99°, isolated from shorea acuminata. Tschesche et al[27,28] assigned a 2β, 3β-configuration to this diol and an 2α, 3α-configuration to the one with lower m.p. (258-60°), from consideration of the infrared absorption due to O-H stretching. Cheung et al demonstrated that the configurations assigned by Tschesche et al should be reversed. By comparing the methyl resonance frequencies from published substitution effects[36] with those observed for the two cis diols and their acetate derivatives Cheung et al suggested that, contrary to the views of Tschesche et al[27,28], the diol m.p. 296-99° must have the 2α, 3α-and the diol m.p. 258-60° the 2β, 3β-configuration.



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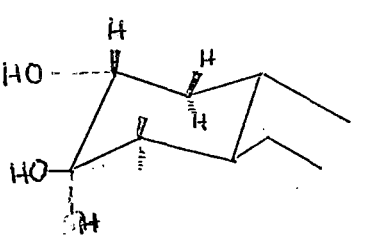


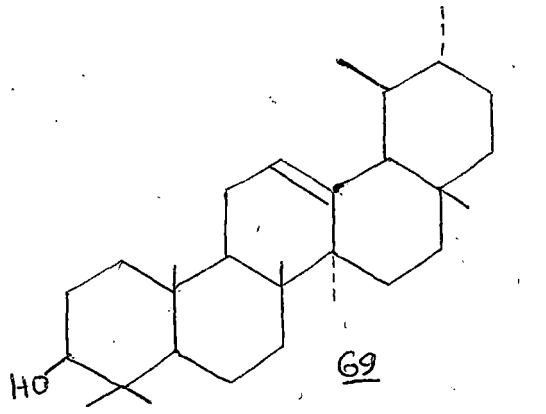
68

In the 2α, 3α-diol, with a chair ring A, 68, the 2β-proton is axial and is expected[39] to be subject to a large ax-ax coupling with the 1-α proton and to small ax-eq coupling with the 3β and 1β -protons. Of the two cis diols from osmium tetroxide only one with m.p. 296-99°, showed a signal due to C-2 proton of sufficient width at half-height (Wh/2 21Hz) to be compatible with a 2α, 3α-diol structure. The other diol m.p. 258-60° having a corresponding signal of Wh/2 8Hz should have a 2β, 3β- arrangement.

2. The 2α, 3β -dihydroxyolean-12-en-28-oic acid (Crategolic/maslinic acid):

Bachler[40] was the first to isolate an amorphous acid "Crategus acid" from the leaves of Crategus Oxyacantha L . He, however, erroneously assigned the molecular formula $C_{32}H_{54}O_4$ to it. The acid was also observed to occur in the leaves of Psidium guaijava by Arthur and Hui[40]. This acid was subsequently studied by a number of workers[40]. However a more detailed study of the acid was made by Tschesche et al[40,41], who succeeded in obtaining the acid in crystalline form and establishing the correct molecular formula $C_{30}H_{48}O_4$. They designated the acid as crategolic acid, established the presence of a double bond resistant to catalytic hydrogenation and suggested the presence of two hydroxyl groups, although they could not prepare a diacetate. From a consideration of the behaviour of the acid towards acylation, decarboxylation and lactonisation, they erroneously

concluded that crategolic acid was an α-amyrin derivative and even suggested the revision of the acdepted structure 69 of α-amyrin to the δ-amyrin structure 70. On the basis of their proposed new formula of α-amyrin 70, they suggested without much valid reason that crategolic acid had the structure 71.



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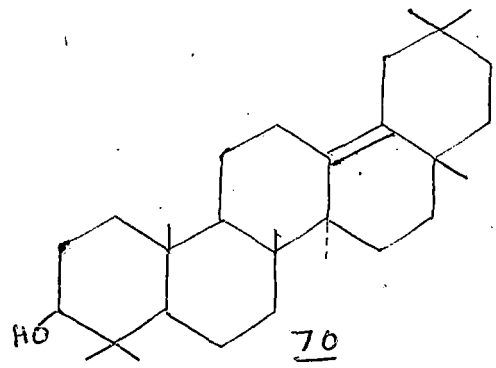
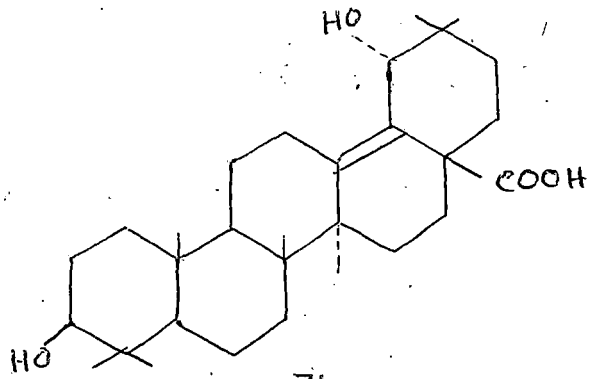
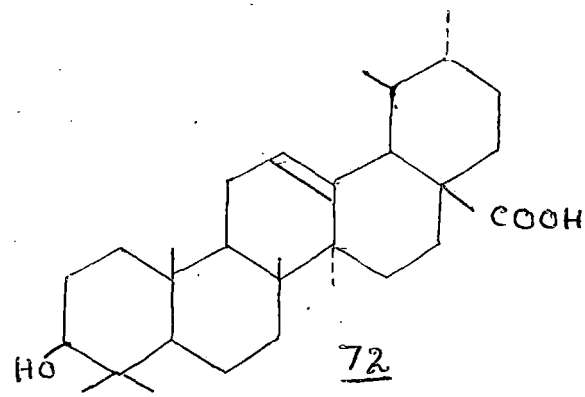


70



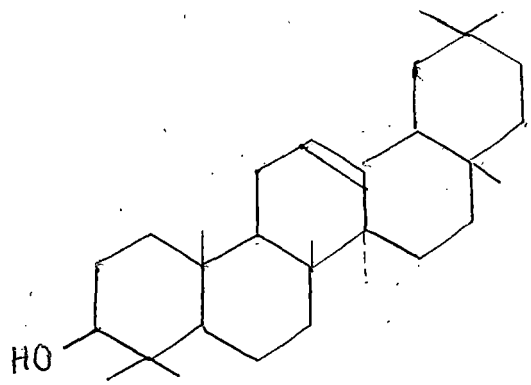
71



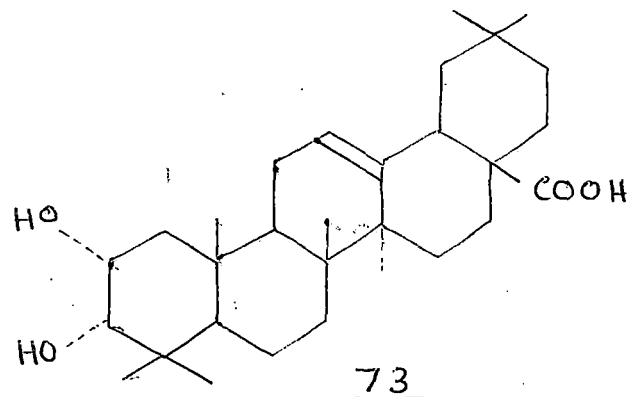
72

However, they themselves latter showed[41] that their 'crategolic acid' was impure, being contaminated with 60-65% of ursolic acid 72 which could not be easily separated. Arthur et al[42] drew attention

to this fact and suggested further work. Tschesche et al in a subsequent paper[43] correctly recognised crategolic acid 73 as a derivative of β-amyrin 74. The impure acid mixture could be



74



73

resolved by them by paper chromatography or column chromatography of the methyl esters derived from it. They were also able to prepare a diacetate, a monoacetate and a keto-monoacetate from methyl ester of crategolic acid 73.

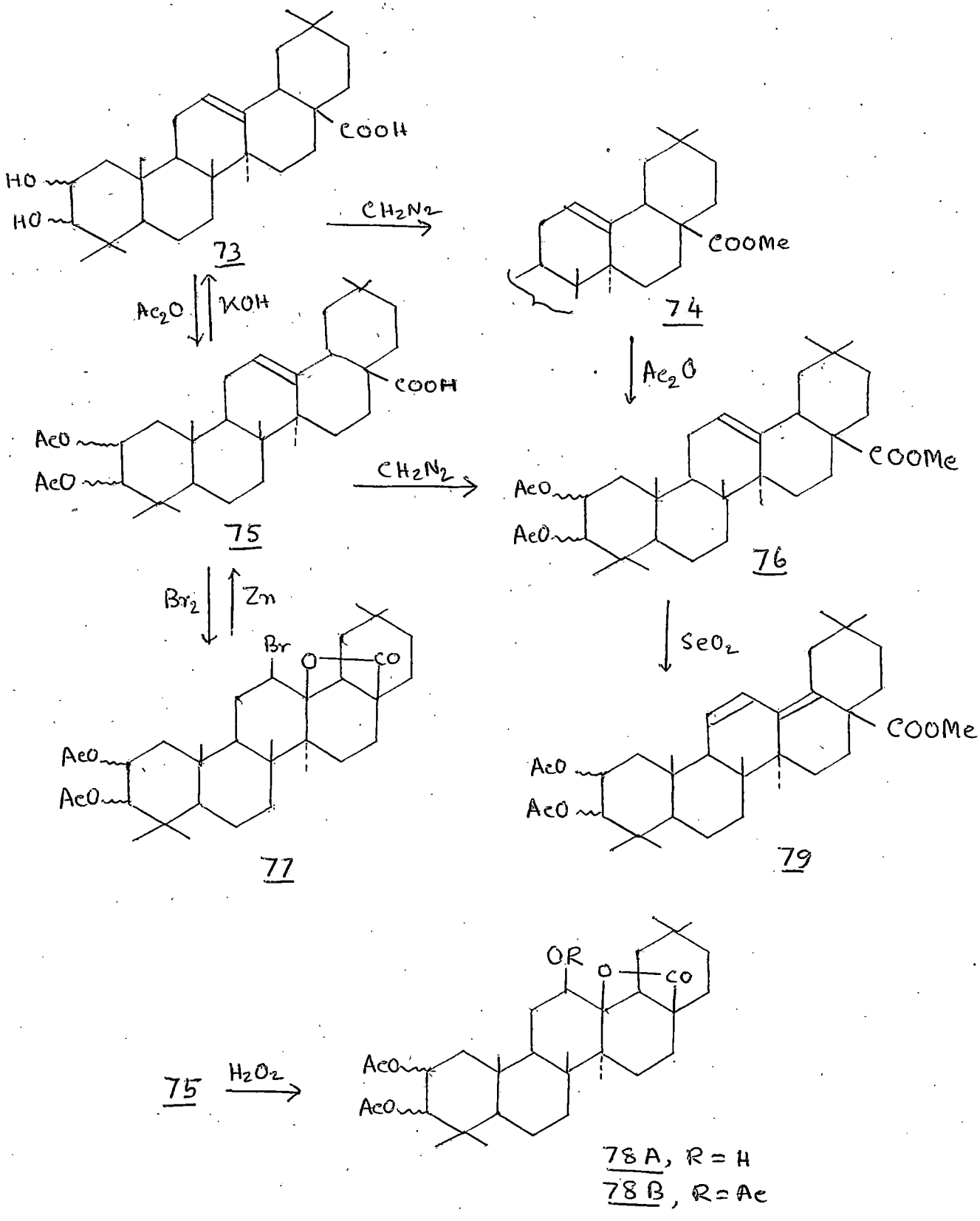
However, in the mean time, Caglioti et al reported[30] the isolation from the cakes of Olean europa of a new acid, maslinic acid, which latter proved identical with crategolic acid 73 of Tschesche et al. The Italian workers[30] were able to show that maslinic acid was a pentacyclic triterpenic acid, probably belonging to the β-amyrin group, containing two acylable hydroxyl groups and a non-hydrogenizable double bond γ- to the carboxyl group.

In their subsequent investigations[44,45] Caglioti et al were

able to elucidate the complete structure of the acid as a 2, 3-dihydroxy olean-12-en-28-oic acid. Their work of structure elucidation of crategolic acid 73 is shown schematically in Chart VI. Crategolic acid 73 formed a methyl ester 74, a diacetate 75 and a methyl ester diacetate 76. The diacetoxy acid 75 with bromine gave a bromolactone 77 which on treatment with zinc and acetic acid regenerated the diacetoxy acid 75. The latter 75 with hydrogen peroxide gave a hydroxy-diacetoxy-lactone 78A which gave a triacetoxy lactone 78B on acetylation. Sclenium dioxide oxidation of the methyl ester diacetate 76 gave a conjugated diene ester 79 showing U.V absorption maxima at 260, 251, 243 mμ, characteristics of β-amyrin derivatives. The presence of an α glycol system was shown by the consumption of one mole of periodic acid of methyl crategolate 74.

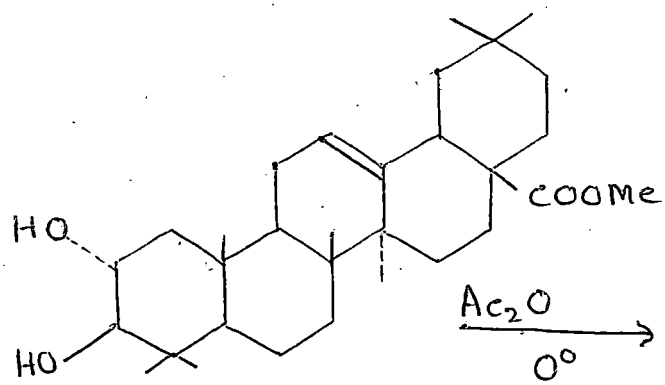
Finally crategolic acid 73 was correlated with the β-amyrin group by its elegant conversion into methyl oleanonate 81 and methyl olean-12-en-28 oate 82. This conversion inter alia settled the position of the two hydroxyl group at C-2 and C-3. When methyl crategolate 74 was acetylated at 0^{o}, the major product was the 2-acetate 79, which could be oxidised with chromic acid to give the 3-keto-2-acetate 80. The latter 80 on treatment with calcium in liquid ammonia was converted to methyl oleanonate 81, a substance of known structure methyl crategolate 74 itself on oxidation with chromic acid followed by Huang Minlon reduction furnished the known ester 82. These transformations are shown schematically in Chart VI.

Chart VI



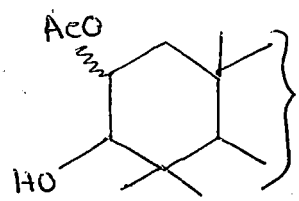
78 A, R = H

78 B, R = Ac



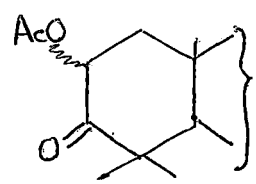
74

Ac_2O, 0°



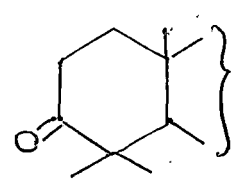
79

CrO_3



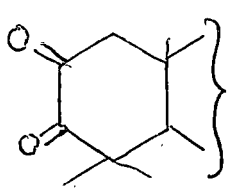
80

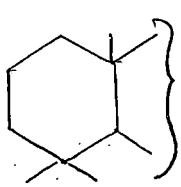
Ca / liq. NH_3



81

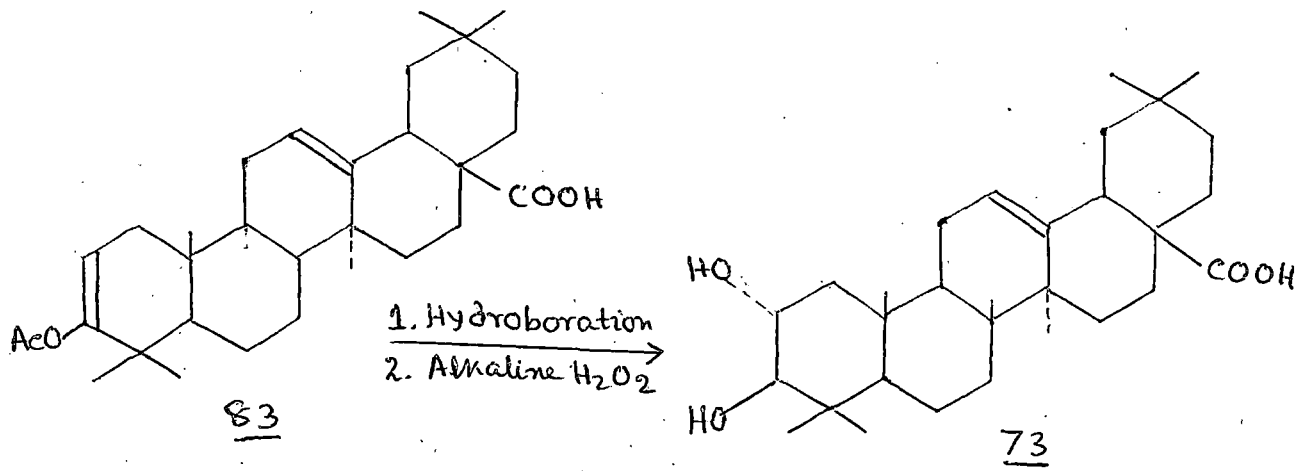
CrO_3





82

Subsequently, the configuration of the two hydroxyl groups was elucidated by Caglioti et al[46]. Preferential formation of the 2-monoacetate 79 suggested the 2α, 3β-trans diequatorial configuration of the diol moiety, which was confirmed by successful synthesis[46] of crategolic acid 73 from the enol-acetate 83 of oleanonic acid by hydroboration, which was known to be a stereospecific process[47,48].



83 73

The correctness of the above assignment of configuration of the two hydroxyl groups of crategolic acid 73 was further supported by the work of Tschesche et al[38] on the structure of bredemolic acid (discussed in page 118, Part II of this thesis).

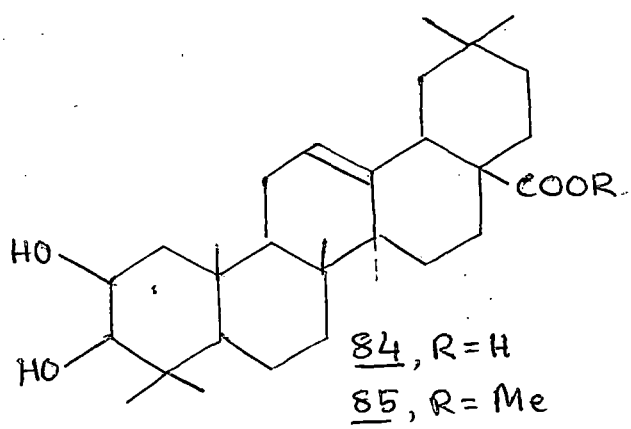
Sengupta et al[49] has also isolated crategolic acid (maslinic acid) 73 from the flowers of Eugenia jambolana Lam as its methyl ester along with oleanolic acid.

The present author has also synthesised methyl crategolate and the details have been discussed on page 163 Part II of this thesis.

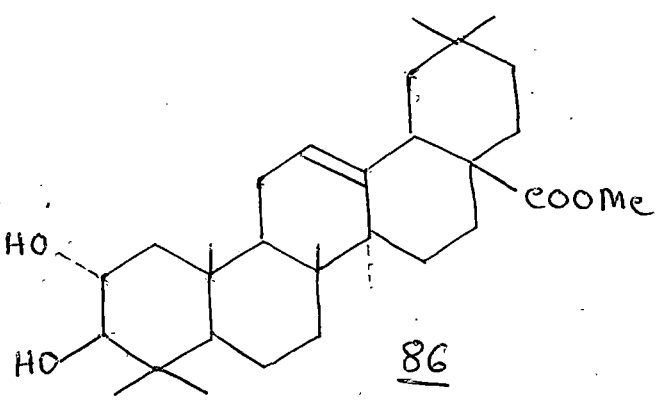
3. The 2β, 3β -dihydroxyolean-12-en-28 oate.

Bannon et al[31] recently reported the isolation of 2β, 3β -dihydroxyolean-12-en-28-oic acid 84 from the sapogenin mixture prepared from the extract of the wood of Castanosperum australe Cunn and Fras. These authors established the identity of 84 (2β, 3β-dihydroxyolean-12-en-28 oic acid) by a high yielding stereospecific synthesis (Chart VII) from methyl crategolate 86.

Bannon et al isolated 84 as its methyl ester 85. The structure was suggested by its IR, NMR, mass spectrum[29b] . The melting point of the methyl ester 276-80°, 85 was in agreement with that published previously for methyl 2β, 3β-dihydroxyolean-12-en-28 oate (lit 278-82°[37] and 276-84°[28]). These authors carried out a stereospecific synthesis of methyl 2β, 3β dihydroxyolean-12-en-28-oate 85 in high yield from methyl crategolate 86 and thereby concluded that the product islated from C. australe is in fact methyl 2β, 3β-dihydroxy-olean-12-en-28 oate.

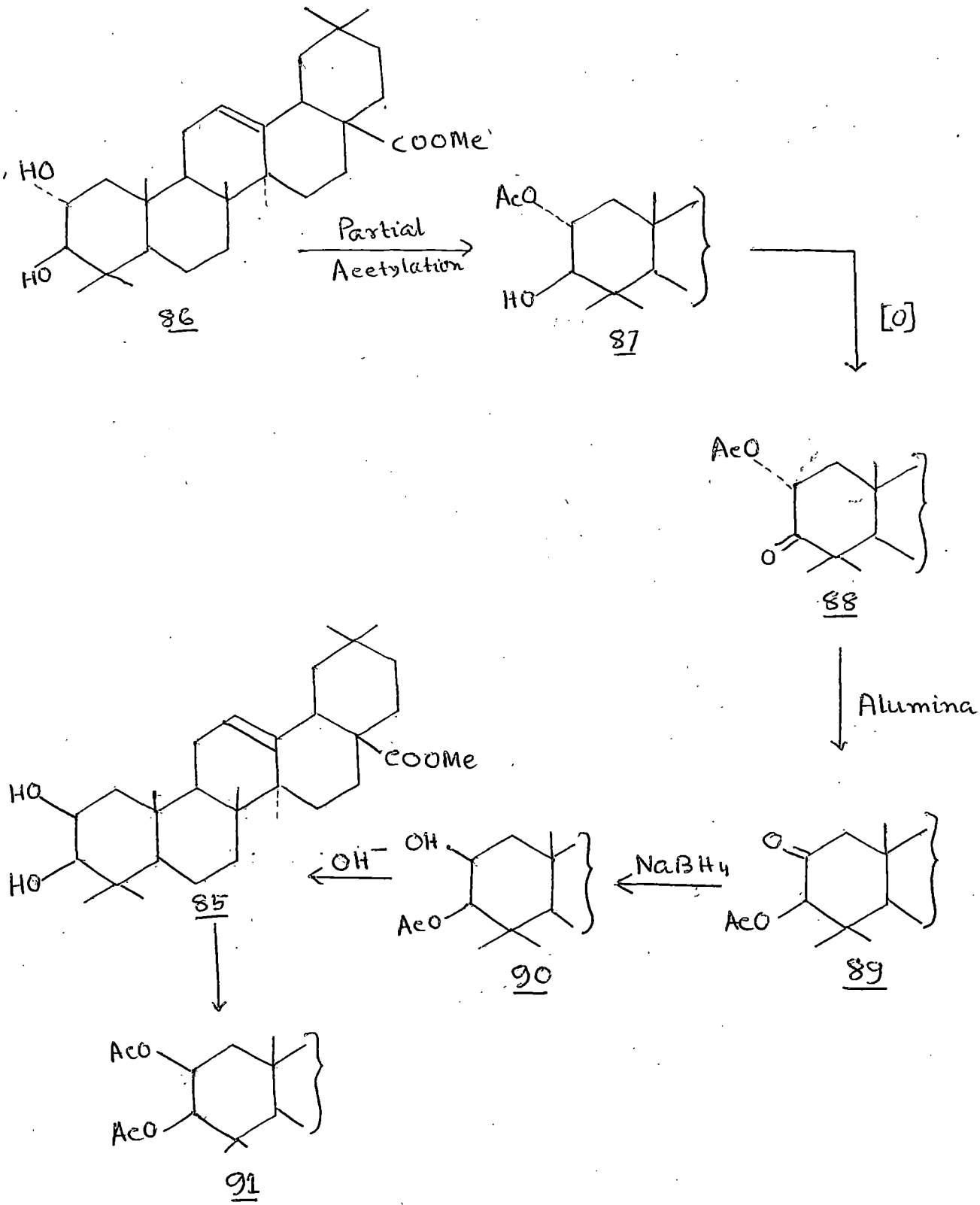


84, R = H
85, R = Me



86

Chart VII


HO
COOMe
HO
Partial
Acetylation
AcO
HO
[O]
AcO
O
Alumina
COOMe
HO
HO
OH⁻
OH
AcO
NaBH4
O
AcO
AcO
AcO
86
87
88
85
90
89
91

Methyl crategolate 86 on partial acetylation yielded the 2α-acetoxy-3β-alcohol[43] 87 which was oxidised with dimethyl sulfoxide in acetic anhydride to give 2α-acetoxy-3-ketone 88. The latter 88 on isomerisation on alumina gave the 3β-acetoxy-2-ketone 89. The structure of this rearranged acetoxy ketone followed from analogy with the rearrangements of similar groups in lupane[51], lanostane and 4, 4-dimethyl cholestane derivatives[52] and from its NMR and IR spectra. Reduction of the 3β-acetoxy-2-ketone 89 with sodium borohydride proceeded quantitatively to give a single product 90 in which the introduced hydroxyl group at C-2 could be assigned the β-configuration on the assumption that attack has occured from less hindered α-side of the molecule. Mild alkaline hydrolysis of 3β-acetoxy-2β-hydroxyolean-12-en-28-oate 90 gave the diol 85 m.p. 278-80°, $(\alpha)_D$ 88 (lit. m.p. 258-60°, $(\alpha)_D$ 97°[43], m.p. 258-62°[28], m.p. 258-62° $(\alpha)_D$ 85°[37], m.p. 258-61°[29b]). 85 on acetylation afforded the diacetate, methyl 2β, 3β-diacetoxyolean-12-en-28-oate 91 m.p. 232-4°, $(\alpha)_D$ 82°, (lit[29b] m.p. 227-31°).

We have also synthesised the 2β, 3β-dihydroxyolean-12-en-28-oic acid from diosphenol of methyl oleanonate (details discussed on page 162 Part II of this thesis).

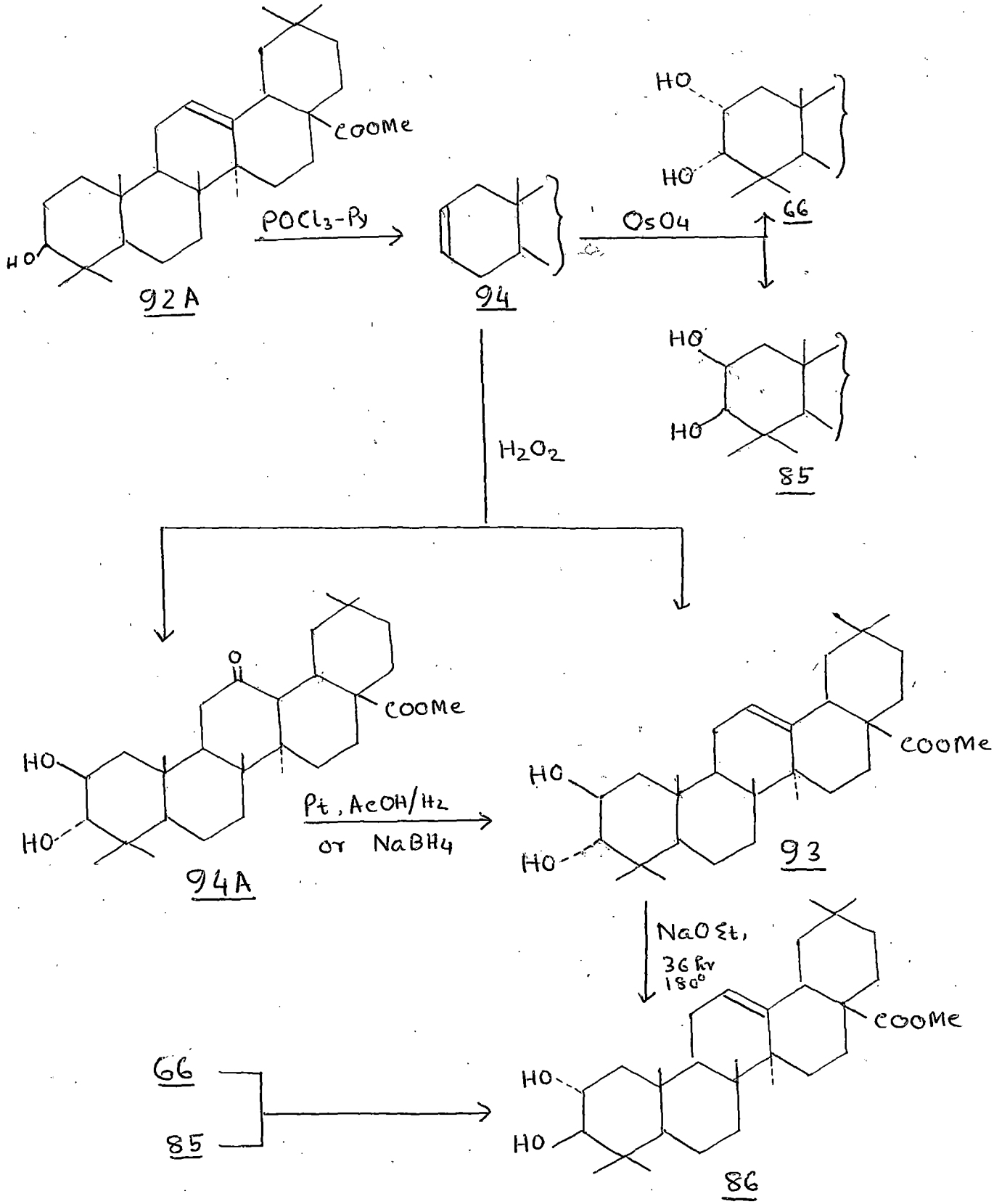
4. The 2β, 3α-dihydroxyolean-12-en-28-oic acid (bredemolic acid):

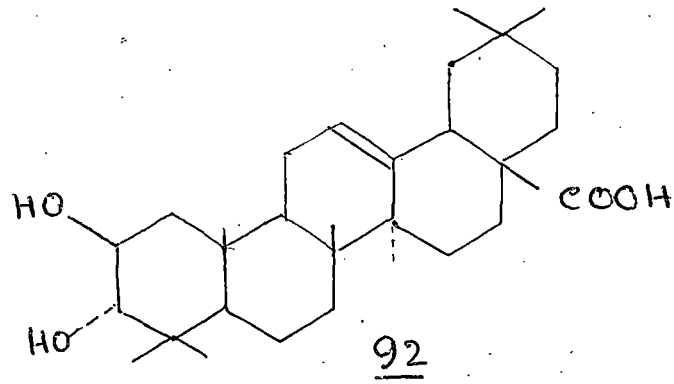
Bredemolic acid 92, isolated from Bredemeyera floribunda willd., was also a 2,3 -dihydroxyolean-12-en-28-oic acid and hence

must be an epimer of crategolic acid 73. Since the 2β, 3β-84, and the 2α, 3α-65A isomers were already known by synthesis[37] and crategolic acid 73 was shown to be the 2α, 3β-dihydroxyolean-12-en-28-oic acid by Caglioti et al[44] bredemolie acid 92 must then be the remaining 2β, 3α-dihydroxy isomer. However bredemolic acid 92 was found to form an acetonide, a somewhat unexpected behaviour on the part of a normal 1,2-diaxial trans cyclohexane diol derivative. Therefore Tschesche et al synthesised[27,28] unambiguously all the four stereoisomeric 2,3-dihydroxy olean-12-en-28-oic acids as their respective methyl esters. The synthetic work of Tschesche et al is shown in Chart VIII.

The key compound in the synthesis of the above epimeric methyl esters Chart VIII; methyl oleana-2, 12-dien-28-oate 94 was prepared from methyl oleanolate 92A by dehydration with phosphorous oxychloride and pyridine. The dien-ester 94 gave two cis diols: the 2α, 3α diol 66 and the 2β, 3β diol 85 by treatment with osmium tetroxide. On the other hand treatment of the diene-ester 94 with hydrogen peroxide gave methyl 12-keto-2β, 3α-dihydroxyolean-28-oate 94A as the major product and only a trace of the desired 2β, 3α-diol 93. The above 12-keto-ester 94A, however, was converted by reduction into methyl bredomolate 93. Finally, all the above three diol-esters 66, 85 and 93 on equilibration with base gave methyl crategolate 86, which must consequently have the stablest diequatorial 2α, 3β-configuration of the two hydroxyl groups.

Chart VIII


COOMe
HO
92A
POCl3-Py
94
HO
HO
66
OsO4
HO
HO
85
H2O2
O
COOMe
HO
HO
94A
Pt, AcOH/H2
or NaBH4
COOMe
HO
HO
93
NaOEt,
36 hr
180°
66
85
COOMe
HO
HO
86



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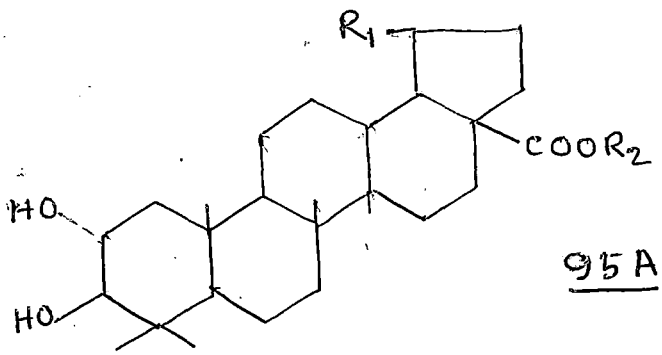
5. Alphitolic Acid:

Guise et al[33] isolated alphitolic acid 95A as its methyl ester, m.p. 233-35° from the wood of Alphitonia petrici Braid and White. The ester, methyl alphitolate 95B (ν_{max} 3634, 3587, 1735, 887 cm^{-1}) formed a diacetate (no hydroxyl absorption in the infrared) and a dihydroderivative 95C (no absorption in the infrared at 887 cm^{-1}) namely methyl dihydroalphitolate. The ester 95B consumed one mole of lead tetra-acetate. Guise et al converted dihydromethyl alphitolate 95C to a dialdehyde 96 by treatment with 1 mole equivalent of sodium metaperiodate. The latter on oxidation with chromic anhydride in acetic acid followed by methylation gave a trimethyl ester 97 identical with trimethyl ester of the Seco-A acid derived from dihydrobetulic acid[54]. From the above physical and chemical evidences Guise et al confirmed the structure of methyl

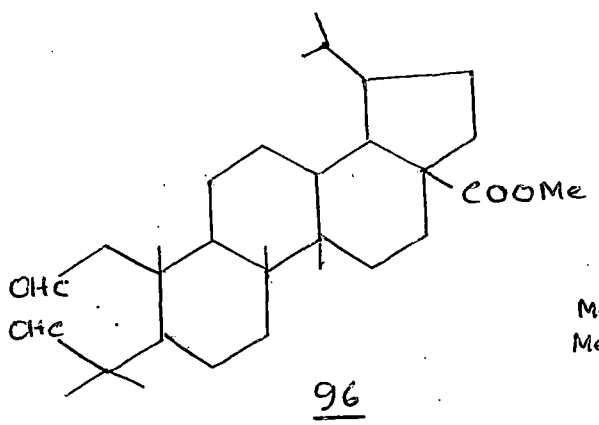
alphitolate as depicted in 95B. The stereochemistry of 1,2-glycol grouping in 95B was based on quantitative lead tetra-acetate titra-



95A, $R_1 = C(CH_3)=CH_2$; $R_2 = H$

95B, $R_1 = C(CH_3)=CH_2$; $R_2 = Me$

95C, $R_1 = HC(CH_3)-CH_3$; $R_2 = Me$



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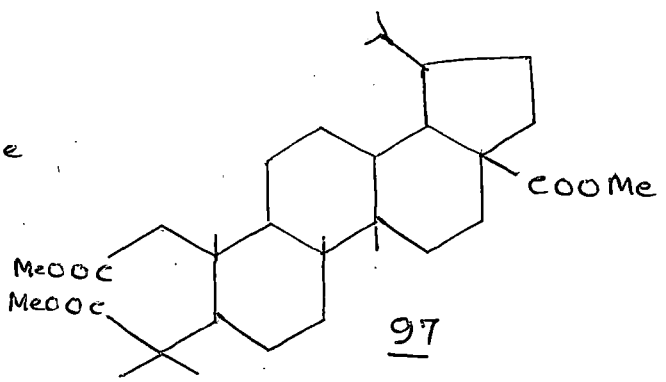
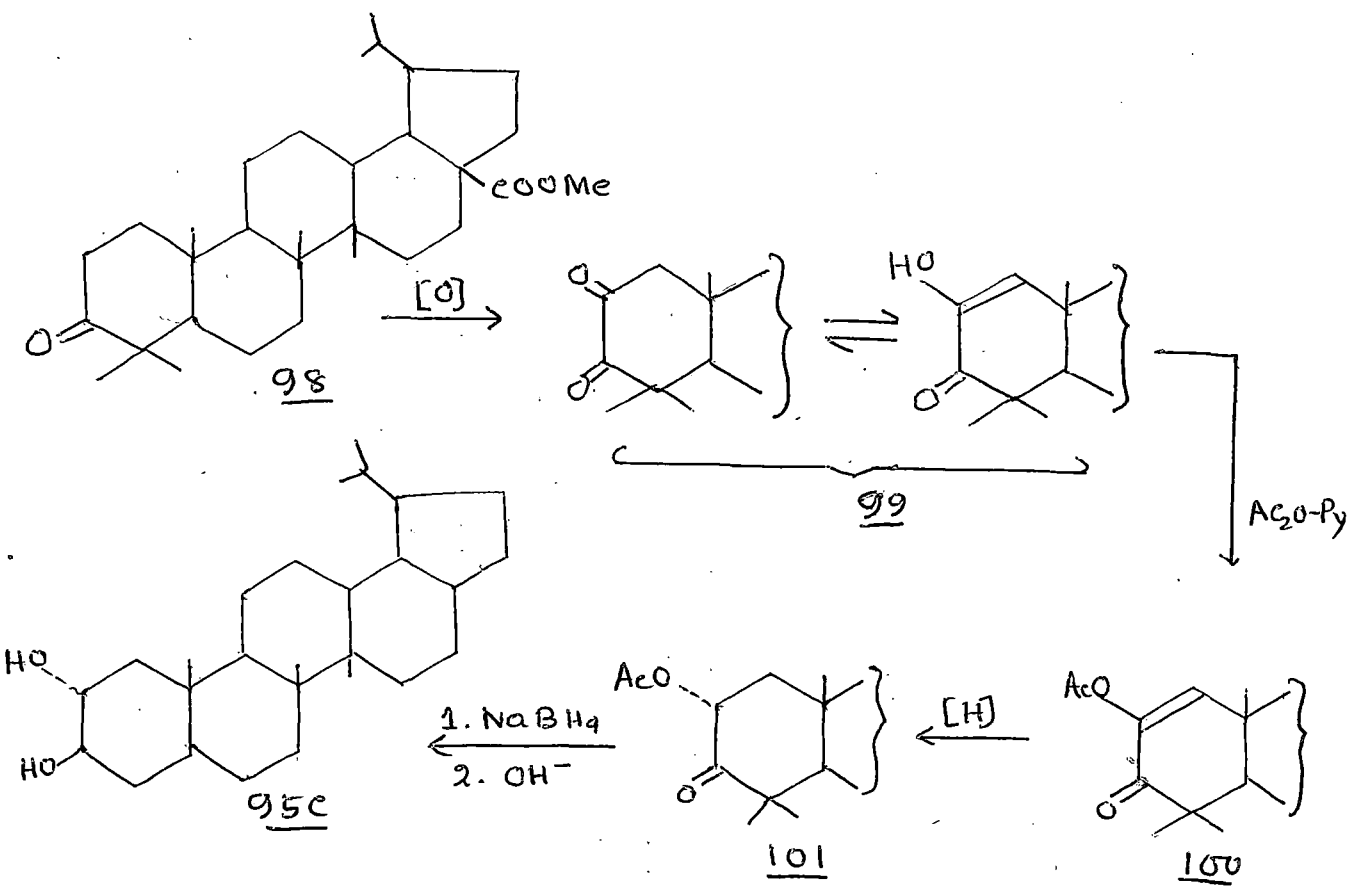


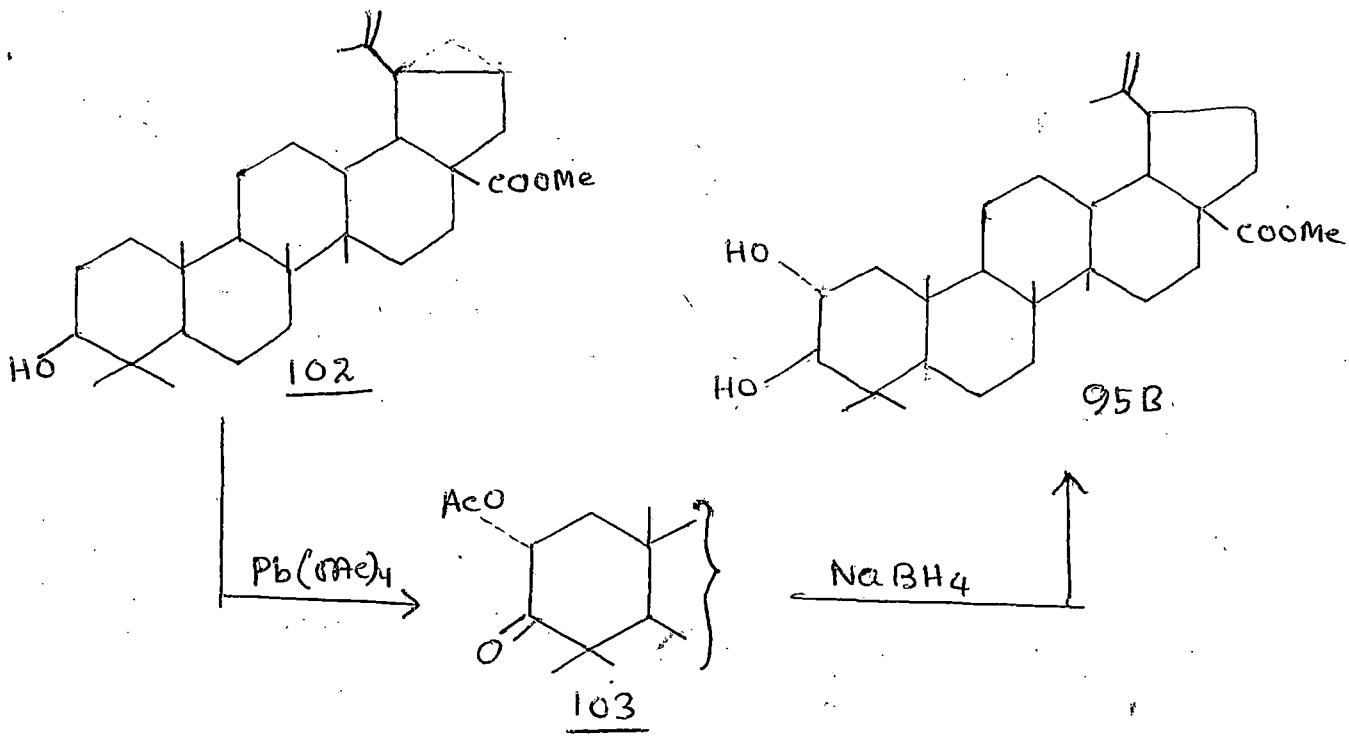
97

tions under the defined conditions of Djerassi and Ehrlich[55], where a value $k = 2.7 \times 10^{-3}$ L mole^{-1} Sec^{-1} was obtained, identical for triterpene 2α, 3β-glycols.

Khastgir et al[56] also reported the synthesis of methyl dihydroalphitolate 95C. Methyl dihydrobetulonate 98 prepared from betulinic acid, was oxidised by passing oxygen through a suspension of K -tertiary butoxide in dry tertiary-butanol to give diosphenol 99, m.p. 131-33°, $\lambda_{max}^{m\mu}$ 269 (ε, 7532) which on acetylation gave the corresponding acetate 100 m.p. 194-96°, λ_{max} 237 mμ (ε, 9968). This acetate 100 on hydrogenation in presence of 10% palladium-on-charcoal catalyst gave 2α-acetoxy methyl dihydrobetulonate m.p. 223-25°, 101. Reduction of 101 with sodium borohydride at PH 8 gave 2α-acetoxy dihydrobetulinate which on alkaline hydrolysis gave methyl dihydroalphitolate 95C identical with an authentic sample of methyl dihydroalphitolate.

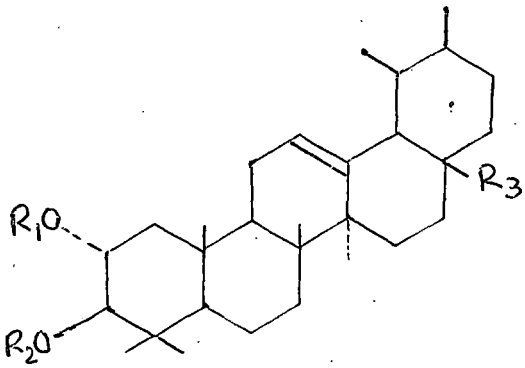


Rastogi et al[57] have very recently described the synthesis of methyl alphitolate 95B from methyl betulonate 102. Methyl betulonate 102 on treatment with lead tetra-acetate yielded 2α-acetoxy-3-ketoderivative 103 m.p. 191° as the major product. That the acetoxy group on C-2 is equatorial was shown by the NMR signal at 2.12 ppm for the acetoxy group and a quartet at 5.6 ppm (J = 13 and 6 Hz) for axial proton at C-2 containing the acetoxy group. The acetoxy ketone 103 on reduction with sodium borohydride gave the glycol namely methyl alphitolate 95B m.p. 235°. NMR signal at 3.66 ppm (multiplet about 26 Hz wide) confirmed the position and 2α, 3β configuration of the hydroxy groups in methyl alphitolate 95B.



6. 2α-hydroxy ursolic acid:

Glen et al[34] reported the isolation of 2α-hydroxy ursolic acid 104A as its methyl ester from the leaves of Rose-bay Willow-herb (chamaenorion angustifolium). That the methyl ester 104B was an ursane derivate was indicated by the presence of three peaks in the region 1400-1350 cm^{-1} and two peaks in the region 1320-1240 cm^{-1} in the infrared spectrum[59]. NMR spectrum revealed the presence of one olefinic proton (τ 4.73) and one methyl ester group (τ 6.38). Further evidence came from mass spectrum of 104B which showed intense peaks at m/e 263, 203 and at 223 corresponding to ions A, B and C, a pattern frequently associated with Δ^{12}-triterpenoids[35]. A similar mass fragmentation pattern was observed for the 2α-hydroxy-uvaol 104C formed by lithium aluminium hydride reduction of the dihydroxy methyl ester 104B. The methyl ester 104B formed a diacetate 104D (twin distinct peaks in the NMR at τ 7.97 and 8.04[60]). This physical evidence for the presence of a 2,3-diol system in 104B was further substantiated by the preparation of an isopropylidene derivative and also by the formation of the diosphenol 105 by chromium trioxide-pyridine oxidation. The presence of an ursane skeleton in the ester 104B was shown by the Wolff Kishner reduction of the diosphenol 105 to give urs-12-en-28 oic acid 106 identical

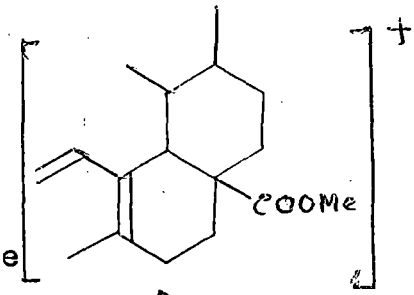


104A $R_1 = R_2 = H$; $R_3 = COOH$

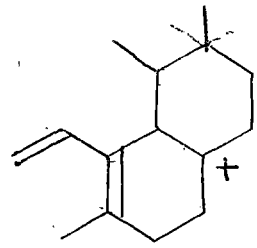
104B $R_1 = R_2 = H$; $R_3 = COOMe$

104C $R_1 = R_2 = H$; $R_3 = CH_2OH$

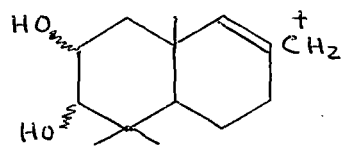
104D $R_1 = R_2 = Ac$; $R_3 = COOMe$



A

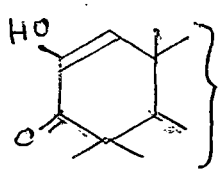


B



C

with an authentic sample. Cleavage of the diosphenol 105 with alkaline hydrogen peroxide gave 2,3 seco-urs-12-en-2,3,28-trioic acid-28 methyl ester 107 which confirmed the presence of the ursane-skeleton in the ester 104B



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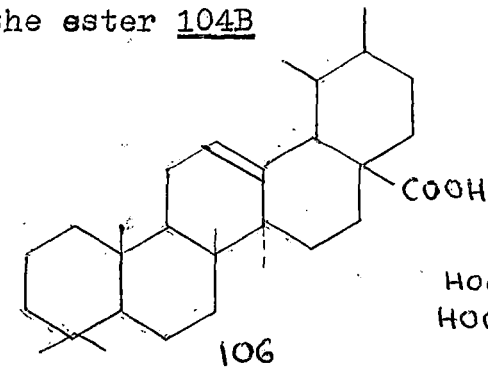

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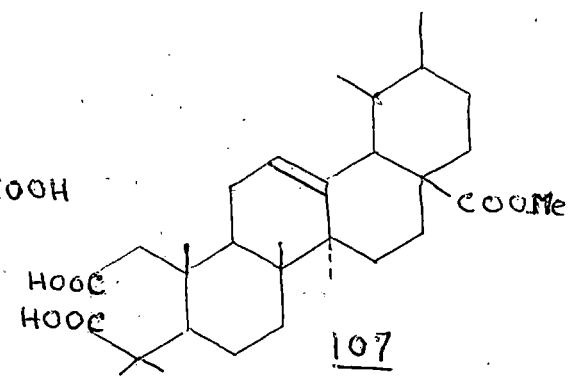

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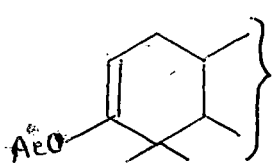

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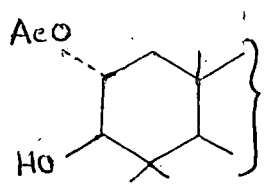

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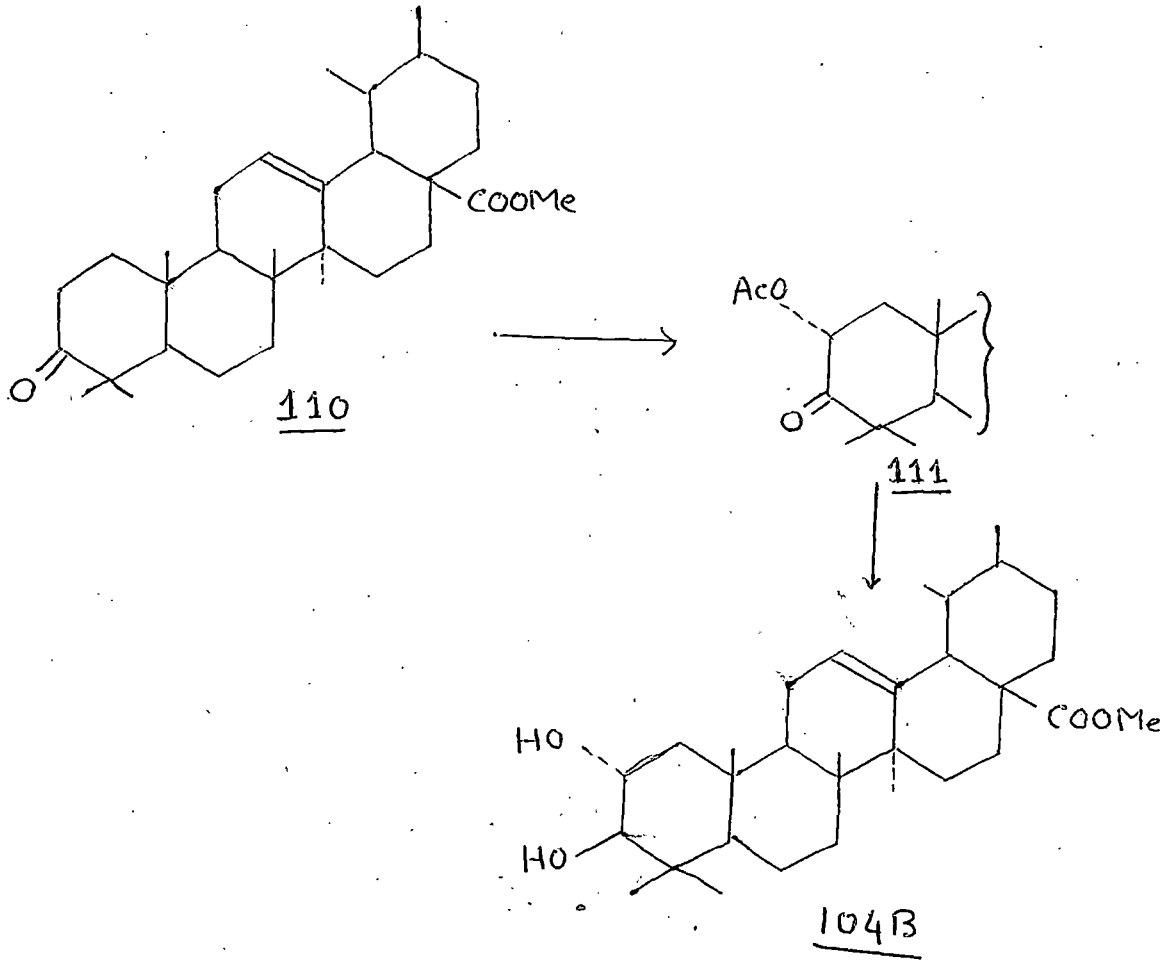
The confiiguration of the hydroxyl group in the dihydroxy ester 104B was settled by the synthesis of the trans diequatorial diol 104B. The enol acetate 108 on treatment with diborane and then subsequent oxidation of the intermediate afforded three products viz. methyl ursonate, methyl ursolate and methyl 2α-, 3β-dihydroxy urs-12-en-28-oate 104B, the latter was identical with that obtained from the natural dihydroxy acid 104A.

Further support for the aissignment of theα-configuration to the hydroxyl group at C-2 in 104B was revealed by the NMR spectrum of the monoacetate, methyl 2α-acetoxy-3β-hydroxy urs-12-en-28-oate 109. The NMR spectrum showed signals in the region τ4.9 - 5.4 due to C-2 proton. The breadth of the resonance indicated that at least two couplings of about 8 c/sec are present and this can only occur when the proton at C-2 occupies an axial conformation allowing an axial interaction with the proton at C-3 and a similar interaction with the axial proton at C-1. In addition, there is an axial-equatorial interaction with the other proton at C-1. This indicated that the proton at C-2 has the axial conformation and acetoxy group at C-2 has the equatorial (α) conformation.

Rastogi et al[57] repeated Glen's[34] work and obtained the enol acetate m.p. 198^{o} in 46%. Yield but subsequent steps of hydroboration and oxidation yielded only the starting material methyl ursolate.

Rastogi et al employed lead tetra-acetate oxidation on methyl ursolate 110 and obtained 2α-acetoxy-3-keto derivative 111 as an amorphous powder, the latter was characterized by NMR spectrum. Reduction of 110 with sodium borohydride gave the methyl 2α-hydroxy ursolate 104B in an overall yield of 26.7%, characterized by IR and NMR data.



CHAPTER - II

Studies on Autoxidation: Synthesis of isomeric 2,3-diols of of isohopane (moretane)(Section B) and methyl, Olean-12-en-28-oates (Section C).

Section A: Introduction:

Khastgir et al[10] have been able to prepare the three isomeric diols (2α , 3α-; 2α, 3β-; 2β, 3β-) of Δ^{12}-oleanene out of the four possible isomers, using diosphenol obtained by the autoxidation of β-amyrone (See Chart IV, page 95).

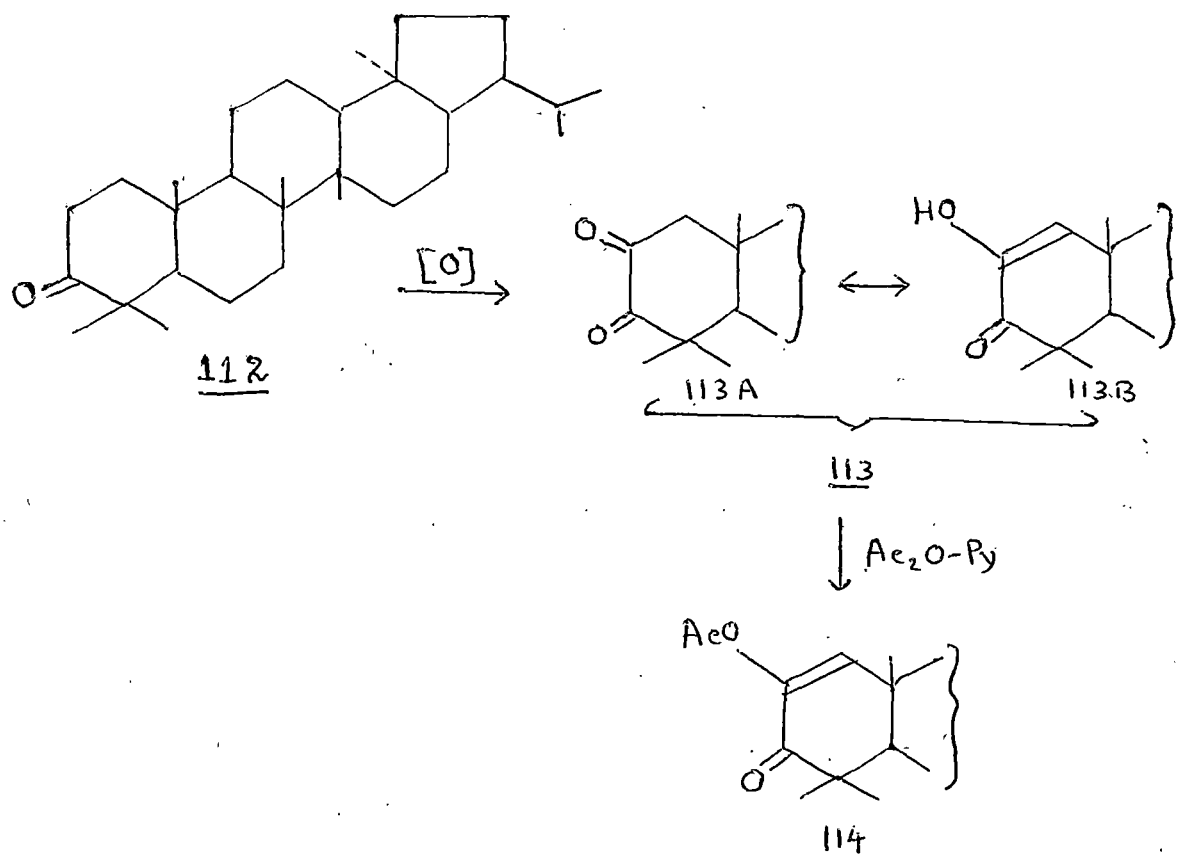
In order to examine the generality of the interesting reactions encountered during the studies on autoxidation and the method of synthesis of isomeric Δ^{12}-oleanene 2,3-diols, we have extended our studies in (A) Isohopane (moretane) series and (B) oleanolic acid series. The synthesis of the isomeric 2,3 -diols in the oleanolic acid series is described in Section C (page 160 of this thesis).

Section B: Synthesis of isomeric 2,3-diols of isohopane (moretane)

1. Synthesis of 2α , 3β-dihydroxy isohopane (moretane) 121

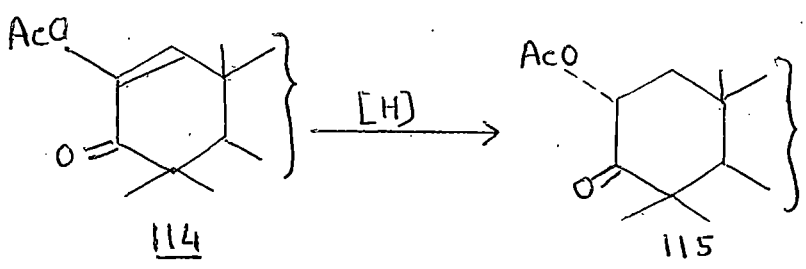
Moretanone 112 obtained by the hydrogenation of moretenone[32] was oxidised by passing oxygen through a suspension of 112 in dry tertiary butanol- containing potassium tertiary butoxide[11,12,13,22]. One mole of oxygen was rapidly absorbed by the compound giving a α-diketone derivative 113 m.p. 190-92° $(\alpha)_D$ 40.00°. The compound

showed two spots on the chromatoplate indicating the presence of a mixture of two compounds. The compound 113 showed positive ferric chloride coloration. Its UV spectra exhibited maxima at 269 mμ (ε, 5104). IR spectra showed peaks at 1100, 1645, 1670, 1715, 2960 and 3560 cm^{-1}. These data were in complete agreement with the assignments shown in 113A and 113B



Acetylation of 113 with acetic anhydride and pyridine at room temperature gave the corresponding acetate 114 as a viscous oil which could not be induced to crystallisation. The latter was found to be

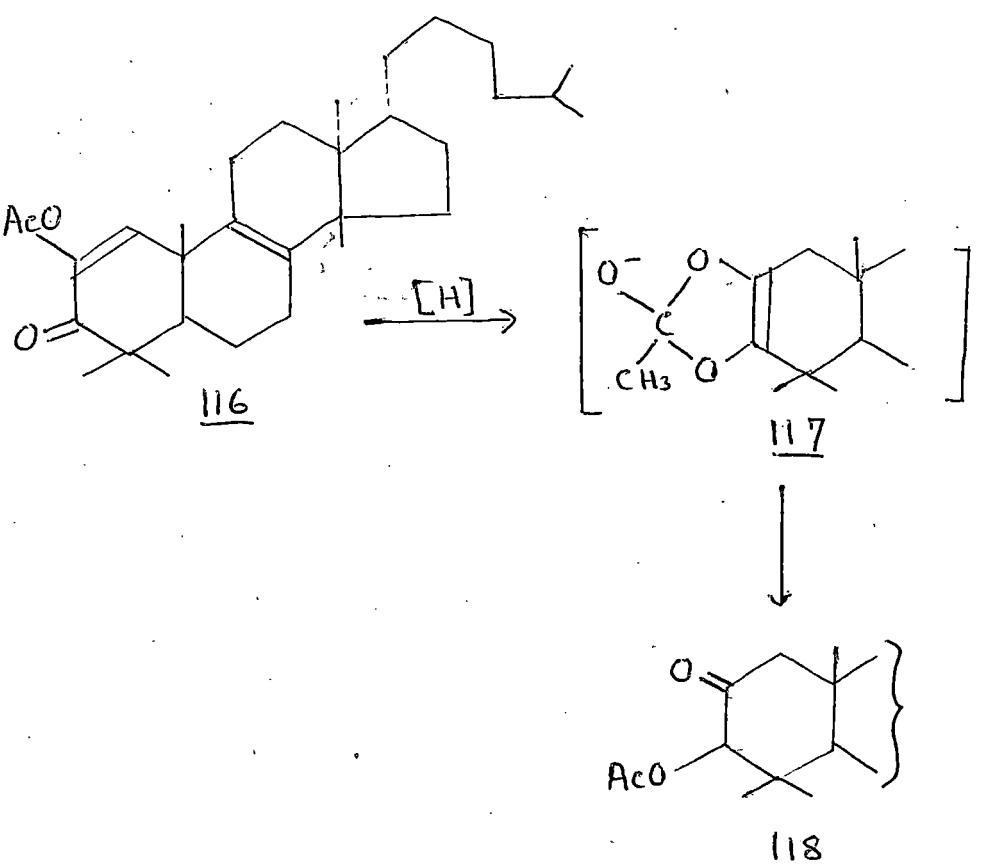
a homogeneous compound as it showed a single round spot in TLC. The acetate showed an UV absorption at λ_{max} 236 mμ (ϵ, 6514). These spectral data clearly established the structure 113B and 114 for the diosphenol and the diosphenol acetate respectively. Diosphenol acetate 114 on hydrogenation in presence of 10% palladium-on-charcoal catalyst in ethanol solution gave a product of m.p. 179-81°, $(\alpha)_D$ 86.31°, λ_{max} 276 mμ (ϵ, 82). NMR spectrum of the compound was



114 115

consistent with the structure 115, the acetoxy group being at C-2 and its configuration as α. The proton at C-2 displayed a quartet of lines centered at 5.4 ppm, no signals were detected in the region 4.95 ppm characteristics for proton α-to a keto group (-CO-C-$\underline{H_2}$). The low field signal, forming X part of an ABX system may be assigned to a methine proton α-both to an acetoxy and a carbonyl group. The coupling constants (J_{ae} = 8 Hz and J_{aa} = 12 Hz) suggest an axial configuration for this hydrogen[38,39]. The coupling constants indicated that it is predominately in a single conformation-probably chair conformation.

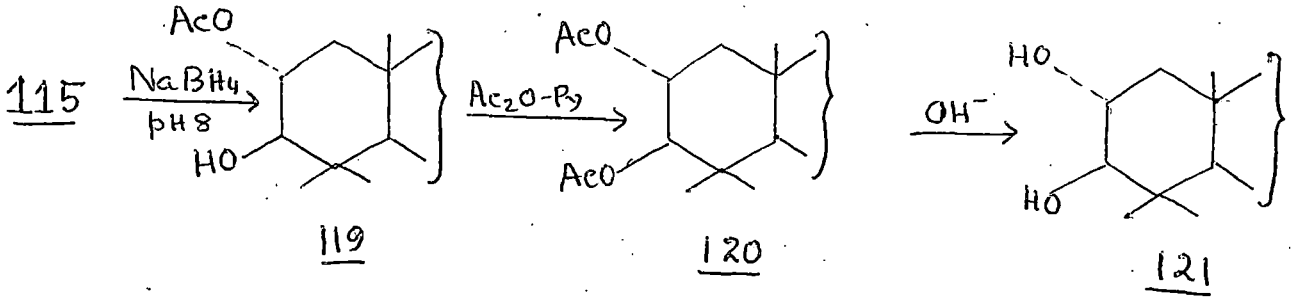
In this connection it is necessary to metnion that Lavie and co-workers during their studies on oxidation in euphol series claimed that hydrogenation of diosphenol acetate of dihydro Euphone 116 gave a product which was identical with C-3 acetate 118 i.e. a 2-keto-3-acetoxy derivative. Migration of the acetoxy group from C-2 to C-3 position was proposed through the cyclic intermediate 117[15] . Their results are contrary to the observations reported in the β-amyrone series[10], and also our experience in moretanone series where we obtained a 2α-acetoxy-3-keto compound 115. In our case 1, 2-addition of hydrogen takes place leading to a stable 2α-acetoxy-3-keto compound.



116 117 118

In order to provide a reasonable explanation for the different behaviour shown by these compounds (dihydro euphone, β-amyrone and moretanone) we have examined the drieding models of euphol, β-amyrin and moretane derivatives. In the euphol series the presence of a double bond in 8,9 position causes deformation[50] and has a modified chair conformation which confers additional strain in the molecule whereas in the β-amyrin series as well as in moretane series (having A/B chair-chair conformation) this strain is not present. Most probably the 2α-acetoxy-3 keto-compound which presumably is formed at first on hydrogenation of 116 isomerises to 118 via 117 to release the additional strain in the molecule. Hence 2α-acetoxy moretanone has the structure 115 and the acetoxy group being at C-2 and its configuration as α.

Sodium borohydride reduction of 115 in dioxan solution buffered at pH-8 to reduce isomerisation gave the crystalline 2α-acetoxy-3 β-hydroxy compound 119, m.p. 199-200°, $(\alpha)_D$ 95.35. The latter on acetylation with pyridine and acetic anhydride afforded the 2α, 3β-diacetate 120, m.p. 228-30°, $(\alpha)_D$ 50.60°, which on alkaline hydrolysis afforded the corresponding 2α, 3β-diol 121, m.p. 242-3°, $(\alpha)_D$ 82.86°. The diequatorial 2α, 3β-configuration of the hydroxyl groups in the diol 121 was unequivocally confirmed by examination of the NMR spectra of the diol 121 and its diacetate 120. The NMR spectra of the diol 121 give rise to an unsymmetrical

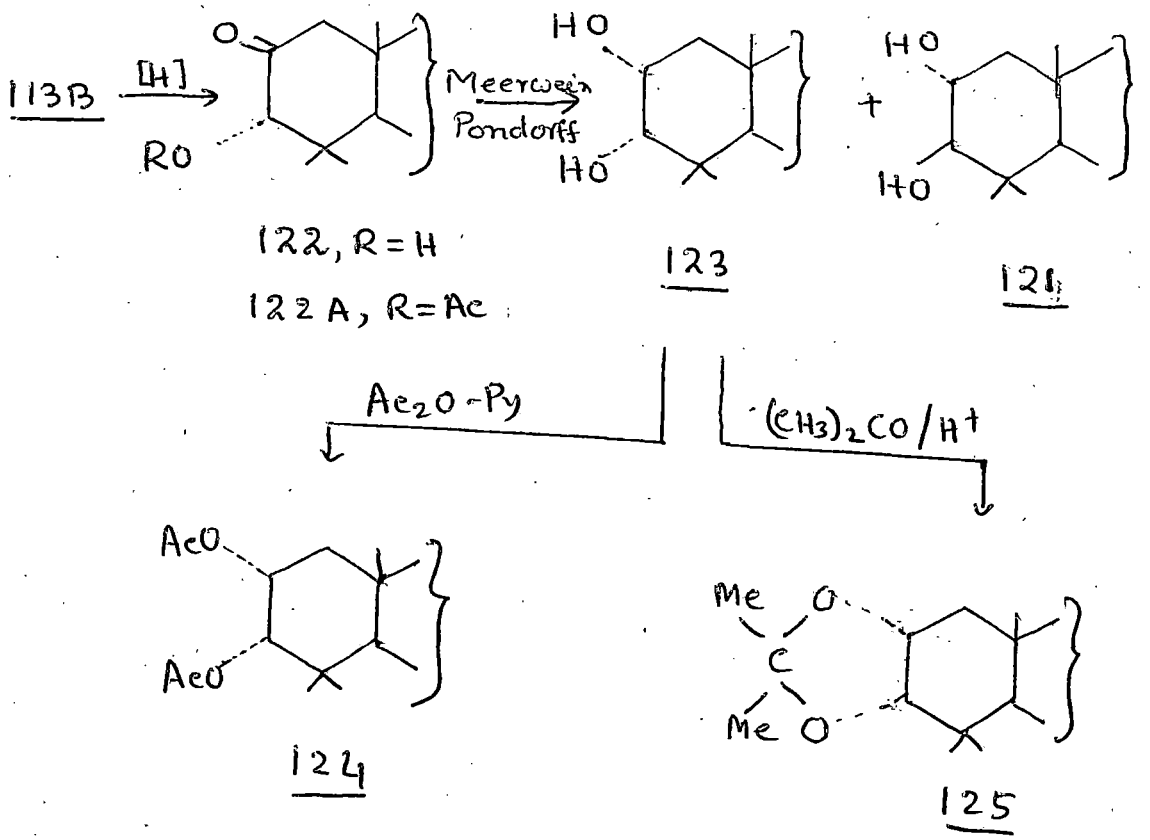


doublet near 2.90 and 3.2 ppm (Jaa = 10 Cps). This unsymmetrical doublet arises due to coupling with C-2 proton. The 10 cps coupling between the proton implies a trans diaxial arrangement of the C-2 and C-3 proton[39]. The C-2 proton is further coupled to methylene protons at C-1 and the signal for this is discerned as a quartet of doublets centered at 3.76 ppm (X part of an ABXY). A similar pattern of δ-values have been observed for methyl crategolate and other triterpenoids with identical ring A[53]. The NMR spectrum of its diacetate 120 showed the same pattern shifted to the lower field due to acetate group.

2. Synthesis of 2α, 3α-dihydroxy isohopane (moretane) 123:

Diosphenol 113B on hydrogenation in presence of 10% palladium-on-charcoal catalyst in ethanol solution afforded 2 keto-moretanol 122, m.p. 181-3°, $(\alpha)_D$ 29.41°, which on acetylation with

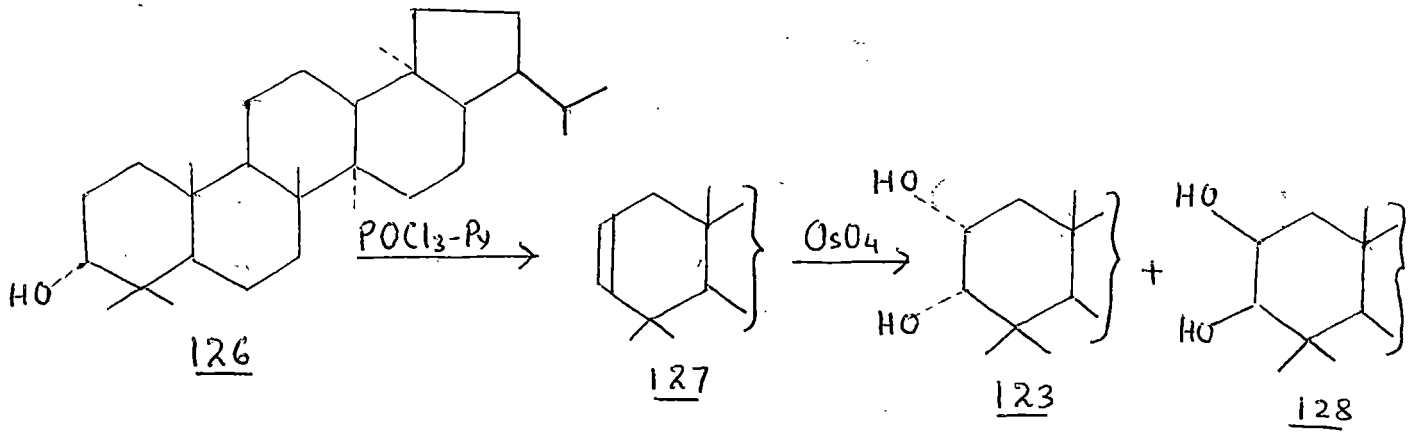
pyridine and acetic anhydride afforded the corresponding acetate 122A m.p. 264-7°, $(\alpha)_D$ 82.61°. NMR spectrum of the compound 122 showed a broad peak at 3.88 ppm accounting for one proton associated with the hydroxyl group containing no neighbouring proton (-CO-CH-OH-C-), broad peak at 3.44 ppm due to proton associated with the hydroxyl group (-CH-OH) which disappeared upon D_2O exchange and two AB type doublets at 2.42 and 2.55 ppm (J_{AB} = 12 cps) accounting for two hydrogens (-CO-CH_2). The corresponding acetate 122A in the



NMR spectrum had a sharp singlet at 4.95 ppm ascribed to the C-3 proton (-CH-OAc) and a broad doublet at 2.49 and 2.37 ppm for two protons adjacent to a carbonyl group (-CO-CH_2).

Meerwein-Pondorff reduction of 2-keto moretanol 122 furnished a crystalline solid which exhibited two distinct spots on chromatoplate. Chromatography of the solid, first eluted a solid compound which after crystallisation from methanol and chloroform mixture afforded a crystalline solid 123, m.p. 250-51°, $(\alpha)_D$ 9.37°, ν_{max} 3420, 2960, 1450, 1370, 1350, 1042 cm^{-1}, in 92% yield. The latter on acetylation with pyridine and acetic anhydride afforded an acetate 124 m.p. 185-7°. The more polar solid obtained from the chromatogram in about 5% yield had the m.p. 242-3° and was found to be identical with 2α, 3β-diol 121, by m.m.p. and IR comparison. The stereochemistry of the hydroxyl groups in the diol has been assigned as 2α, 3α as depicted in 123 on the basis of chemical and physical evidences described below.

Δ^2-moretane 127 prepared by dehydration of epi-moretanol 126 with phosphorousoxychloride and pyridine, was treated with osmium tetroxide in pyridine solvent and the product obtained after chromatography melted at 235-40° and showed two spots on chromatoplate. The separation of the two components could not be successfully accomplished by column chromatography.



126 127 123 128

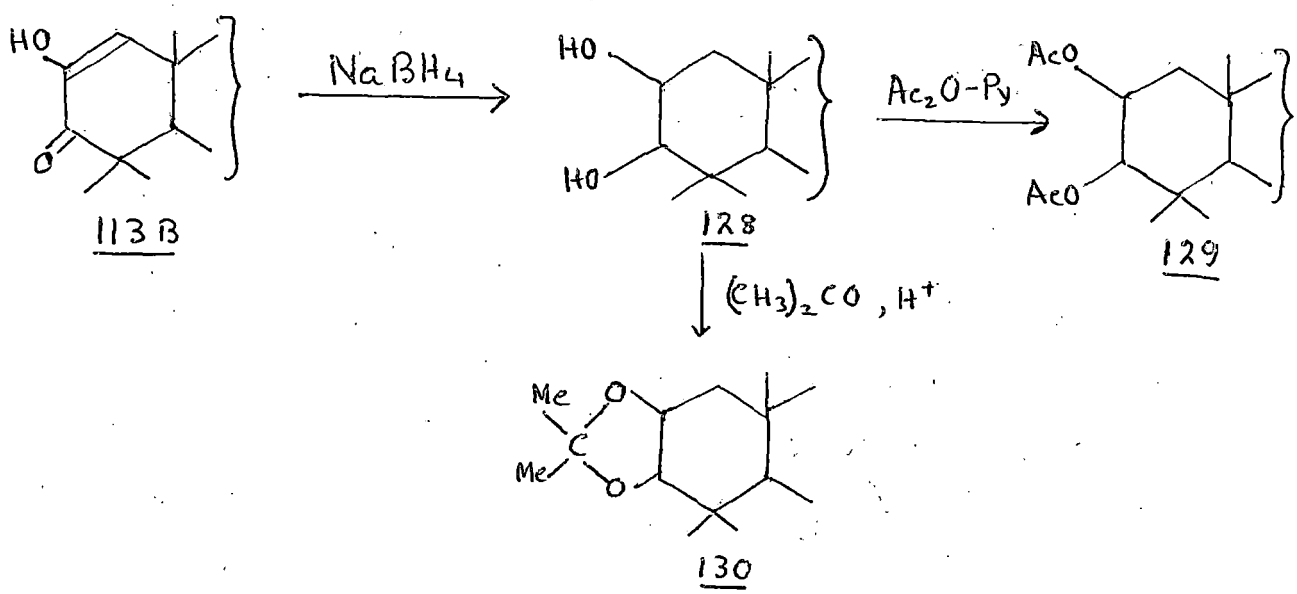
However, careful fractional crystallisation of the mixture from chloroform methanol mixture afforded first a diol having m.p. 250-51° $(\alpha)_D$ 9.83, as the major component (87%). From the mother liquor a second diol, m.p. 261-3°, $(\alpha)_D$ 23.68° was isolated as a minor product (8%). The latter compound has been identified as the 2β, 3β-diol 128 from its m.m.p. and IR comparison with a~~d~~ sample of 2β, 3β-diol (described on page 138). The major diol 250-51°, $(\alpha)_D$ 9.83° afforded a diacetate, m.p. 185-7°, 124. The diol and the diacetate were found to be identical with the diol and diacetate respectively obtained by the Meerwein-Pondorff reduction of 2 keto-moretanol 122 described above. Since osmylation can only afford two cis isomers, the diol 123 having m.p. 250-51° and its diacetate 124, m.p. 185-7° must have the 2α, 3α-configuration as depicted in formulas 123 and 124 respectively.

NMR spectrum of the diol 123 is in good accord with the 2α, 3α-orientation of the hydroxyl groups. NMR of 123 showed a narrow doublet (Jea- 3Hz) multiplet at 3.90 ppm associated with C-3 proton (Hα). The C-2 proton was discerned as a multiplet centered at 4.05 ppm (Jaa 12Hz, Jea 3, 2·5 Hz). In the NMR spectrum of its diacetate 124, the peak associated with C-3 ~~carbinol~~ proton is shifted to the lower fields (doublet 4.63 ppm) and the C-2 proton give rise to a ~~quartet~~ multiplet at 5.32 ppm. All these are in good agreement with the stereochemical assignments 123 and 124 .

The diol 123 on treatment with acetone in presence of catalytic amount of p-toluene sulfonic acid gave an acetonide derivative 125, m.p. 186-88°, $(\alpha)_D$ 19.04.

3. Synthesis of 2β, 3β-dihydroxy isohopane (moretane) 128:

Diosphenol 113B on sodium borohydride reduction gave a compound 128, m.p. 262-4°, $(\alpha)_D$ 23.68°, ν_{max}^{nujol} 3420, 2950, 1460, 1275, 1250 cm^{-1}. The latter on acetylation with acetic anhydride-pyridine

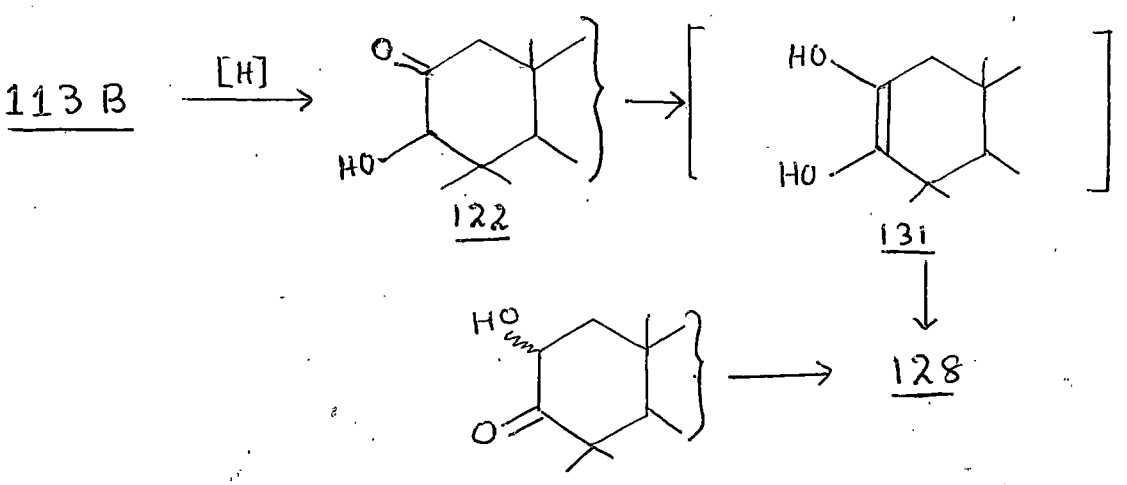


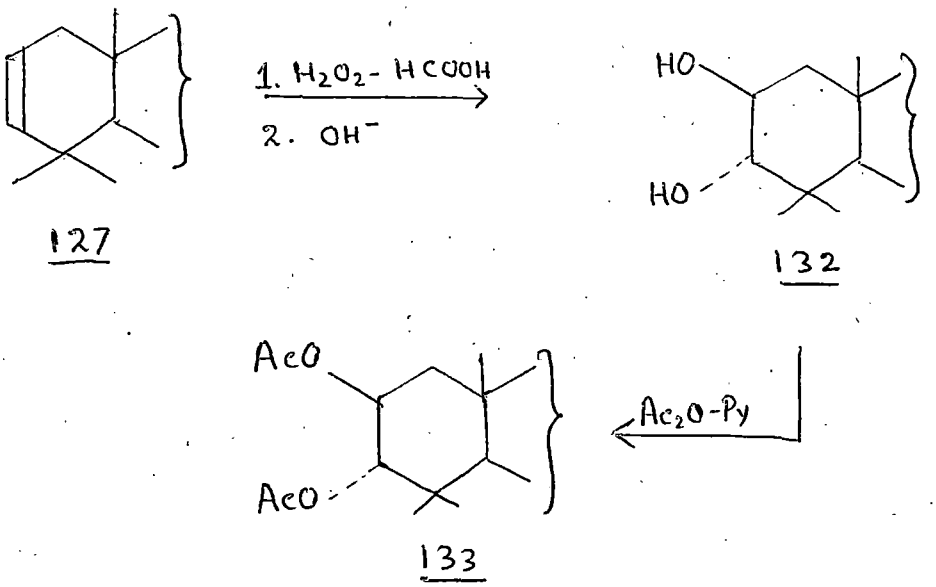
113B 128 129 130

afforded a crystalline diacetate 129, m.p. 214-15°, $(\alpha)_D$ 31.25°. NMR of 128 showed a multiplet at 3.23 ppm and a broad unresolved multiplet at about 4.60 ppm which collapsed to a doublet (J = 3.6 Hz) and a multiplet (J = 16 Hz) respectively upon exchange of hydroxyl proton with D_2O. Thus the hydroxyl group at C-3 is equatorial (Hα) and the one at C-2 is axial (Hβ). In the NMR spectra of the diacetate 129 these signals were shifted downfield to 4.97 ppm (doublet, J = 3 Hz) and at about 5.25 ppm (broad multiplet).

Compound 128 on treatment with acetone in presence of catalytic amount of p-toluene sulfonic acid gave an acetonide derivative 133 m.p. 239-41°, $(\alpha)_D$ 29.85°.

Sodium borohydride reduction would be expected to furnish a 2α, 3α-diol or 2β, 3β-diol or a mixture of 2α, 3β-and 2α, 3α-diols. However, 2β, 3β-diol 128 could only be isolated which can be explained if it is assumed that the reduction proceeds via





127 132 133

the intermediate 131.

4. Synthesis of 2β, 3α-dihydroxy isohopane (moretane) 132:

The sterically most unstable 2β, 3α-diol (axial-axial) was prepared by the known method[1,2,3] described in literature. The method involved the oxidation of Δ^2-moretane 127 with performic acid and subsequent hydrolysis of the ester with alkali solution. By following the above procedure a crystalline diol 132, m.p. 221-4°, $(\alpha)_D$ 21.18° was obtained. IR spectrum of the diol 132 showed peaks at

3560, 2980, 1455 cm^{-1}. Acetylation of the diol with acetic anhydride and pyridine afforded the crystalline 2β, 3α-diacetate 133, m.p. 145-7°, $(\alpha)_D$ 31.40.

The 2β, 3α-stereochemistry of the diol has been assigned by analogy with previous work reported in the literature[10,28]. The NMR spectrum of the diacetate 133, is shown in Fig. 4.

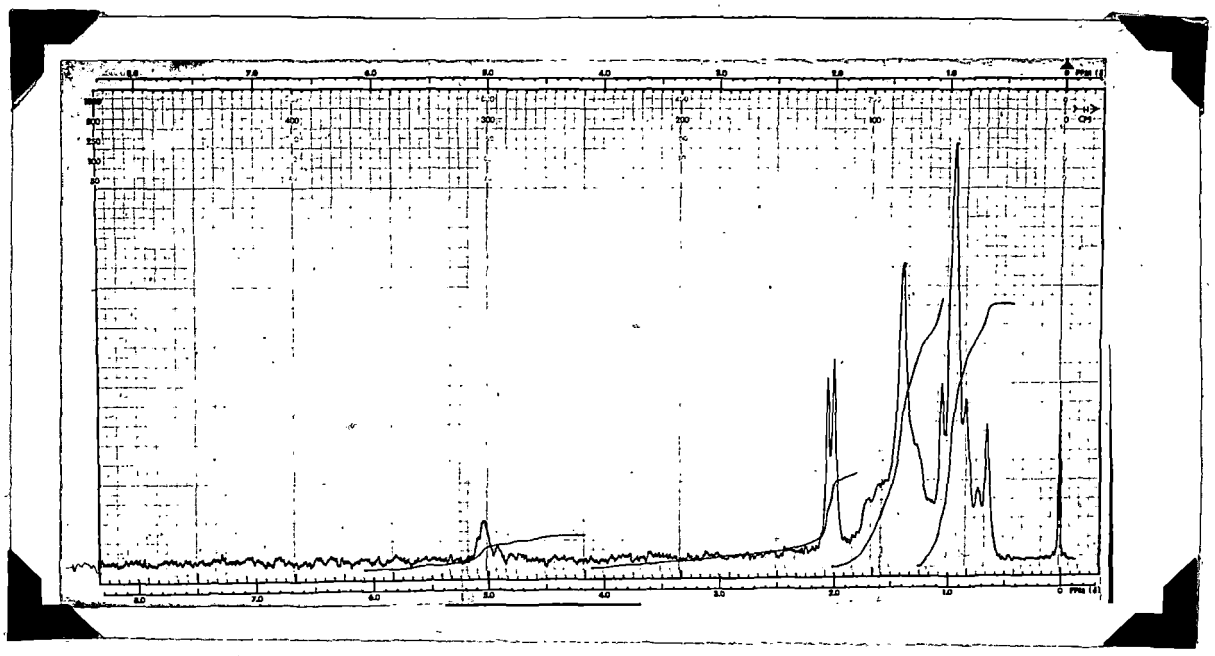


Fig. 4. NMR spectrum of 2β, 3α-diacetate 133

EXPERIMENTAL

Melting points are uncorrected. The petroleum ether used throughout the investigation had b.p. 60-80°. All optical rotations were determined in chloroform solution unless stated otherwise. NMR spectra were determined on Varian A-60 and HA-100 spectrometers using chloroform-d solution containing tetramethyl-silane as reference. The IR spectra were recorded in Perkin-Elmer 337 and 221 and Beckmann I.R. - 20 spectrophotometers. UV absorption spectra were taken in Ziess VSU-1 and UV Beckmann DU-2 spectrophotometers in 95% ethanol solution unless otherwise stated.

Preparation of moretanone 112:

Moretenone (3 gms) (obtained from the bark of Sapium Sebiferum Roxb. by the method of Khastgir et al[32]) dissolved in ethyl acetate (400 ml) was stirred in presence of 10% palladim-on-charcoal catalysts (300 mg) in an atmosphere of hydrogen till the absorption ceased. After working up in the usual manner it gave a solid (2.8 gm). The latter on crystallisation from chloroform and methanol mixture gave crystals of 112, m.p. 190-92° $(\alpha)_D$ 33°, which was found to be identical with an authentic sample of moretanone (m.m.p. and $(\alpha)_D$).

Autoxidation of isohopanone (moretanone) 112: Preparation of diosphenol 113.

Moretanone (2 gm) suspended in potassium-tertiary butoxide in tertiary butanol (prepared from 6 gm of potassium and 160 ml of tertiary butanol) was shaken in a stream of oxygen for three hours. The reaction mixture was diluted with water and then 6N hydrochloric acid was added till the solution was acidic. It was then extracted with chloroform (150 ml), washed with water till neutral and the combined extract was dried (Na_2SO_4). On removal ofthe solvent under reduced pressure, a yellowish gummy foam was obtained (1.8 gm). The latter on crystallisation from acetone-methanol gave colorless crystals (1 gm), m.p. 190-2°, $(\alpha)_D$ 40.00°. It gave a positive ferric chloride test for diosphenol. TLC of the compound showed two spots on chromatoplate (using benzene as solvent), an upper spot R_f = 0.76 of slightly weaker intensity than the lower spot R_f = 0.73. These were assumed to be due to the tautomeric mixture of the diketone 113A and the diosphenol 113B.

Found: C, 81.62; H, 10.56%

Calculated for $C_{30}H_{48}O_2$: C, 81.81; H, 10.90%

UV : λ_{max} 269 mμ (∈, 5104)

Acetylation of diosphenol 113 : Preparation of diosphenol acetate 114

Diosphenol 113 (500 mg) was acetylated by treatment with acetic anhydride (10 ml) and pyridine (10 ml) overnight at room temperature. After working up in the usual manner a highly viscous

oil was obtained which could not be crystallised. The compound showed a single spot on a chromatoplate and did not give any coloration with ferric chloride solution.

UV: λ_{max} 236 mμ(ε, 6514)

Hydrogenation of diosphenol acetate 114: Preparation of 2α-acetoxy isohopanone (moretanone)115

To diosphenol acetate 114 (200 mg) dissolved in absolute ethanol was added 10% palladium-on-charcoal catalyst (25 mg) and the mixture was shaken in an atmosphere of hydrogen till the absorption ceased. The catalyst was removed by filtration and the solvent was distilled off under reduced pressure from the filtrate. A solid residue was obtained which after three crystallisation from methanol furnished a solid 115, m.p. 179-81°, $(\alpha)_D$ 86.31°.

Found: C, 79.59; H, 10.62%

Calculated for $C_{32}H_{52}O_3$: C, 79.33; H, 10.74%

UV: λ_{max} 278 mμ(ε, 82)

IR: ν_{max}^{nujol} 1720 (-C=O), 3500 (-OH) cm^{-1}

Fig. 1

Preparation of 2α-acetoxy-3β-hydroxy isohopane (moretane) 119: Sodium borohydride reduction of 2α-acetoxy isohopanone (moretanone) 115:

To 2α-acetoxy isohopanone 115 (300 mg) dissolved in dry dioxan (25 ml) was added, with cooling a slurry of sodium borohydride

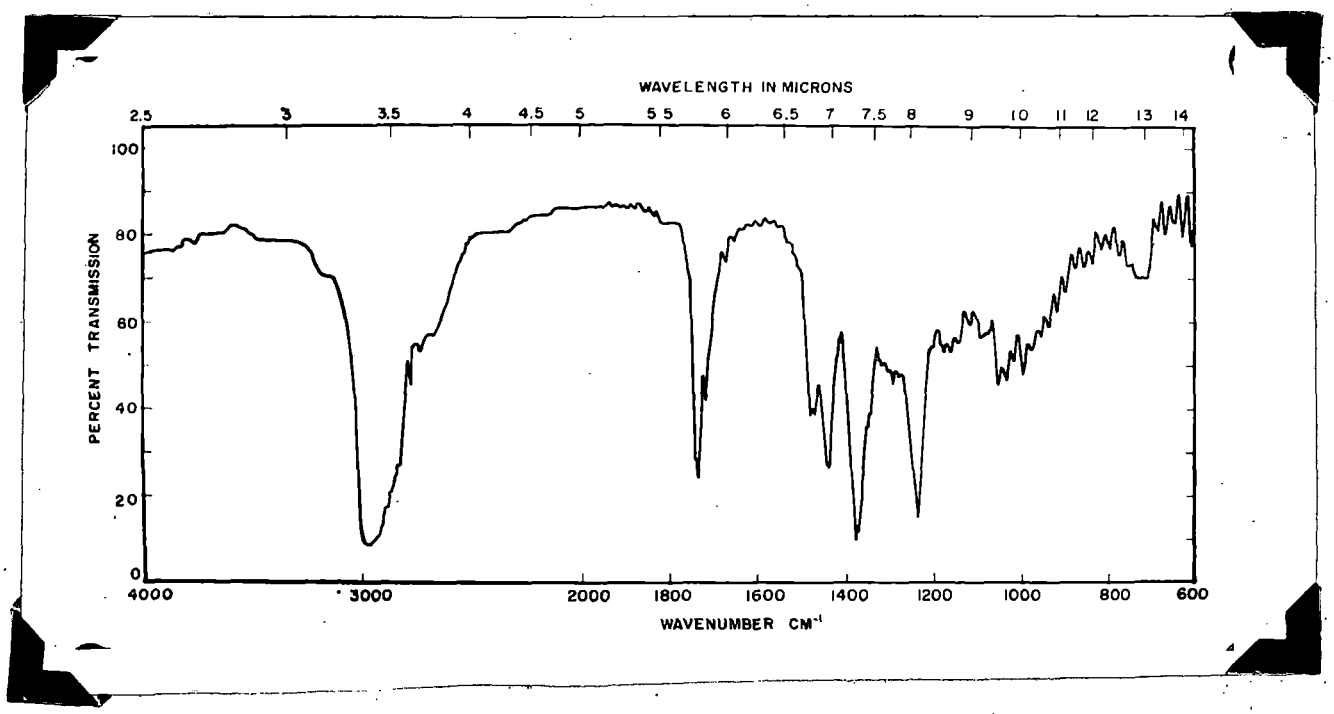


Fig. 1. IR spectrum of 2α-acetoxy isohopanone (moretanone) 115

(300 mg) prepared in an NH_4Cl - NH_4OH buffer (PH = 8, 4 ml). The mixture was stirred at room temperature for two hours. A portion of the solvent was removed by distillation, cooled and acidified with dilute hydrochloric acid and extracted with ether. The ethereal layer was washed with water till neutral and dried (Na_2SO_4). Removal of ether gave a solid residue (250 mg) which was chromatographed over a column of alumina (30 gm, deactivated with 1.2 ml of 10% aqueous acetic acid) developed with petroleum ether. The residue was dissolved in benzene, poured on the column and was eluted with following solvents. (Table I)

Table I

Eluent	Fractions 50 ml each	Residue
Petroleum ether	1-4	Oil solid small (15 mg)
Petroleum ether: benzene (4:1)	5-7	Nil
Petroleum ether: benzene (3:2)	8-14	Solid (200 mg) m.p. 196-7°

Further elution with more polar solvents did not afford any solid.

The solid from fractions 8-14 (Table I) were combined and crystallised from chloroform-methanol mixture. After two crystallisation

pure 2α-acetoxy-3β-hydroxy isohopane (moretane) 119, m.p. 199-200°, $(\alpha)_D$ 95.35 was obtained.

Found:	C, 78.59; H, 11.01%
Calculated for $C_{32}H_{54}O_3$:	C, 78.96; H, 11.18%

Acetylation of 2α-acetoxy-3β-hydroxy isohopane 119: Isolation of 2α, 3β-diacetate 120:

The solid (200 mg) m.p. 199-200°, 119 was acetylated by heating with pyridine (2 ml) and acetic anhydride (5 ml) on a water bath for six hours. After working up in the usual manner it gave crystals of 2α, 3β-diol diacetate 120, m.p. 228-30°, $(\alpha)_D$ 50.60°.

Found:	C, 77.33; H, 10.76%
Calculated for $C_{34}H_{56}O_4$:	C, 77.27; H, 10.6%

No UV absorption in the region 200-300 mμ

IR: ν_{max}^{nujol} 1750, 1480, 1465, 1355 cm^{-1} (Fig. 2)

NMR (100 Mc/s): Peaks at 1.97, 2.03 (6H, 2-O-COC$\underline{H}_3$), 4.65 4.82 (2H, $\underline{H}$ -C-OCOCH$_3$) ppm.

Hydrolysis of 2α, 3β-diacetate 120: Preparation of 2α, 3β-dihydroxy isohopane (moretane) 121:

To the above diacetate m.p. 228-30° (150 mg) in dioxan (40 ml) was added 10% sodium hydroxide solution (10 ml) and the mixture was heated under reflux for three hours. The reaction mixture was cooled, diluted with water and then extracted with ether. The ethereal layer after washing with water till neutral, was dried (Na_2SO_4). The solvent was removed and a solid m.p. 238-40° was obtained. After three crystallisations from methanol it afforded pure 2α, 3β-dihydroxy isohopane

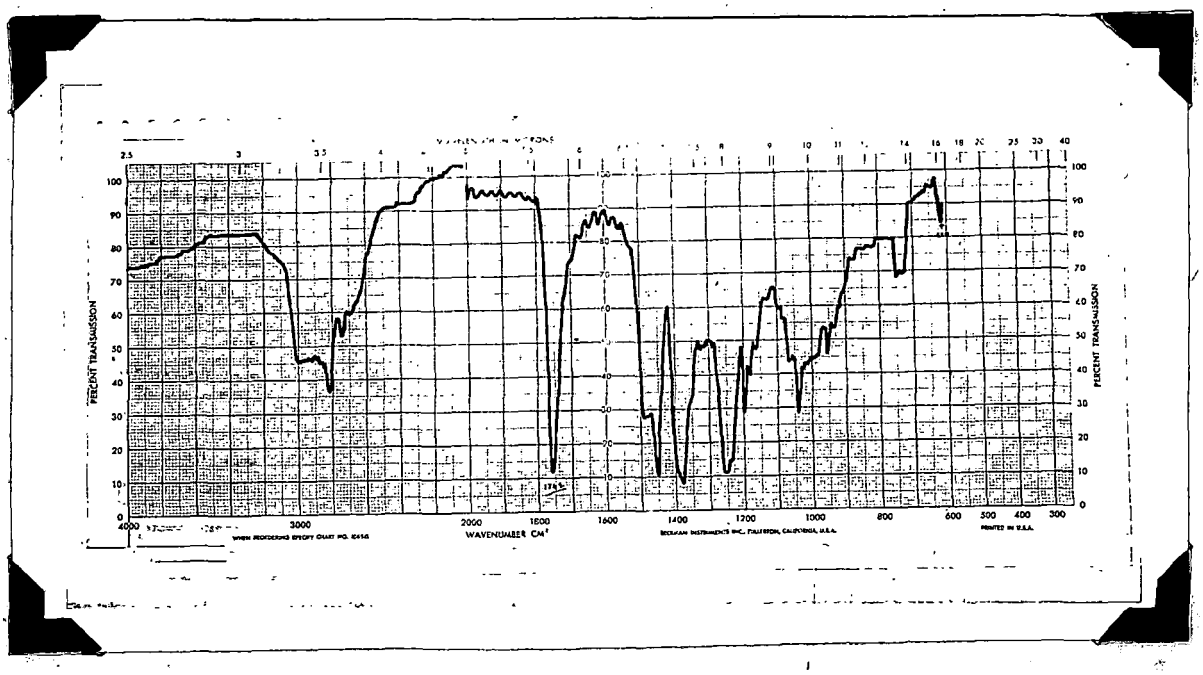


Fig. 2. IR spectrum of 2α, 3β-diacetate 120

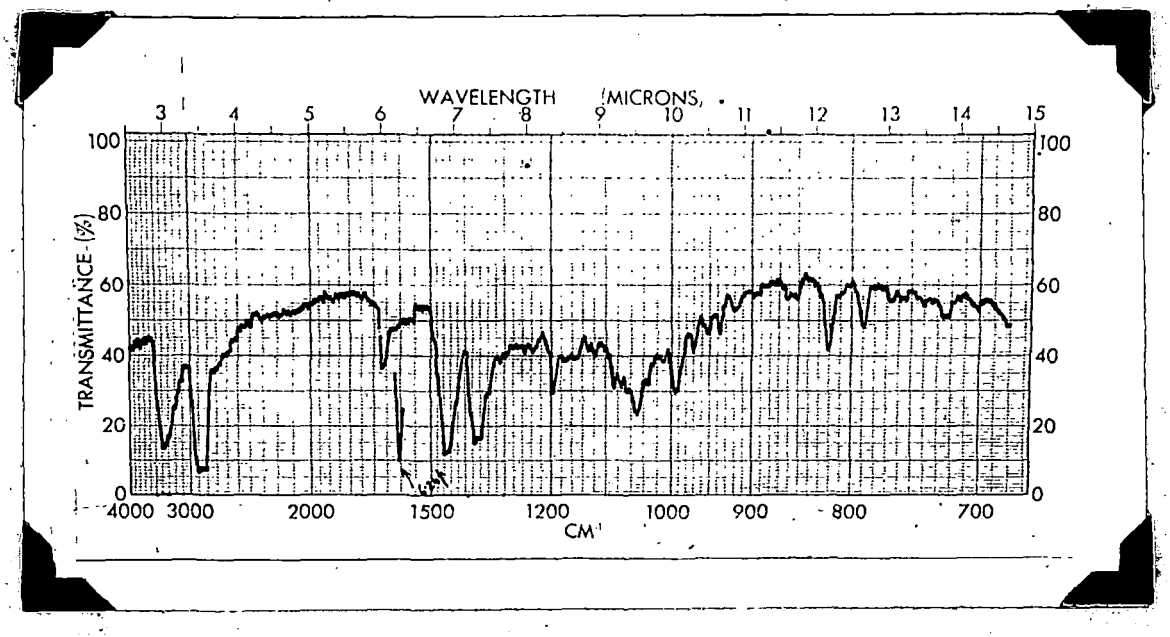


Fig. 3. IR spectrum of 2α, 3β-diol 121

(moretane) 121, m.p. 240-42° $(\alpha)_D$ 82.86°.

Found: C, 80.72; H, 11.35%

Calculated for $C_{30}H_{52}O_2$: C, 81.08; H, 11.70%

UV : No absorption in the region 220-300 mμ

IR : ν_{max}^{nujol} 3350, 2960, 1460, 1360, 1030 cm^{-1} Fig. 3

NMR (100 Mc/S): Peaks at 2.90, 3.2 (1H, doublet, H $-C_3-OH$) 3.76 (quartet of a doublet H $-C_2-OH$) ppm.

2. Preparation of 2α, 3α-dihydroxy isohopane (moretane) 123: Hydrogenation of diosphenol 113: Preparation of 2 keto-moretanol 122:

Diosphenol 113 (200 mg) dissolved in absolute ethanol (80 ml) was stirred in presence of 10% palladium-on-charcoal catalyst (25 mg) in an atmosphere of hydrogen till the absorption of hydrogen ceased. After working up in the usual manner and crystallisation from chloroform-methanol mixture it afforded a crystalline solid, m.p 181-3°, $(\alpha)_D$ 29.41°.

Found: C, 81.02; H, 11.62%

Calculated for $C_{30}H_{50}O_2$: C, 81.44; H, 11.31%

UV : λ_{max} 278 mμ (ε, 78)

NMR (100 Mcs): 3.88 (broad, -CO-CHOH -C), 3.44 (broad), 2.42 and 2.55 ppm ($CO-CH_2$).

Acetylation of 2 keto-moretanol 122: Isolation of 2 keto-moretanyl acetate 122A:

2 keto-moretanol (200 mg) was treated with acetic anhydride (5 ml) and pyridine (5 ml) and the mixture was kept at room temperature overnight. After working up in the usual manner and crystallisation from chloroform-methanol mixture it afforded a crystalline solid 122A, m.p. 264-67°, $(\alpha)_D$ 82.61°.

Found:	C, 79.62; H, 10.36%
Calculated for $C_{32}H_{52}O_3$:	C, 79.33; H, 10.74%

NMR (100 Mcs): 4.95 ($-C_3H-OAc$), 2.49, 2.37 (C_6-CH_2) ppm.

Meerwein-Pondorff reduction of 2 keto-moretanol 122: Isolation of 2α, 3α-dihydroxy isohopane (moretane) 123 :

A mixture of 2 keto-moretanol 122, Al-isopropoxide (650 mg) in dry isopropanol (12.5 ml) was distilled slowly with the addition of isopropanol to maintain constant volume. After 5 hours the distillate no longer contained acetone and the solution was concentrated to a small volume. The reaction mixture was diluted with water followed by 10% sulphuric acid solution (20 ml) and then extracted with ether. The product obtained after removal of the ether was dissolved in benzene (6 ml) and poured on a column of alumina, (25 gm deactivated with 1 ml of 10% aqueous acetic acid) developed with petroleum ether. The following solvents were used for elution (Table II).

Table- II

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1-3	Nil
Petroleum ether: benzene (4:1)	4-6	Nil
Petroleum ether: benzene (3:2)	7-9	Nil
Petroleum ether: benzene (1:4)	10-13	Solid m.p. 245-8° (400 mg)
Benzene	14-15	Nil
Benzene: ether (4:1)	16-20	Solid (40 mg) m.p. 239-40°

Elution with more polar solvents did not give any solid material.

Solids from fraction 10-13 (Table II) were combined which after crystallisation from methanol afforded the crystalline 2α, 3α-dihydroxy isohopane (moretane) 123 (350 mg), m.p. 250-51°, $(\alpha)_D$ 9.37°.

Found: C, 81.80; H, 11.6%

Calculated for $C_{30}H_{52}O_2$: C, 81.08; H, 11.70%

UV : No absorption in the region 220-300 mμ

IR : ν_{max} 3420, 2960, 1450, 1370, 1350, 1040 cm^{-1}

NMR (100 MC/S): Peaks at 3.20 (~~doublet~~ multiplet, C-3H), 4.0, 4.1 (~~doublet~~ multiplet 1H, H - C_2- OH) ppm.

The solids from fractions 16-20 (Table II) after crystallisation from methanol afforded a crystalline solid (28 mg), m.p. 242-3°, identical with the 2α, 3β-dihydroxy isohopane (moretane) described earlier.

Acetylation of 2α, 3α-dihydroxy isohopane (moretane)123: Preparation of 2α, 3α-diol diacetate 124:

200 mg of the 2α, 3α-diol 123 was treated with pyridine (5 ml) and acetic anhydride (5 ml) and the mixture was heated on a water bath for four hours. After working up in the usual manner, it gave a solid which after crystallisation from chloroform-methanol mixture afforded the 2α, 3α-diacetate 124, m.p. 185-7°.

Found: C, 76.80; H, 10.40%

Calculated for $C_{34}H_{56}O_4$: C, 77.27; H, 10.60%

UV: No absorption in the region 200-300 mμ

IR: ν_{max} 1725, 1430, 1360, 1340, 1240 cm^{-1}

NMR (100 MC/S): Peaks at 4.63 (doublet C-3H (e), centred at 5.32 ppm (~~quartet~~ multiplet, C-2H (a))

Preparation of acetonide derivative of 2α, 3α-diol 123:

To the diol 123 (100 mg) dissolved in dry acetone was added catalytic amount of p-toluene sulfonic acid. The mixture was shaken for 10 minutes and then kept overnight. After usual work up a solid

(86 mg) was obtained which after crystallisation from methanol afforded pure crystals of the acetonide derivative 125, m.p. 186-88°, $(\alpha)_D$ 19.04°.

Found:	C, 81.48; H, 11.45%
Calculated for $C_{33}H_{56}O_2$:	C, 81.81; H, 11.57%

Preparation of epi-moretanol 126:

Epi-moretenol (2 gm) (isolated from the bark of Sapium sebiferum Roxb. by the method of Khastgir et al[61]), dissolved in ethyl acetate (350 ml) was stirred in presence of Adam's catalyst (200 mg) in an atmosphere of hydrogen till the absorption ceased. After working up in the usual manner, it gave a solid which on crystallisation from chloroform-methanol gave crystals 126, m.p. 184-6°, $(\alpha)_D$ -2.30° identical with an authentic sample (m.m.p. and IR comparison).

Preparation of Δ^2-moretane 127: $POCl_3$ - Pyridine dehydration of epi-moretanol 126:

To an ice cold solution of epi-moretanol 126 (600 mg) in pyridine (10 ml), phosphorous oxychloride (8 ml) was added and the mixture was kept overnight. It was then poured on crushed ice cautiously and extracted with ether. The ether extract was washed with water and dried (Na_2SO_4). On removal of the solvent a solid residue

(540 mg) was obtained. The latter was dissolved in benzene (5 ml) and poured on a column of active alumina (30 gm) developed with petroleum ether. The following solvents were used for elution.

Table- III

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1-3	Solid (510 mg) m.p. 158-60°

Further elution with more polar solvents did not afford any solid material.

The solid from fractions 1-3 (Table III) were combined and on crystallisation from chloroform-methanol mixture afforded Δ^2- moretane, 127 m.p. 162-4°.

Osmium tetroxide Oxidation of Δ^2 -moretane 127:

A solution of Δ^2 -moretane 127 (1.0 gm) and osmium tetroxide (650 mg) in pyridine (10 ml) and dry ether (5 ml) was stirred for 12 hours at room temperature and then kept in the dark for eight days. After this period the solvents were ~~reduced~~ removed under reduced pressure and a black residue was obtained. It was dissolved in a mixture of benzene (25 ml) and 95% ethanol (25 ml) and refluxed for six hours after the addition of a solution of mannitol (5.6 gm) and potassium

hydroxide (5.6 gm) in ethanol (25 ml) and water (12.5 ml). The reaction mixture was diluted with water and extracted with ether. The organic layer was washed with water, dried (Na_2SO_4) and evaporated to yield a crude solid (960 mg), which was chromatographed over a column of alumina (50 gm, deactivated with 2 ml of 10% aq. acetic acid) developed with petroleum ether. The residue dissolved in benzene (8 ml) was poured on the column and eluted with the following solvents. (Table IV)

Table-IV

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1-3	Oil
Petroleum ether: benzene (3:1)	4-6	Oil
Petroleum ether: benzene (1:1)	7-9	Nil
Petroleum ether: benzene (1:3)	10-11	Nil
Benzene	13-14	Oil
Benzene: ether (4:1)	15-22	Solid (500 mg) m.p. 235-40°

Further elution with more polar solvents did not give any solid material.

The solid (500 mg) from fraction 15-22 (Table IV) were combined and crystallised from chloroform-methanol to furnish a solid m.p. 236-40°. This solid showed two spots on a chromatoplate and could not be separated even after repeated column chromatography. However crystallisation from methanol at first deposited a homogeneous solid (TLC) (460 mg), m.p. 250-51°, $(\alpha)_D$ 9.83°. The IR spectrum of this solid was found to be identical with 123 prepared by Meerwein-Pondorff reduction of 122.

From the mother liquor, a second diol m.p. 261-3°, $(\alpha)_D$ 23.68° was isolated and was found to be identical with 2β, 3β-dihydroxy-isohopane (moretane) 128 (m.m.p. and IR comparison), described below.

Preparation of 2β, 3β-dihydroxy isohopane (moretane) 128: Sodium borohydride reduction of diosphenol 113:

To a solution of diosphenol 113 (200 mg) in 100 ml of methanol, sodium borohydride (100 mg) was added and the mixture was stirred with a magnetic stirrer for one hour. The reaction mixture was concentrated, diluted with water and then acidified with dilute hydrochloric acid (6 ml). It was then taken up in ether and washed with water until neutral and dried (Na_2SO_4). The solvent was removed. The solid residue (200 mg) was dissolved in benzene and was poured on a column of alumina (10 gm, deactivated with 0.4 ml of 10% aqueous acetic acid) developed with petroleum ether. The chromatogram was eluted with following solvents. (Table V)

Table-V

Eluent	Fractions 50 ml each	Residue on evaporation.
Petroleum ether	1-2	Nil
Petroleum ether: benzene (3:1)	3-4	Nil
Petroleum ether: benzene (1:1)	5-6	Nil
Petroleum ether: benzene (1:3)	7-8	Nil
Benzene	9-14	Solids (180 mg) m.p. 255-8°

Further elution with more polar solvents did not afford any solid material.

The solid from fraction 9-14 (Table V) were collected and after crystallisation from chloroform-methanol mixture gave pure crystalline 2β, 3β-dihydroxy isohopane (moretane), 128, m.p. 262-4°, $(\alpha)_D$ 23.68°.

Found: C, 81.02; H, 11.20%

Calculated for $C_{30}H_{52}O_2$: C, 81.08, H, 11.70%

UV : No absorption in the region 200-300 mμ

IR : ν_{max} 3410, 2945, 1455, 1380, 1350, 1040 cm^{-1}

Preparation of 2β, 3β-diacetate 129: Acetylation of 2β, 3β-dihydroxy isohopane (moretane) 128:

200 mg of the 2β, 3β-diol 128 was acetylated by heating with pyridine (3 ml) and acetic anhydride (5 ml) on a water bath for 4 hours. After working up in the usual manner it gave a solid which after several crystallisation from chloroform-methanol afforded pure diacetate 129, m.p. 214-5°, $(\alpha)_D$ 31.25°.

Found: C, 77.01; H, 10.31%

Calculated for $C_{34}H_{56}O_4$: C, 77.27; H, 10.60%

UV: No absorption in the region 220-300 mμ.

IR: ν_{max} 2960, 1725, 1485, 1455, 1380, 1370, 1255 cm^{-1}.

Preparation of acetonide derivative 130 of 2β, 3β diol 128:

100 mg of 2β, 3β-diol 128 was dissolved in dry acetone (10 ml) and to this a few crystals of p-toluene sulfonic acid was added. The reaction mixture was shaken for a few minutes and kept overnight. To this reaction mixture 5% sodium bicarbonate solution (2 ml) was added and a part of the solvent was removed by distillation and then diluted with water. The cloudy precipitate which appeared was extracted with ether. The ethereal layer after being washed with water till neutral was dried (Na_2SO_4). The ether was then removed and the solid residue after several crystallisations from chloroform-methanol mixture afforded the pure acetonide derivative 130, m.p. 239-41°, $(\alpha)_D$ 29.85°.

Found:	C, 81.50; H, 11.04%
Calculated for $C_{33}H_{56}O_2$:	C, 81.81; H, 11.57%

Preparation of 2β, 3α-dihydroxy isohopane (moretane) 132 and its diacetate 133:

To a solution of Δ^2-moretane 127 (200 mg) dissolved in hexane (10 ml) in an erlenmeyer flask, was added formic acid (98-100%) 50 ml, water (4 ml) and hydrogen peroxide (30%, 0.5 ml) and the mixture was stirred for eight hours at 55-60°. The reaction mixture was then kept at room temperature for sixteen hours. The solvents were removed under reduced pressure and the residue was extracted with ethyl acetate. The ethyl acetate layer was washed with water, dried (Na_2SO_4) and the solvent removed. To the residue (170 mg) was added a 20% sodium hydroxide solution (20 ml) and the mixture was heated on a water bath for half an hour. The reaction mixture was then cooled, acidified with dilute hydrochloric acid and extracted with ether. The ethereal layer after being washed with water was dried (Na_2SO_4) and the solvent removed. The residue (120 mg) was dissolved in benzene and poured on a column of alumina (15 gm, deactivated with 0.6 ml of 10% aqueous acetic acid) developed with petroleum ether. The chromatogram was eluted with the following solvents. (Table VI)

Table - VI

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1-3	Oil
Petroleum ether: benzene (3:1)	4-6	Nil
Petroleum ether: benzene (1:1)	7-10	Nil
Petroleum ether: benzene (1:3)	11-13	Nil
Benzene	14-18	Solid (50 mg) m.p. 219-21°

Further elution with more polar solvents did not give any solid material.

The solids (50 mg) from fractions 14-18 (Table VI) were combined and after crystallisation from methanol afforded crystals of 2β, 3α-dihydroxy isohopane (moretane) 132, m.p. 221-4°, $(\alpha)_D$ 31.18°.

Found: C, 80.71%; H, 11.73%

Calculated for $C_{30}H_{52}O_2$: C, 81.08; H, 11.70%

UV: No absorption in the region 220-300 mμ.

IR: ν_{max} 3440, 2965, 1475, 1375, 1044, 1007 cm^{-1}

The above diol 132, m.p. 221-4° on being treated with acetic anhydride

pyridine in the usual manner gave crystalline 2β, 3α-diol diacetate 133, m.p. 145-7°, $(\alpha)_D$ 31.40°.

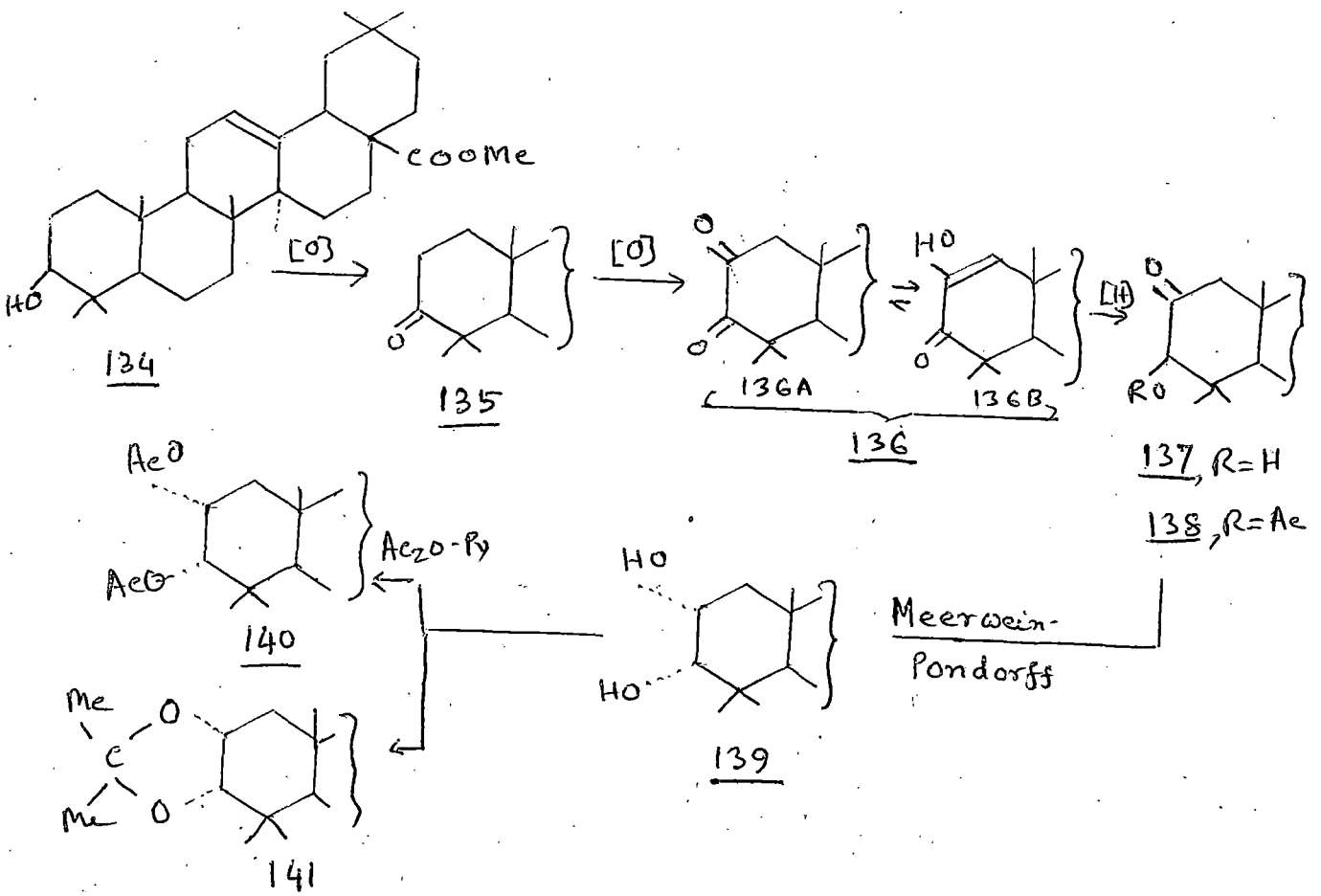
Found:	C, 77.00; H, 10.21%
Calculated for $C_{34}H_{56}O_4$:	C, 77.27; H, 10.60%

UV: No absorption in the region 200-300 mμ.

Section C: Synthesis of isomeric 2, 3-diols of methyl Olean-12-en-28-Oate.

1. Synthesis of methyl 2α, 3α-dihydroxy-Olean-12-en-28-Oate 139:

Methyl oleanonate 135 m.p. 182-4°, $(\alpha)_D$ 89°, prepared by Jones oxidation of methyl oleanolate[58] 134, was oxidised by passing oxygen through a suspension of 135 in dry t-butanol containing potassium tertiary butoxide[11,12,13,22]. Absorption of one mole of oxygen led to a diketone derivative 136, m.p. 130-35°, $(\alpha)_D$ 104 ± 4°. The compound showed two spots on a chromatoplate indicating the presence of two compounds.



This compound 136 showed UV absorption at 269.5 mμ (ϵ,5700), ν_{max}^{KBr} 3420, 2960, 1730, 1670, 1650 cm^{-1} (Fig. 5) and it gave a positive ferric chloride coloration. These data were in complete agreement with the assignments shown in 136A and 136B.

Hydrogenation of diosphenol 136 in presence of 10% palladium-on-charcoal catalyst gave the corresponding reduced product 137, m.p. 129-31°, $(\alpha)_D$ 109°, λ_{max} 270 mμ (ϵ = 43), ν_{max}^{nujol} 3450 (-OH) 1710 (C = O), 1730 (-COOMe) cm^{-1} (Fig. 6) It showed negative ferric chloride coloration. During this hydrogenation 1,4-addition of hydrogen took place giving the ketol 137 (TLC homogenous). Acetylation of 137 with acetic anhydride and pyridine gave the corresponding acetate 138, m.p. 182-4°, $(\alpha)_D$ 85°, λ_{max} 275 mμ (ϵ,80), ν_{max}^{KBr} 1725, 1740, 1235 cm^{-1}, Fig. 7. Meerwein-Pondorff reduction of 137 furnished a crystalline solid 139 which exhibited a single spot on chromatoplate. Chromatography of the solid gave 139 m.p. 286-7°, $(\alpha)_D$ 71°, ν_{max} 3340 (-OH), 1725 (-COOMe) cm^{-1}. This compound 139 was found to be identical with methyl 2α, 3α-dihydroxy-olean-12-en-28-oate kindly supplied by H.T.Cheung (by m.m.p. and Co-TLC). The NMR spectrum of the diol 139 revealed that the C-3 proton give rise to a doublet (J, 3 Hz) and δ 3.4 due to vicinal coupling of equatorial and axial protons. The signal for C-2H appeared as a double triplet at δ 3.9 with splittings of 12 , 3 and 3Hz indicating it as an axial proton with one axial-axial and two axial-equatorial splittings. Thus 139 is the

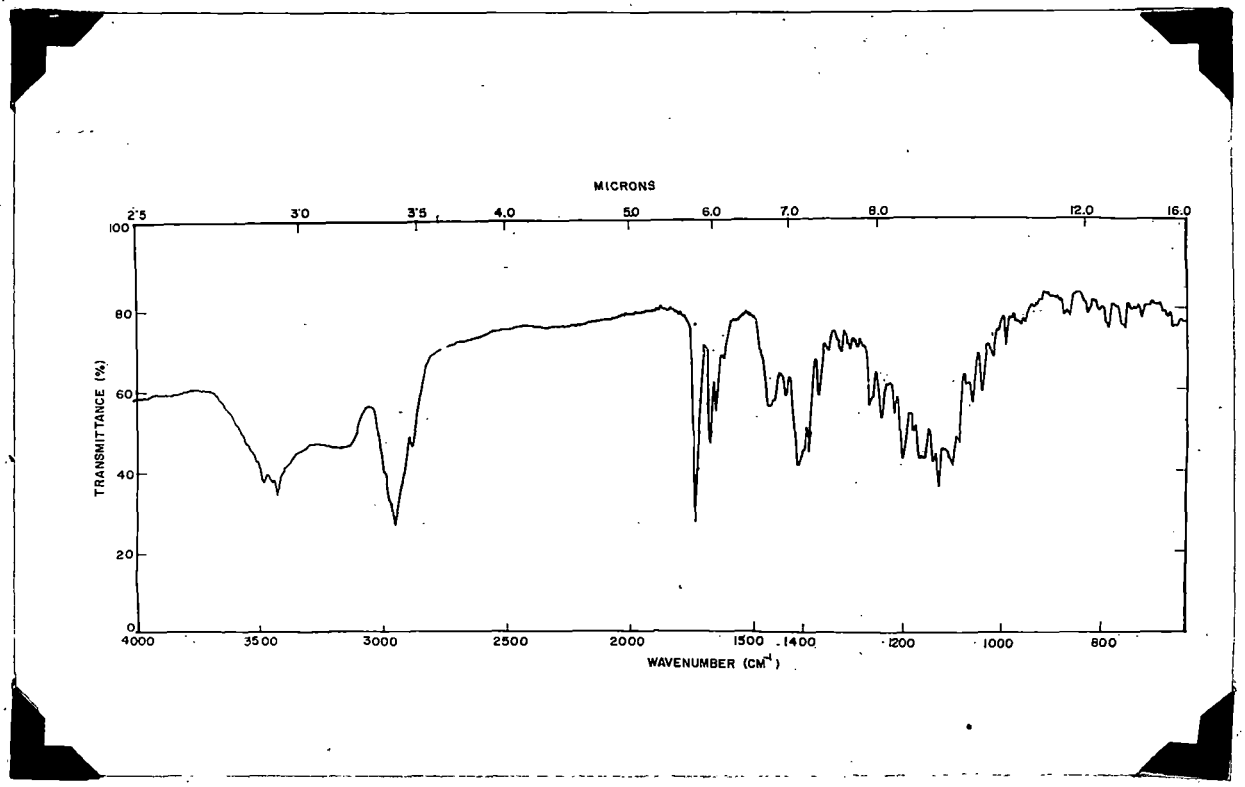


Fig. 5. IR spectrum of diosphenol 136

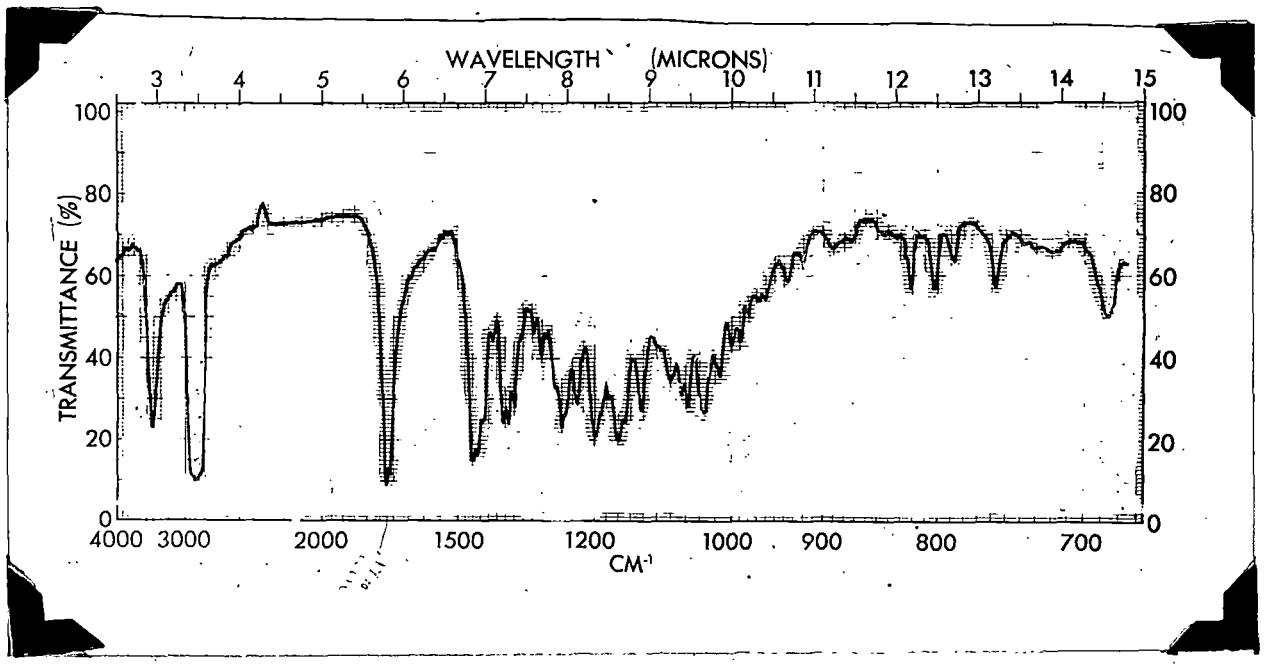


Fig. 6. IR spectrum methyl-3 β -hydroxy-2-keto-olean-12-en-28-oate 137

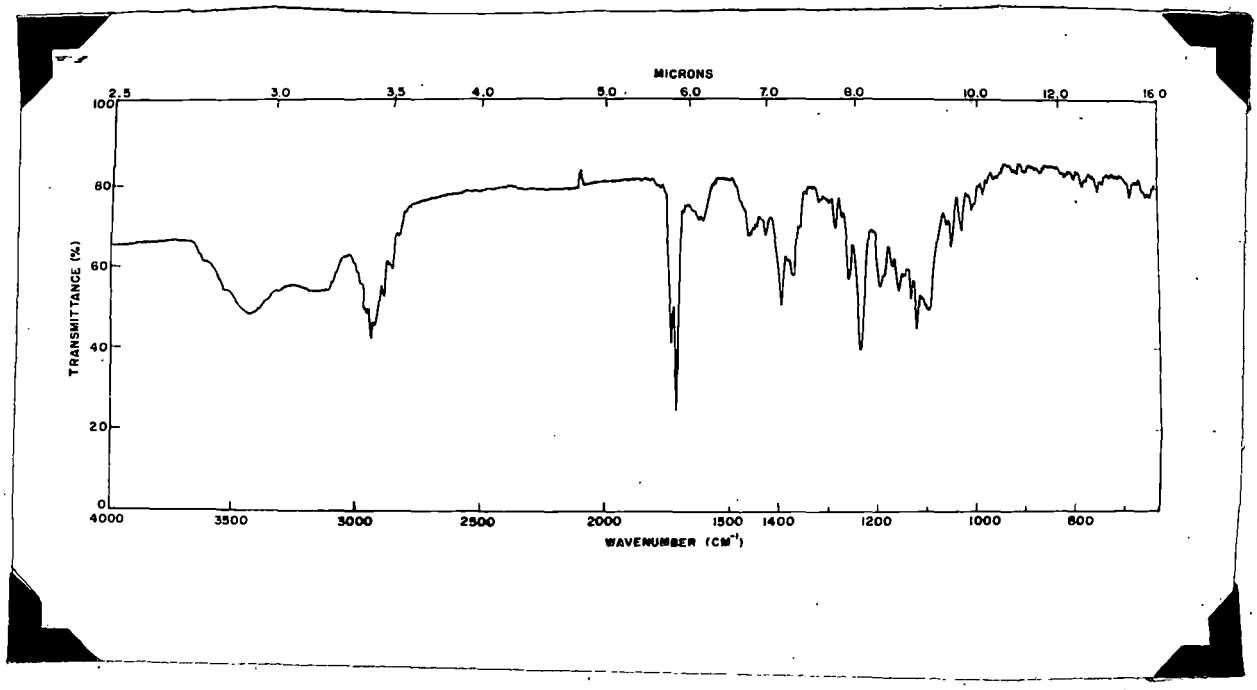


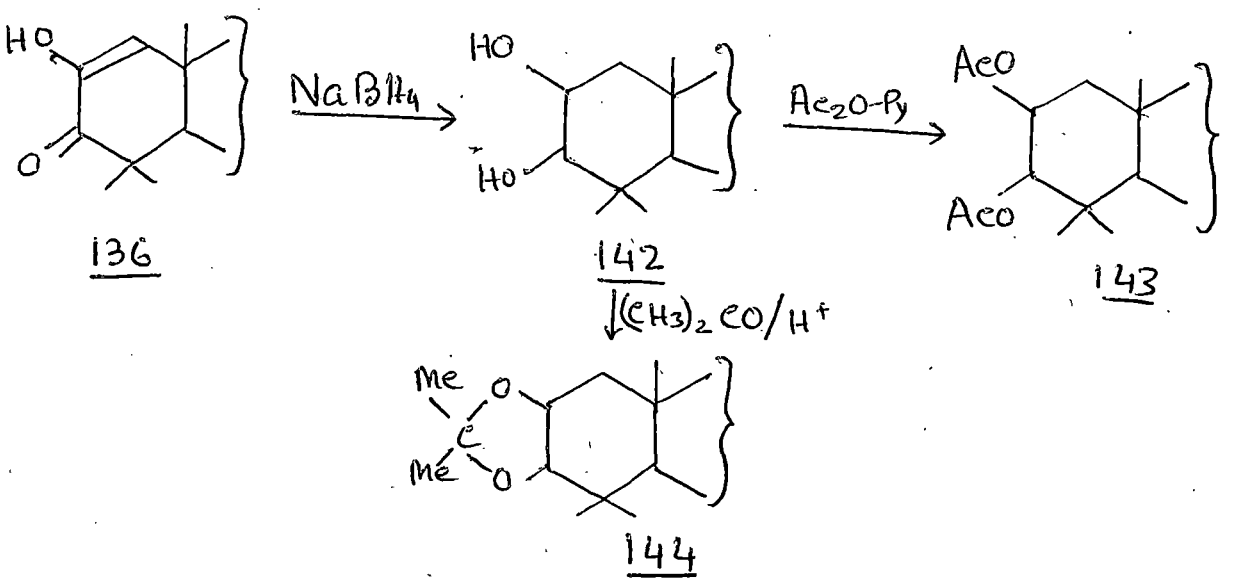
Fig. 7. IR spectrum of methyl 3 β-acetoxy-2 keto-olean-12-en-28-oate 138

2α, 3α-diol. Acetylation of 139 with acetic anhydride-pyridine gave the diacetate 140 m.p. 226-8° $(\alpha)_D$ 95.20°. This diacetate showed lower field NMR signals which were in good agreement with the expected structure 140.

The diol 139 on treatment with acetone in presence of catalytic amount of p-toluene sulfonic acid gave an acetonide derivative 141, m.p. 235-9°.

2. Synthesis of methyl 2β, 3β-dihydroxy-olean-12-en-28-oate 142:

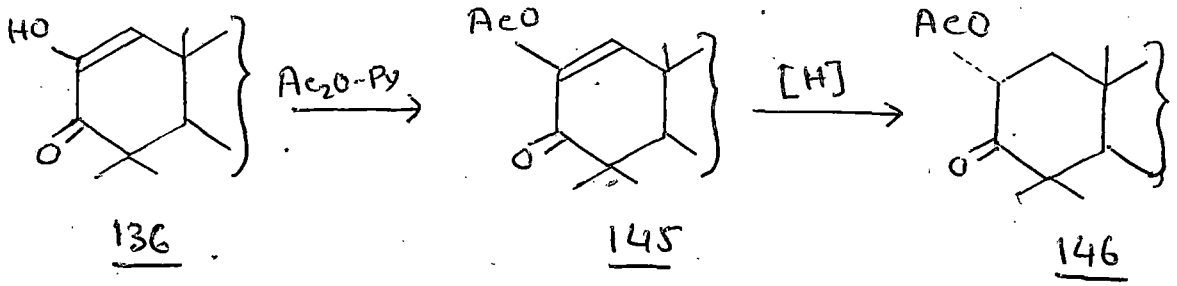
Diosphenol 136 on sodium borohydride reduction in methanol solution gave a compound 142, m.p. 269-72°, $(\alpha)_D$ 88.88° (lit.[43,37,29b] m.p. 258-60°, $(\alpha)_D$ 97°; m.p. 258-62°, $(\alpha)_D$ 85°, m.p. 258-61°), no UV abosorption in the region 220-300 mμ, ν_{max} 3525, 3360, 1720 cm^{-1}.



Treatment of 142 with pyridine and acetic anhydride gave the diacetate 143, m.p. 220-22°, $(\alpha)_D$ 86.20°, ν_{max}^{KBr} 1745, 1720 (C = O), 1258 (C - O) cm^{-1}. Examination of the NMR spectrum of 142 showed (Fig. 8) two unresolved multiplets, one at 3.15 ppm assigned to C-3H and the other at about 4.4 ppm (C-2H) in addition to the olefinic proton at 5.15 ppm. Thus the hydroxyl group at C-3 is axial (H_e) and the one at C-2 is equatorial (H_a). In the NMR spectrum of its diacetate Fig. 9, these signals were shifted to a downfield to 4.6 (J = 4Hz)ppm and about 5.4 (broad multiplet). The signals for the ester group showed a singlet at 3.65 and the acetate 2.06 (singlet 6H) ppm. The diol 142 on treatment with acetone in presence of catalytic amount of p-toluene sulfonic acid gave an acetonide derivative 144, sintering at 75-80°.

3. Synthesis of methyl -2α, 3β -dihydroxy-olean-12-en-28 oate 148:

Diosphenol 136 on a acetylation with a acetic anhydride-pyridine gave the corresponding acetate 145 m.p. 168-70°, $(\alpha)_D$ 93°, having λ_{max} 237 mμ (ε, 8500), ν_{max}^{nujol} 1205, 1685, 1720, 1738 cm^{-1}, (Fig. 10). Hydrogenation of 145 with 10% palladium-on-charcoal catalyst gave a solid 146 m.p. 208-9°, $(\alpha)_D$ 52°, ν_{max} 1225, 1730, 1750 cm^{-1}. The



136 145 146

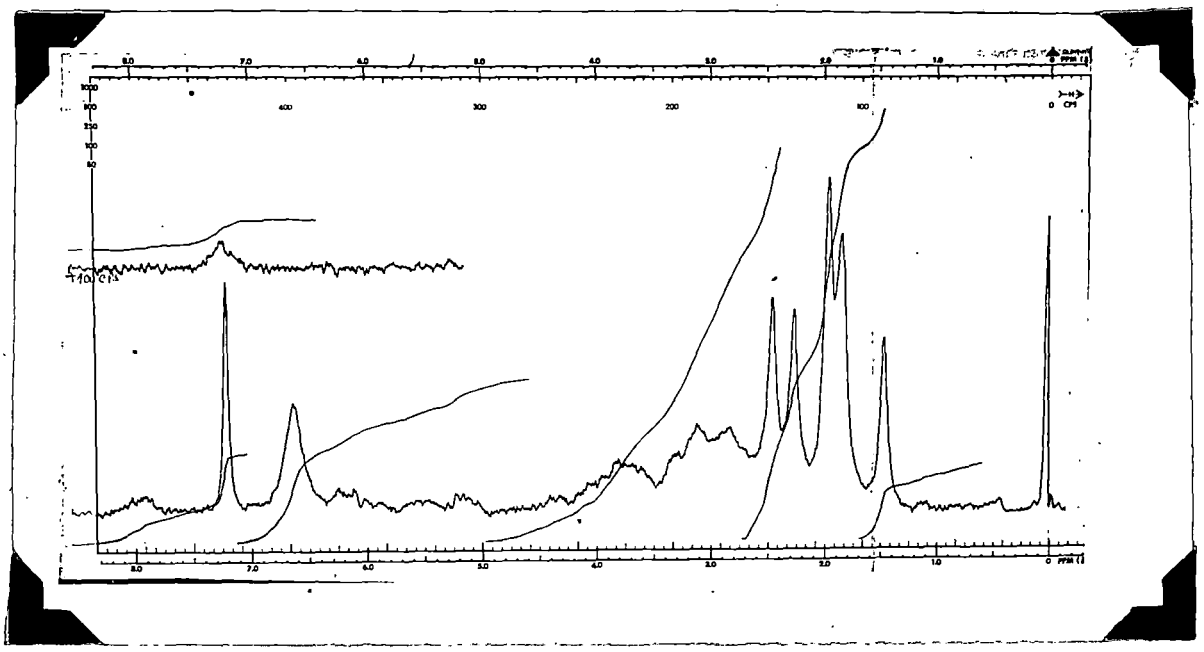


Fig. 8. NMR spectrum of 2β, 3β -diacetate 142

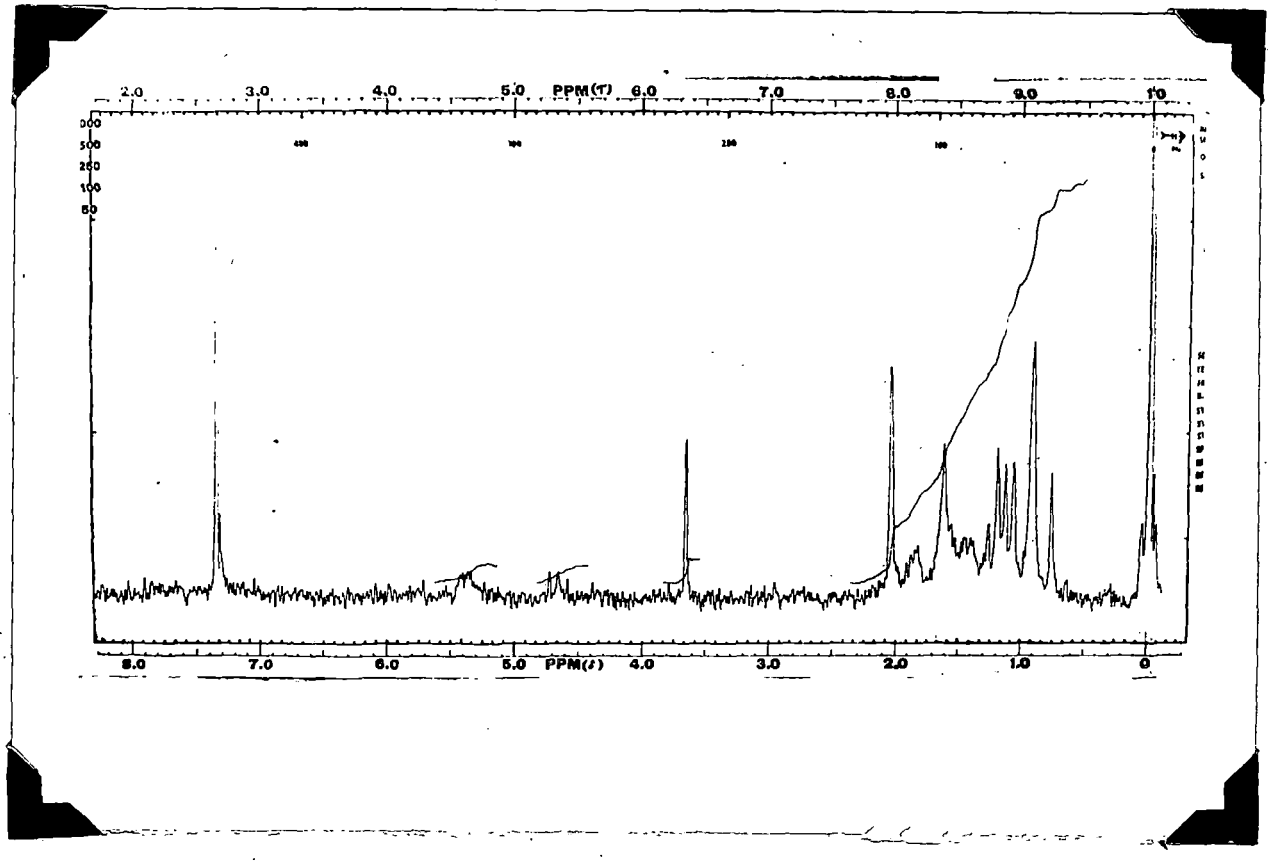
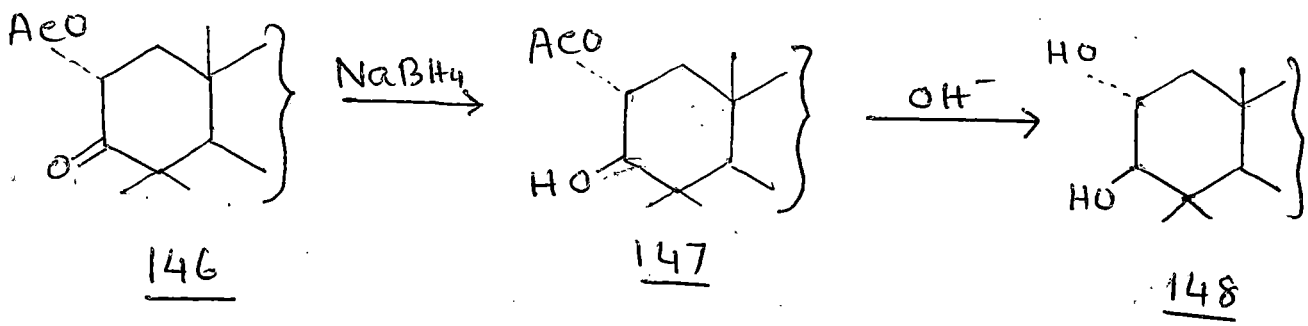


Fig. 9. NMR spectrum of 2β, 3β-diol 143

ketoacetate 146 on reduction with sodium-borohydride at pH 8 to reduce isomerisation in methanol solution gave a solid 147 m.p. 199-204°, $(\alpha)_D$ 27.9°. The latter 147 was directly hydrolysed by 10% sodium hydroxide solution to afford a solid 148 m.p. 220-22°, $(\alpha)_D$ 36°. This solid 148 was found to be identical with an authentic sample of methyl 2α, 3β-dihydroxy-olean-12-en-28-oate (methyl crategolate) by m.m.p. and Co - TLC.



146 147 148

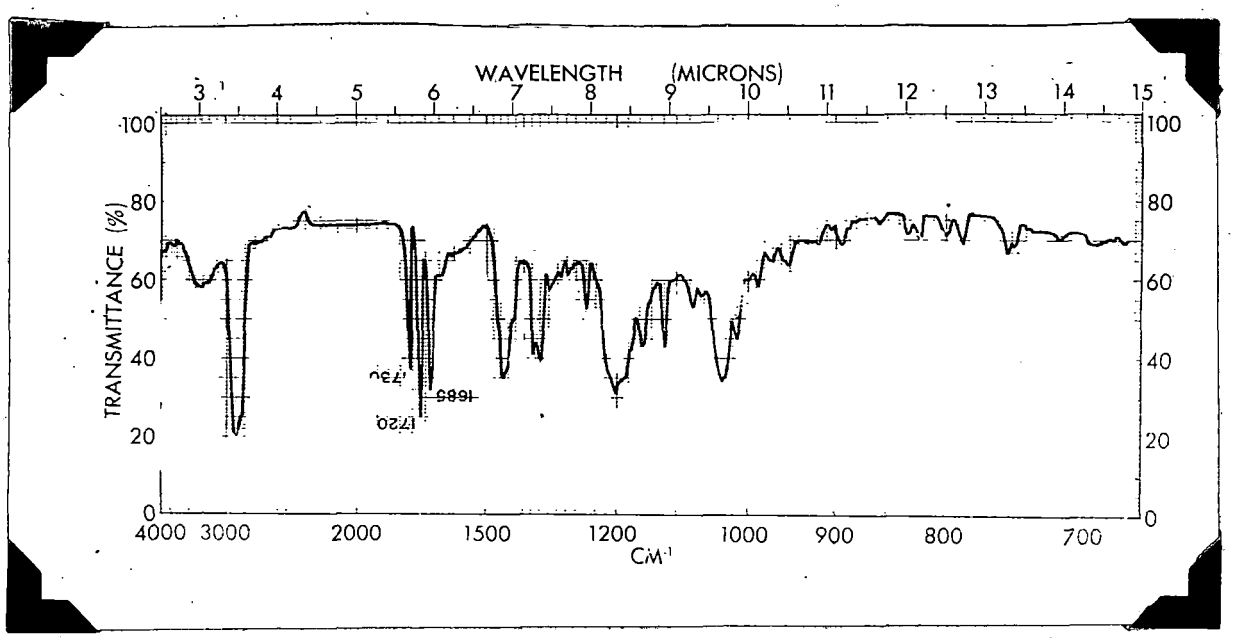


Fig. 10. IR spectrum of diosphenol acetate 145

EXPERIMENTAL

Melting points are uncorrected. The petroleum ether used throughout the investigation had b.p. 60-80°. All optical rotations were determined in chloroform solution unless stated otherwise. NMR spectra were determined on Varian A-60 and HA-100 spectrometers using chloroform-d solution containing tetramethyl-silane as reference. The IR spectra were recorded in Perkin-Elmer 337 and 221 and Beckmann I.R - 20 spectrophotometers. UV absorption spectra were taken in Ziess VSU-1 and UV Beckmann DU-2 spectrophotometers in 95% ethanol solution unless otherwise stated.

Isolation of methyl Oleanolate[58] 134 from Achyranthes aspera Linn.

800 gm of powdered seed of Achyranthes aspera Linn was extracted for 30 hours in a soxhlet apparatus with 95% ethanol. A thick syrup was obtained on concentration and this was shaken with a 200 ml portion of petroleum ether. The petroleum ether layer was decanted and the residual syrup was again shaken with petroleum ether (200 ml). Petroleum ether was decanted and the residue was hydrolysed with a mixture of 95% alcohol (130 ml), conc. hydrochloric acid (95 ml) and water (65 ml) for four hours. The mixture was diluted with water after cooling and then filtered. The solid residue was washed with water till neutral and air dried. The solid was extracted with ether in a soxhlet apparatus for four hours. The resulting ethereal solution was shaken with 50 ml portion of 3% aq. potassium hydroxide. The precipitated potassium salt was collected on a Buchner funnel. The solid

potassium salt was suspended in cold water, acidified with hydrochloric acid (50%) and extracted with ether. The ether extract was washed with water and dried over anhydrous Na_2SO_4. To the dry ether solution was added a solution of diazomethane in ether prepared from nitroso methyl urea (700 mg) and was kept overnight. Next day excess of diazomethane was destroyed with acetic acid. The ether solution was washed with water till neutral and then dried (Na_2SO_4). Evaporation of the ether yielded a solid (2 gm). This crude ester dissolved in 15 ml benzene was placed over a column of alumina (120 gm deactivated with 4.8 ml of 10% aqueous acetic acid). The chromatogram was developed with petroleum ether and eluted with following solvents (Table VII).

Table - VII

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether (500 ml)	1-10	Solid on digestion with methanol, m.p. 193-4°

Elution with more polar solvents did not afford any solid material.

The combined solid from fraction 1 to 10 were collected and after crystallisation from chloroform-methanol mixture afforded pure crystals of 134, m.p. 196°, $(\alpha)_D$ 73° which was found to be identical with an authentic sample of methyl oleanolate (m.m.p. and rotation).

Oxidation of methyl oleanolate 134: Preparation of methyl Oleanonate 135:

To a solution of methyl oleanolate 134 (200 mg) in pure acetone (30 ml) was added Jones reagent dropwise (5-6 drops) with shaking until a faint orange colour persisted. The mixture was kept at room temperature for 1 hour, diluted with water and extracted with ether. The ether layer was washed thoroughly with water, dried (Na_2SO_4) and the ether evaporated. The residue (150 mg) dissolved in benzene (3 ml) was chromatographed over a column of active alumina (5 gm). The chromatogram was developed with petroleum ether and then eluted with the following solvents. (Table VIII)

Table - VIII

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1-2	Nil
Petroleum ether: benzene (4:1)	3-6	Solid, m.p. 179-80° (120 mg)

Further elution with more polar solvent did not yield any solid.

Fractions 3-6 (120 mg) were combined and on crystallisation from chloroform-methanol furnished needle shaped crystals of 135, m.p. 182-4°, $(\alpha)_D$ 89°, identical with authentic sample of methyl oleanonate (m.m.p. and I.R. comparison).

Found: C, 79.01; H, 10.02%

Calculated for $C_{31}H_{48}O_3$: C, 79.44; H, 10.32%

Autoxidation of methyl oleanonate 135 : Isolation of methyl 2,3 dioxo-olean-12-en-28-oate (diosphenol)136:

To a suspension of (2 gm) methyl oleanonate 135 in potassium tertiary butoxide in tertiary butanol (prepared from 6 gm of potassium and 160 ml of tertiary butanol), oxygen was passed for three hours with stirring. The reaction mixture was diluted with water and 6N hydrochloric acid was added till the solution was acidic. It was then extracted with chloroform (200 ml) and the combined extract was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. A yellowish gummy foam was obtained (1 gm), which after crystallisation from methanol gave an amorphous solid, 136, m.p. 130-5°, $(\alpha)_D$ 104°. It gave a positive ferric chloride colouration for diosphenol. Two spots on chromatoplate, an upper spot at R_f = 0.79 of slightly weaker intensity than the lower spot R_f = 0.76. These were assumed to be due to tautomeric mixture of the diketone 136A and 136 B.

Found: C, 76.62; H, 9.56%

Calculated for $C_{31}H_{48}O_4$: C, 76.82; H, 9.95%

UV : λ_{max} 269.5 mμ (ϵ ,5700)

IR : ν_{max}^{KBr} 3420, 2960, 1730, 1670, 1650 cm^{-1} Fig. 5

Hydrogenation of methyl 2,3-dioxo-olean-12-en-28-oate 136: Preparation of methyl-3β hydroxy-2-keto-olean-12-en-28-oate 137 :

Diosphenol 136 (500 mg) dissolved in absolute ethyl alcohol (50 ml) was stirred in presence of 10% palladium-on-charcoal catalyst (50 mg) in an atmosphere of hydrogen till the absorption ceased. The catalyst was removed by filtration and the solvent was evaporated to dryness under reduced pressure. A solid residue (460 mg) was obtained which after crystallisation from methanol furnished a solid 137, m.p. 129-31°, $(\alpha)_D$ 109.09°. This solid did not give ferric chloride colouration and showed one single spot on chromatoplate (R_f = 0.42 in benzene).

Found: C, 76.54; H, 10.28%

Calculated for $C_{31}H_{50}O_4$: C, 76.50; H, 10.31%

UV : λ_{max} 270 mμ (ε ,43)

IR : ν_{max}^{nujol} 3450 (-OH), 1710 (-C = O), 1730 (-COOMe) cm^{-1}.

Fig. 6.

Acetylation of methyl 3β-hydroxy-2-keto-olean-12-en-28-oate 137: Preparation of methyl 3β-acetoxy-2-keto-olean-12-en-28-oate 138:

The hydroxy ketone 137 (200 mg) was treated with acetic anhydride (5 ml) and pyridine (5 ml) and heated on a water bath for 5 hours. After usual work up it gave a crystalline solid 138, m.p. 182-4°, $(\alpha)_D$ 84.85°.

Found: C, 74.36; H, 9.52%

Calculated for $C_{33}H_{52}O_5$: C, 74.76; H, 9.91%

UV : λ_{max} 275 mμ (ϵ ,80)

IR : ν_{max}^{KBr} 1725, 1740, 1235 cm^{-1} Fig. 7

Preparation of methyl 2α, 3α-dihydroxy-oleanø-12-en-28-oate 139: Meerwein-Pondorff reduction of 137:

A mixture of methyl 3β-hydroxy-2-keto-olean-12-en-28-oate 137 (500 mg) aluminium isopropoxide (650 mg) in dry isopropanol (12.5 ml) was distilled slowly with the addition of isopropanol to maintain constant volume. After 6 hours the distillate no longer contained acetone and the solution was concentrated to a small volume. The reaction mixture was diluted with water followed by 10% sulphuric acid (20 ml) and then extracted with ether. The product obtained after removal of ether was dissolved in benzene (6 ml) and poured on a column of alumina (25 gm deactivated with 1 ml of 10% aqueous acetic acid) developed with petroleum ether. The following solvents were used as eluent. (Table IX)

Table - IX

Eluent	Fractions 50 ml each	Residue
Petroleum ether	1-3	Nil
Petroleum ether: benzene (3:1)	4-6	Nil
Petroleum ether: benzene (1:1)	7-9	Nil

Contd..

Table - IX (Contd.)

Eluent	Fractions 50 ml each	Residue
Petroleum ether: benzene (1:3)	10-12	Nil
Benzene	13-15	Nil
Benzene: ether (4:1)	16-21	Solid (400 mg) m.p. 285-6°

Further elution with more polar solvent did not give any solid material.

The solid from fraction 16-21 (Table IX) were combined which after crystallisation from methanol afforded methyl 2α, 3α-dihyroxy-olean-12-en-28-oate 139, m.p. 286-7°, $(\alpha)_D$ 71.11° identical with an authentic sample of methyl 2α, 3α -dihydroxy-olean-12-en-28-oate (m.m.p. and Co- TLC).

Found: C, 76.40; H, 10.31%

Calculated for $C_{31}H_{50}O_4$: C, 76.50; H, 10.40%

UV : No absorption in the region 220-300 mμ.

IR : ν_{max} 3340 (-OH), 1725 (-COOMe) cm^{-1}

Acetylation of methyl 2α, 3α-dihydroxy-Olean-12-en-28-oate 139: Preparation of methyl 2α, 3α-diacetoxy-olean-12-en-28-oate 140:

The solid 139 (200 mg) was acetylated by heating with acetic anhydride (2 ml) and pyridine (2 ml) on a water bath for four hours. After working up in the usual manner it gave ∅ crystals of methyl 2α, 3α-diacetoxy olean-12-en-28-oate 140, m.p. 226-8°, $(\alpha)_D$ 95.20°.

Found:	C, 73.20; H, 9.40%
Calculated for $C_{35}H_{54}O_6$:	C, 73.60; H, 9.50%

UV : No absorption above 200 mμ.

IR : ν_{max} 1750, 1740 ($-OCOCH_3$), 1725 ($-COOMe$) cm^{-1}.

Preparation of acetonide derivative 141 of methyl 2α, 3α-dihydroxy-Olean-12-en-28-oate 139:

2α, 3α-diol 139 (100 mg) was dissolved in dry acetone (20 ml) and to this a catalytic amount of p-toluene sulfonic acid was added. The reaction mixture was shaken for a few minutes and kept overnight. To the reaction mixture 5% sodium bicarbonate solution was added and part of the solvent was removed by distillation and then diluted with water. The cloudy precipitate that appeared was extracted with ether. The ethereal layer after being washed with water till neutral was dried (Na_2SO_4). The ether was then removed and the solid residue after several crystallisation from chloroform-methanol mixture afforded the pure acetonide derivative 141, m.p. 236-8°.

Found: C, 77.42; H, 10.28%

Calculated for $C_{34}H_{54}O_4$: C, 77.50; H, 10.30%

Preparation of methyl 2β, 3β-dihydroxy-olean-12-en-28-oate 142:

Sodium borohydride reduction of diosphenol 136:

To a solution of diosphenol 136 (200 mg) in methanol (100 ml) sodium borohydride (100 mg) was added and the mixture was stirred for one hour. The reaction mixture was concentrated, diluted with water and then acidified with dilute hydrochloric acid (6 ml) when a solid precipitated out. The latter was collected by filtration and dried. The solid (200 mg) was dissolved in benzene and was poured on a column of alumina (10 gm, deactivated with 0.4 ml of 10% aqueous acetic acid) developed with petroleum ether. The chromatogram was eluted with following solvents. (Table X)

Table - X

Eluent	Fractions 50 ml each	Residue
Petroleum ether	1-2	Nil
Petroleum ether: benzene(3:1)	3-4	Nil
Petroleum ether: benzene(1:1)	5-6	Nil
Petroleum ether: benzene(1:3)	7-8	Nil
Benzene	9-14	Solid (190 mg) m.p. 267-71°

Elution with more polar solvent did not offer any solid material.

The solid from fractions 9-14 (Table X) was collected and after crystallisation from methanol gave pure needle-shaped crystals of 142, m.p. 269-72°, $(\alpha)_D$ 88.88°.

Found: C, 76.48; H, 10.39%

Calculated for $C_{31}H_{50}O_4$: C, 76.50; H, 10.40%

UV : No absorption in the region 220-300 mμ.

NMR (60 MC/S): Peaks at 3.15 (multiplet) and 4.4 (multiplet) 5.15 ppm. Fig. 8

Acetylation of methyl 2β, 3β-dihydroxy-olean-12-en-28 oate 142: Isolation of methyl 2β, 3β-diacetoxy-olean-12-en-28-oate 143:

The diol 142 (200 mg) was acetylated by heating with pyridine (4 ml) and acetic anhydride (4 ml) on a water bath for four hours. After working up in the usual manner it gave a solid which after several crystallisation from chloroform-methanol afforded pure methyl 2β, 3β-diacetoxy-olean-12-en-28-oate 143, m.p. 220-222°, $(\alpha)_D$ 86.20°.

Found: C, 73.87; H, 9.54%

Calculated for $C_{33}H_{54}O_6$: C, 73.68; H, 9.50%

UV: No absorption in the region 220-300 mμ.

IR : ν_{max} 1258, 1720, 1745 cm^{-1}

NMR (60 MC/S) : Peaks at 3.65 (singlet, COOMe) 2.06 (singlet, 6H), 4.6, 5.04 ppm Fig. 9

Preparation of acetonide 144 of methyl 2β, 3β-dihydroxy-olean-12-en-28-oate 142:

To 2β, 3β-diol 142 (100 mg) dissolved in dry acetone (20 ml) was added a few crystals of p-toluene sulfonic acid and the mixture was shaken for 10 minutes and then kept overnight. After usual work up a solid (80 mg) was obtained which after crystallisation from methanol afforded pure crystals of the acetonide derivative 144, m.p. 75-80°.

Found: C, 77.31; H, 10.25%

Calculated for $C_{34}H_{54}O_4$: C, 77.50; H, 10.30%

Synthesis of methyl crategolate 148 : (methyl 2α, 3β-dihydroxy-olean-12-en-28-oate).

Acetylation of diosphenol 136: Preparation of diosphenol acetate 145:

Diosphenol 136 (200 mg) was treated with acetic anhydride (5 ml) and pyridine (5 ml) and kept overnight at room temperature. After working up in the usual manner the crude acetate (180 mg) was obtained. This was chromatographed over a column of alumina (10 gm) deactivated with 0.4 ml of 10% aqueous acetic acid. The compound was dissolved in benzene and the chromatogram was developed with petroleum ether. The following solvents were used as eluent. (Table XI)

Table - XI

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1-2	Oil (trace)
Petroleum ether: benzene (4:1)	3-8	Solid m.p. 165-7° (160 mg)

Elution with more polar solvents did not give any solid material.

The solid (160 mg) from fractions 3-8 (Table XI) were collected which after crystallisation from a mixture of chloroform and methanol afforded needle shaped crystals of 145, m.p. 168-70°, $(\alpha)_D$ 93.33°. It showed a single round spot on a chromatoplate and did not give ferric chloride coloration for diosphenol.

Found: C, 77.50; H, 9.78%

Calculated for $C_{33}H_{50}O_4$: C, 77.60; H, 9.87%

UV : λ_{max} 237 mμ (ϵ, 8500)

IR : ν_{max}^{nujol} 1205, 1685, 1720, 1738 cm^{-1} Fig. 10

Hydrogenation of diosphenol acetate 145: Preparation of methyl 3 Keto-2α-acetoxy olean-12-en-28-oate 146:

To diosphenol acetate 145 (200 mg) dissolved in absolute ethyl alcohol was added 10% palladium-on-charcoal catalyst (50 mg)

and the mixture was shaken in an atmosphere of hydrogen till the absorption of hydrogen ceased (absorption of one mole equivalent of hydrogen within one hour). The solution was filtered and after removing the solvent from the filtrate a semisolid residue (200 mg) was obtained which after crystallisation from methanol afforded 146, m.p. 208-9°, $(\alpha)_D$ 52°.

Found:	C, 77.20; H, 10.02%
Calculated for $C_{33}H_{52}O_4$:	C, 77.30; H, 10.22%
UV :	272 mμ (ε, 84)
IR : ν_{max}^{nujol}	1225, 1730, 1750 cm^{-1}

Preparation of methyl 2α-acetoxy-3β-hydroxy-Olean-12-en-28-oate 147: Sodium borohydride reduction of 146:

To methyl 3-keto-2α-acetoxy-olean-12-en-28-oate 146 (200 mg) dissolved in dry dioxan (20 ml), was added, with cooling a slurry of sodium borohydride (200 mg) prepared in an NH_4Cl-NH_4OH buffer (PH = 8, 3 ml) and the mixture was stirred at room temperature for three hours. A portion of the solvent was removed by distillation, cooled and acidified with dilute hydrochloric acid and then extracted with ether. The ethereal layer was washed with water till neutral and dried (Na_2SO_4). Removal of ether gave a solid residue (150 mg) which was chromatographed over a column of alumina (25 mgm. deactivated with 1 ml of 10% aqueous acetic acid). The residue was dissolved

in benzene, poured on the column and was eluted with the following solvents. (Table XII)

Table- XII

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum	1-4	Oil (15 mg)
Petroleum ether: benzene (4:1)	5-7	Nil
Petroleum ether: benzene (3:2)	8-12	Solid (100 mg) m.p. 199-200°

Further elution with more polar solvents did not give any solid material.

The solid from fractions 8 to 12 (Table XII) were combined and crystallized from methanol. After several crystallisation pure methyl 2α-acetoxy-3β-hydroxy-olean-12-en-28-oate 147, m.p. 199-204°, $(\alpha)_D$ 27.9° was obtained.

Found:	C, 74.8; H, 9.8%
Calculated for $C_{33}H_{52}O_5$:	C, 75.0; H, 9.9%

Hydrolysis of methyl 2α-acetoxy-3β-hydroxy-Olean-12-en-28-oate 147: Preparation of methyl 2α, 3β-dihydroxy-olean-12-en-28-oate (methyl crategolate) 148:

To a solution of 2α-acetoxy-3β-hydroxy olean-12-en-28-oate 147 (100 mg) in benzene (5 ml) was added 10% sodium hydroxide solution (15 ml) and the reaction mixture was refluxed for 3 hours on a water bath. The reaction mixture, after removal of solvent, was diluted with water and then extracted with ether. The ethereal layer was washed with water till neutral and dried (Na_2SO_4). On removal of ether, a solid was obtained, which on crystallisation from methanol gave crystals of methyl crategolate 148 m.p. 220-22°, $(\alpha)_D$ 36°, which was found to be identical with an authentic sample (m.m.p. and Co-TLC, rotation comparison).

Found:	C, 76.49; H, 10.28%
Calculated for $C_{31}H_{50}O_4$:	C, 76.50; H, 10.40%

UV: No absorption in the region 220-300 mμ.

R E F E R E N C E S

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PART-III

ALLYLIC OXIDATION AND BROMINATION STUDIES WITH NBS ON TARAXERYL ACETATE.

Part - III

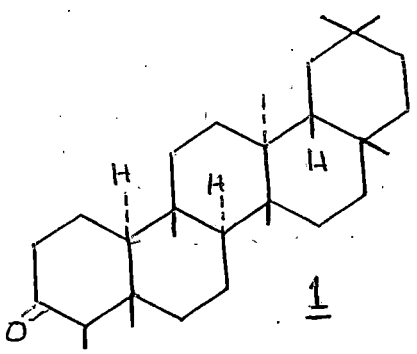
CHAPTER-I

A short review on allylic oxidation and bromination on triterpenoids.

1. Bromination on friedelin with bromine in chloroform:

Corey and Ursprung[1] carried out bromination on friedelin 1 in course of their work for the establishment of the structure of friedelin. These studies also demonstrated clearly the presence of methyl group at C-5 in friedelin 1. Direct bromination of friedelin with one mole of bromine in chloroform gave the 2-bromo derivative 2. From infra-red and ultraviolet absorption of the carbonyl group at 1710 cm^{-1} and 311 mμ compared with those obtained at 1708 cm^{-1} and 295 mμ[2,3] in case of friedelin Corey et al deduced the axial oreintation of bromine atom at C-2 in 2. Furthermore, successive treatment of 2 with sodium borohydride and zinc-acetic acid yielded Δ^2 -friedelene, proving thereby the location of bromine at C-2 in 2-bromo friedelin. Friedelin 1 was converted into an enol-benzoate by heating to 160°, which was predominantly the Δ^2-isomer, whereas Δ^3-enol benzoate was obtained at a higher temperature (180-190°). Bromination of the latter (high temperature enol-benzoate) produced the 4-bromo derivative of friedelin 3. The axial orientation of bromine in this bromo ketone 3 was based on spectral data (carbonyl absorption at 1715 cm^{-1}, 310 mμ) and location of bromine at C-4

followed from conversion in two steps to Δ^3-friedelene. Debromination of both bromoketones with zinc-acetic acid-ether yielded friedelin. Whereas 2-bromofriedelin 2 was relatively inert to silver acetate in acetic acid solution at steam bath temperature, the 4-bromoisomer was dehydrobrominated to an unsaturated unconjugated ketone which was not isomerised to a conjugated structure by prolonged treatment with strong acid. The UV of the substance indicated trisubstituted nature of the double bond. The production of nonconjugated nonisomerisable ketone from 4-bromofriedelin indicated that migration of an alkyl group most probably methyl, from C-5 to C-4 had occured during the dehydrobromination process and on this basis formulation 4 was suggested. Wolff-Kishner reduction of 4 gave a different unsaturated hydrocarbon 5 which was not isomerisable by heating at reflux with dilute ethanolic sulphuric acid. The stability of the olefin 5 to isomerisation as well as its UV indicated that it occupied the position indicated in 5. Corey et al also deduced stereochemical disposition of ring A from studies on 2-bromofriedelin 2 and 4-bromofriedelin 3. The assymetric centre at C-2 in 2 was not epimerizable with hydrobromic acid under conditions which resulted in further bromination and hence the axial orientation of bromine in 2-bromofriedelin was the stable one.



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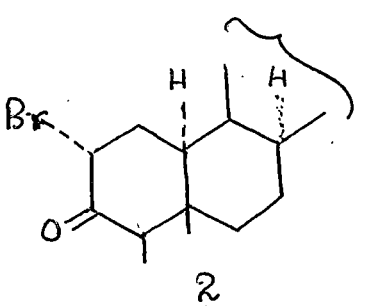

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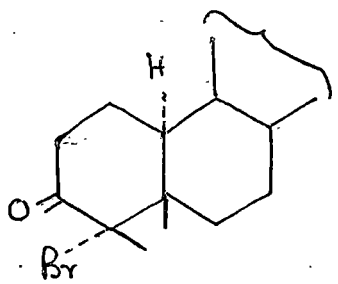

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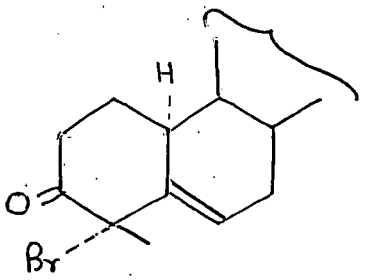

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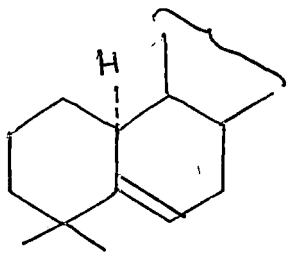
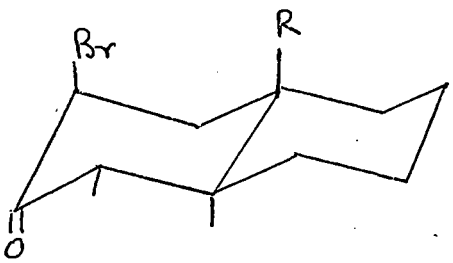


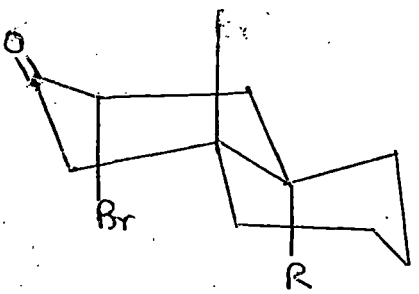
5

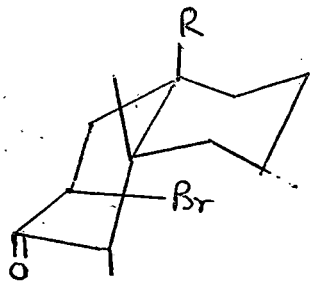
This observation ruled out all but one of the remaining stereochemical possibilities for the A/B ring fusion 6 and 7. In structure 6, C-2 would be epimerizable if R = CH_3 but not R = H, so that only the latter possibility is acceptable. In structure 7, C-2 would be epimerizable to a more stable configuration regardless of whether R = H or CH_3 and so both of these possibilities are inadmissible. From this observation it appeared that friedelin 1 must have a trans A/B juncture with a hydrogen at C-10.

Further the change in rotation due to axial bromine at C-2 (ΔMD - 651°) was opposite in direction to that due to axial bromine at C-4 (ΔMD +614°). The sign of these shifts taken together with molecular rotation data on α-bromoketosteriods of known absolute configuration revealed that bromine was α-oriented in both 2-and 4-bromofriedelin 2 and 3 and that the methyl group at C-5 was β and

the hydrogen at C-10 was α, that is, of the two structures of different absolute configurations 6a and 6c only 6c was acceptable using this criterion.







6a R = H

6b R = CH_3

6c R = H

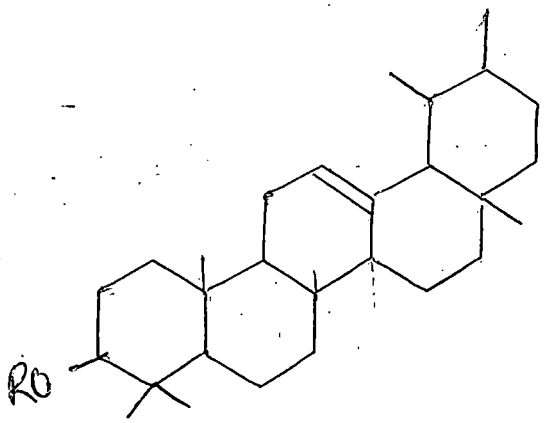
6d R = CH_3

7

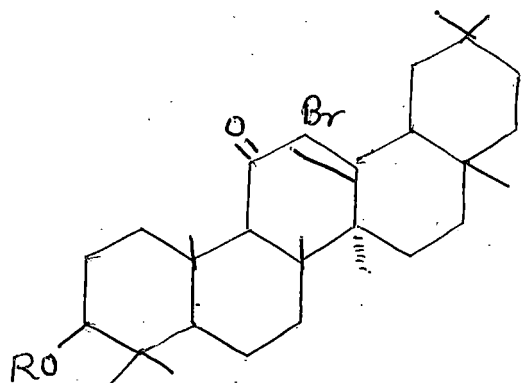
2. Bromination of triterpenoids of the oleanane and ursane series[5].

Vesterberg[6], Zinke et al[7-8] reported that esters of α and β-amyrin could be brominated. Rollet[9,10] also reported bromination studies on triterpenoids. Arya and Cookson[5] observed that α-amyrin acetate or benzoate 8a took only one mole of bromine rapidly and a second more slowly. The resulting bromo-α-amyrin and its acetate agreed in melting point with Vesterberg's compound[6,8] whereas the benzoate roughly corresponded with Zinke, Friedrich and Rollet's ester[7]. Monobromo β-amyrin acetate prepared by Arya et al had the reported melting point of Vesterberg[6,10] but the benzoate was different from any of the monobromo-β-amyrin benzoate reported by

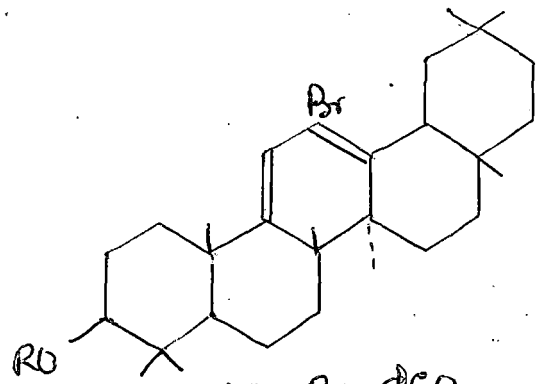
Rollet[10] who may have had mixtures. Reduction of each monobromo amyrin with sodium and alcohol[5] gave back the appropriate amyrin proving thereby that bromination had not been attended with rearrangement of the carbon skeleton and making it probable that the double bond in bromides still occupied its original position. The great stability of the monobromides to base e.g. monobromo-α-amyrin was unchanged by long boiling with 10% potassium hydroxide in diethylene glycol(250°)- at once suggested that the bromine atom might be attached to a doubly bound carbon atom, probably C-12. Further verification was achieved by oxidation of monobromo-β-amyrin benzoate by chromic acid to an $\alpha\beta$-unsaturated ketone with light absorption maxima at 1680 cm^{-1} and 269 mμ. These were the spectral properties expected[11] of the bromoketone 9 (R = benzoate), the ultraviolet maximum of the 12-en-11-one being shifted about 20 mμ to longer wavelength by the 12-bromine atom. Monobromo-11-oxo-β-amyrin benzoate 9 on reduction with lithium aluminium hydride, followed by acetylation, led to a bromodiene 10 characterized as a 9(11): 12-diene by its light absorption (λ_{max} 283 mμ) and high dextrorotation. Reduction of this diene with sodium and alcohol and acetylation produced



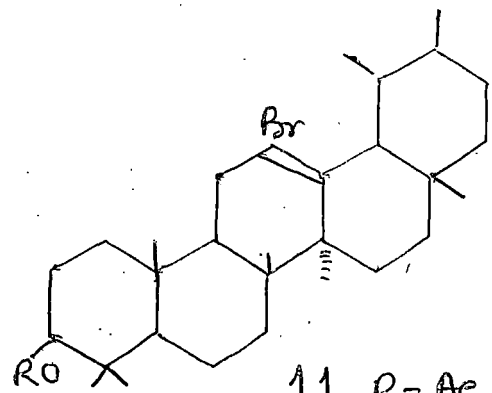
8a



9 R = ϕCO



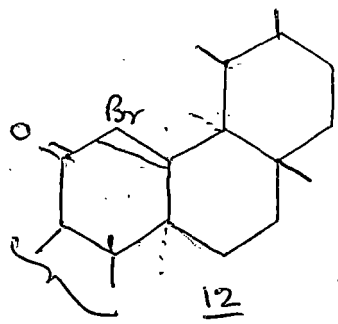
10 R = φCO



11 R = Ac.

3β-acetoxy-β-amyrin- 9(11):12-diene. Preparation of the bromoketone 9 and bromodiene 10 proved the absence of bromine at C-9 and C-11.

Monobromo -α-amyrin acetate and benzoate 11 were converted into the analogous compounds 12 and 13 in the same way. 12-bromo-α-amyrin benzoate did not react further with bromine in acetic acid but 12-bromo-β-amyrin acetate or benzoate took up a second mole of bromine in a few hours at room temperature and yielded a mixture from which was isolated a dibromo-β-amyrin benzoate having identical melting point with that obtained by Zinke et al[7].



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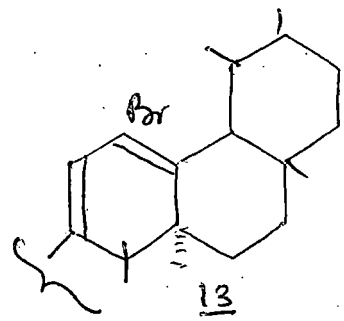

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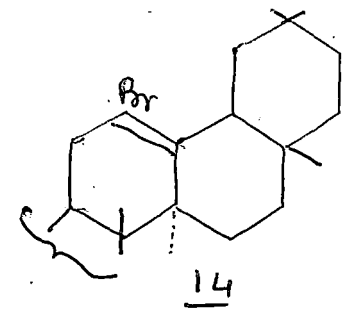

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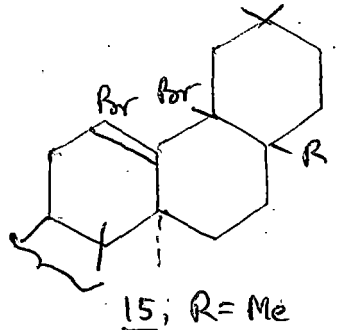
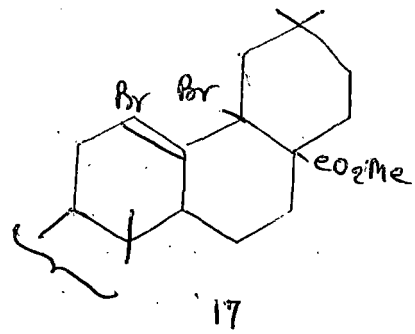


15; R = Me

16, R = CO_2Me



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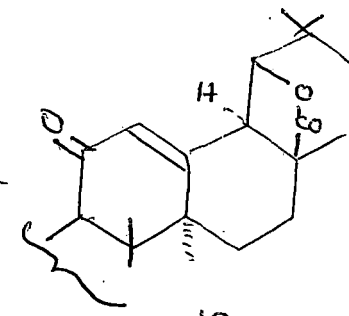


18

Oxidation of dibromo-β-amyrin acetate or benzoate with chromic acid in acetic acid gave the corresponding dibromo-11-ketone (UV absorption at 269 mμ). The latter was also produced by bromination of appropriate ester of 12-bromo-11-oxo-β-amyrin 9. From these observations they concluded that the second bromine should be attached only to C-9 or C-18.

In order to test the latter possibility, the acetate of the dibromide was heated with collidine at 300°, yielding an isomer of 12-bromo-11-oxo-β-amyrin acetate. Here reduction occured instead of expected elimination of bromine atom. Similar treatment with collidine on dibromo-β-amyrin acetate gave an isomer identified as 12-bromo-18α-β-amyrin acetate 14. The latter was also produced by brominating 18α-β amyrin acetate. 12-bromo-18α-β-amyrin acetate was oxidised to 12-bromo-11-oxo-18α-β- amyrin acetate identical with the monobromo ketone formed on reduction of dibromo-11-oxo β-amyrin acetate. Since normal 12-bromo β-amyrin acetate and its 11-oxo derivative were stable to collidine under conditions that reduced the dibromo compounds to the 18α-isomer, the isomerisation was produced by reduction thereby indicating the location of the second bromine atom at C-18 (15, R = Me).

Methyl-O-acetyl oleanolate absorbed one mole of bromine in acetic acid to give the 12-bromo derivative which more slowly took up a second mole of bromine to form the 12:18 dibromide 16 (R = CO_2Me).

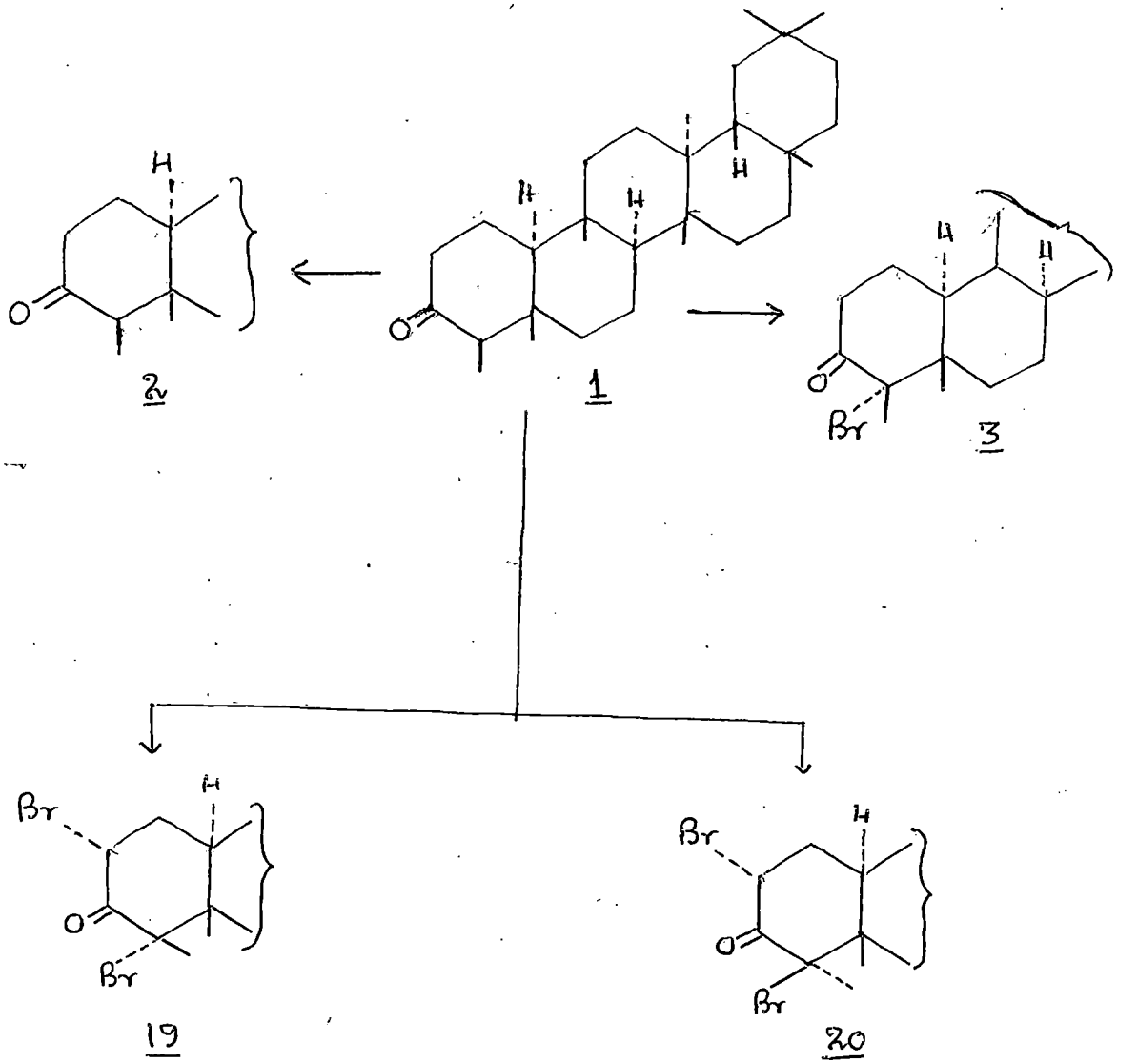
Chromic acid oxidation of two bromides gave the respective 11-ketones. Treatment of dibromoketone 17 with collidine resulted in elimination of elements of methyl bromide, producing in good yield a neutral, high melting methyl free substance, the behaviour of which with alkali showed it to be a lactone. The band at 1790 cm^{-1} in the IR indicated a γ -lactone while the band at 1688 cm^{-1} and UV at 264 mμ showed that the 12-bromo-12-en-11-one chromophore was probably still intact. These structural features could be accommodated by the tentative constitution 18.

The configuration of the 18-bromine atom in the dibromides is uncertain but nearly identical UV spectra of the mono and dibromides in both the long and short wave length bands suggested very strongly that the bromine at C-18 was equatorial[12] to ring D (18β) rather than axial as might have been expected from the operation of purely electronic effects[13].

3. Action of N-Bromosuccinimide (NBS) on friedelin and derivatives[14].

Corey and Ursprung[1] showed that friedelin 1 on direct monobromination gave 2 α-(axial)-bromofriedelin 2 and bromination of appropriate enol benzoate gave the isomeric 4 α-axial bromofriedelin 3. They have also prepared a dibromofriedelin 19 in presence of hydrobromic acid in chloroform solution. The UV absorption at 332 mμ indicated that most probably both the bromine atoms were axially oreinted and has been regarded as the 2α, 4α-dibromofriedelin 19. Djerassi and co-workers[15] prepared a second dibromofriedelin 20 by

bromination of 2α-bromofriedelin in acetic acid. From its UV absorption at 310.5 mμ and also from ORD studies they formulated the compound as 2α, 4β-dibromofriedelin 20.



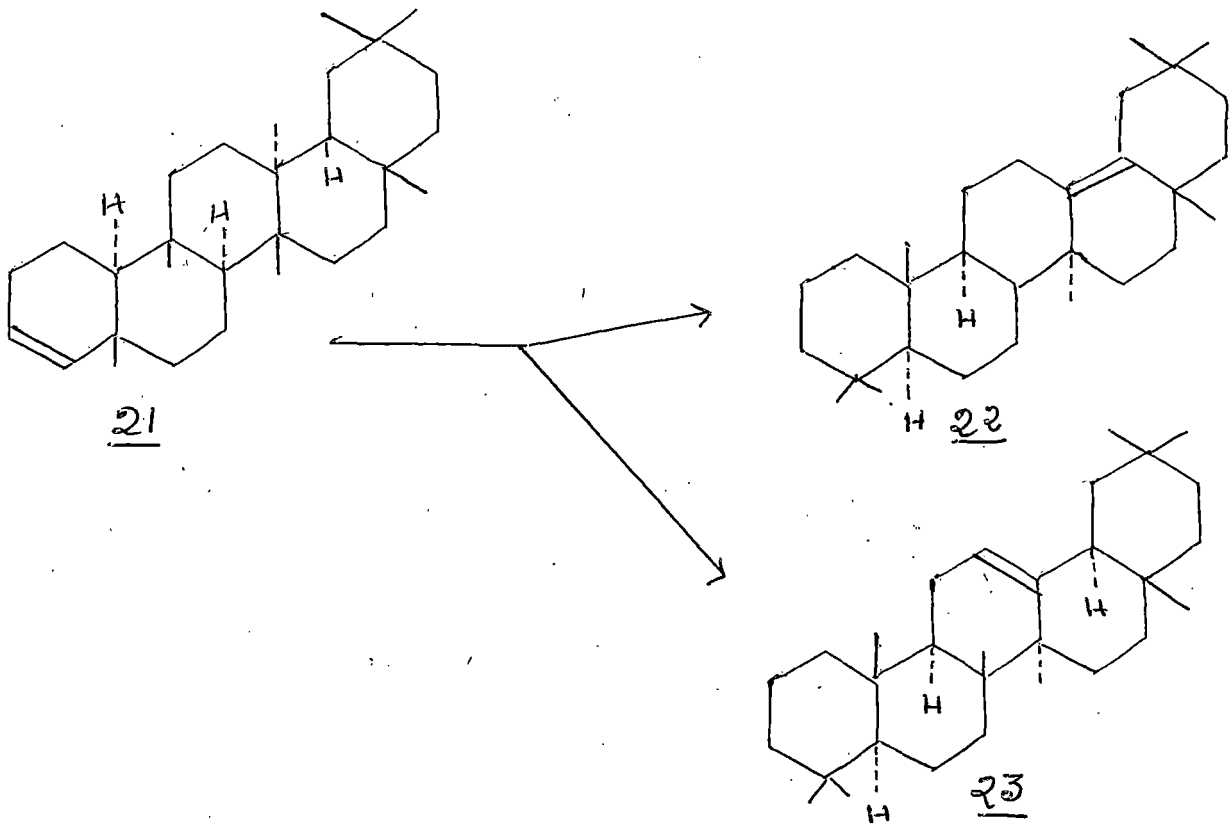
Takahasi and Ourisson[16] also prepared a dibromofriedelin by dibromination of friedelin in chloroform-acetic acid which showed an UV absorption at 320 mμ. These workers did not assign any structure to

this compound.

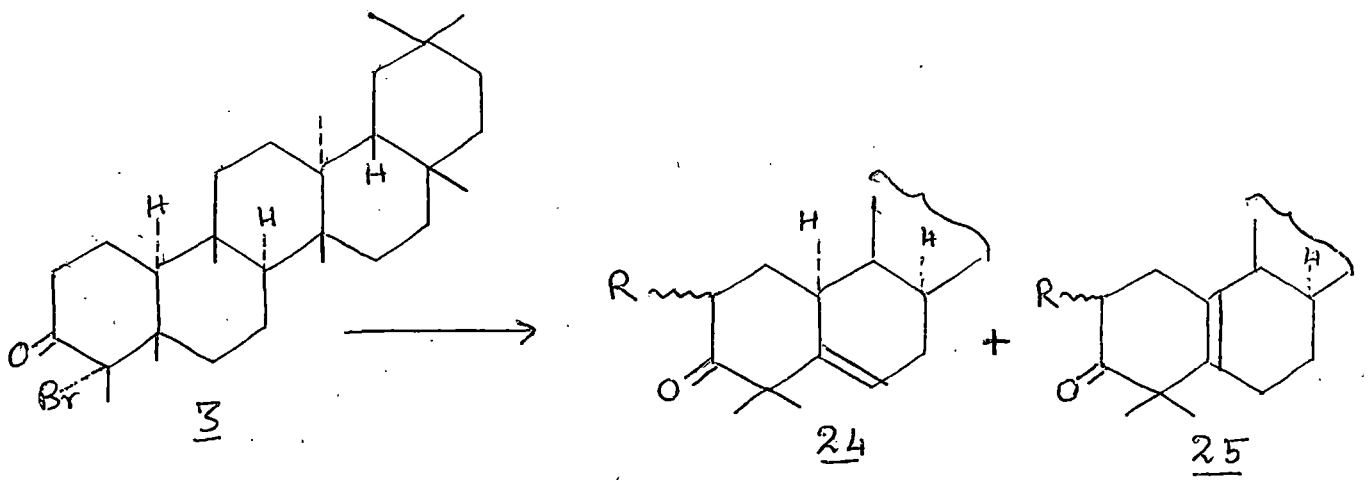
Stevenson et al[14] examined the action of N-bromosuccinimide (NBS) on friedelin and derivatives. Treatment of friedelin 1 in carbontetrachloride with a molar equivalent of NBS gave 4α-bromofriedelin 3 in good yield. By further treatment of 3 with bromine in acetic acid solution, these authors isolated 2α-bromofriedelin 2 -here isomerisation rather than substitution occured. As anticipated from this result, it was found that 4α-bromoketone 3, $(\alpha)_D$ 92° was unstable in chloroform-hydrobromic acid, the presumed equilibrium mixture $(\alpha)_D$ -75° being formed after 24 hours. Similar equilibration of 2α-bromofriedelin gave the same result.

Since this route for obtaining a dibromofriedelin was unsuccessful, the alternative method of treating 2α-bromofriedelin 2 with NBS was examined. 2α-bromofriedelin 2 on treatment with NBS gave an unsaturated monobromoketone, $C_{30}H_{47}OBr$. The latter showed a positive tetranitromethane test indicating the presence of an ethylenic function. UV and IR spectrum of this ketone showed that the double bond was not conjugated to the carbonyl group and that the α-bromine atom retained an axial conformation. Since it is known that acid isomerisation of friedel-3-ene 21 afforded a mixture of olean-13, 18-ene, 22 and 18α-olean-12-ene, 23[17,18]- it was considered that this non-conjugated bromoketone had probably arisen by a molecular rearrangement of 2α, 4-dibromoketone intermediate (or

derived radical or cation) with elimination of hydrobromic acid. A



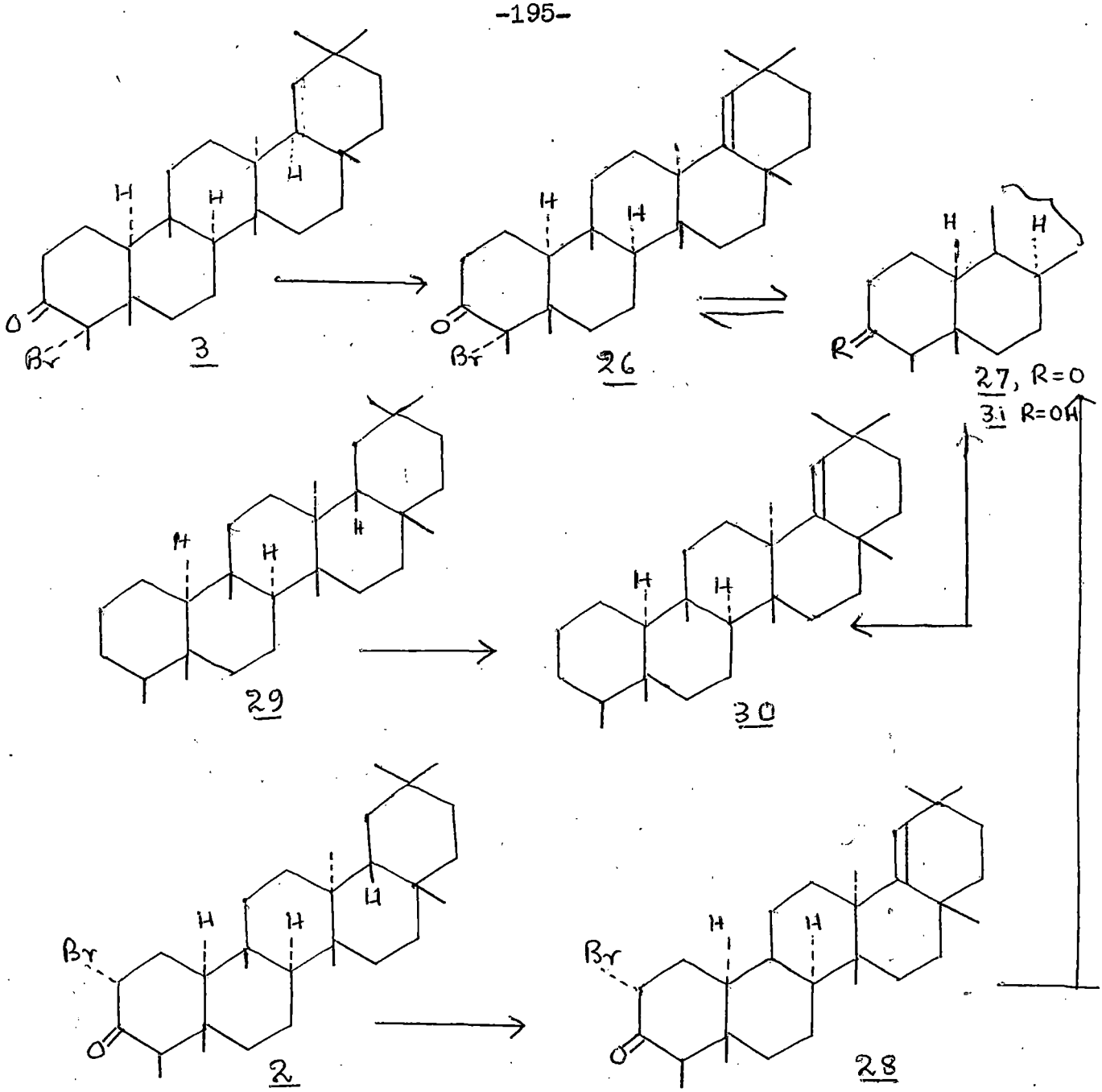
precedent for such a postulated rearrangement was provided by the action of silver-acetate on 4α-bromofriedelin 3 to yield a product[1] which was shown to be a mixture[19] of alnus-5-enone 24 (R = H) and alnus-5(10)-enone, 25 (R = H). The probability that the unsaturated bromoketone derived from 2 could be represented as a 2-bromo-alnus-enone 24 (R = Br) or 25 (R = Br), was excluded from the fact that the zinc-debromination product in neutral solution was different from either alnusenone 24 or 25 (R = H).



3 24 25

Treatment of 4α-bromofriedelin 3 with NBS gave an isomeric non-conjugated axial bromosubstituted ketone $C_{30}H_{47}OBr$, which also on debromination gave the same ketone 27, $C_{30}H_{48}O_3$. Reduction of 27 with lithium aluminium hydride gave an alcohol 31 and reduction by Huang-Minlon method gave the hydrocarbon 30. From these evidences, the isomeric monobromoketones obtained from 2 and 3 by the action of NBS were assigned structures 2α-bromofriedel-18-en-3-one 28 and 4α-bromofriedel-18-en-3-one 26 respectively. These assignments were corroborated by specific rotation and ORD studies.

Action of NBS on saturated hydrocarbon friedelane 29[14,20] was also examined and they isolated an unsaturated hydrocarbon 30 identical in all respects with that obtained from Haung-Minlon reduction of 27. This further suggested that the products obtained by NBS on ketones 2 and 3 were ethylenic non-conjugated ketones and hence the attack has proceeded at a site not activated by carbonyl group. The site of the double bond introduced by NBS was established



by the following way. The unsaturated hydrocarbon 30, resisted catalytic hydrogenation, yielded an oxide $C_{30}H_{50}O$ with perbenzoic acid, indicating that the double bond has a degree of steric hindrance[21] comparable to the Δ^{12}-trisubstituted ethylenic linkage in β-amyrin

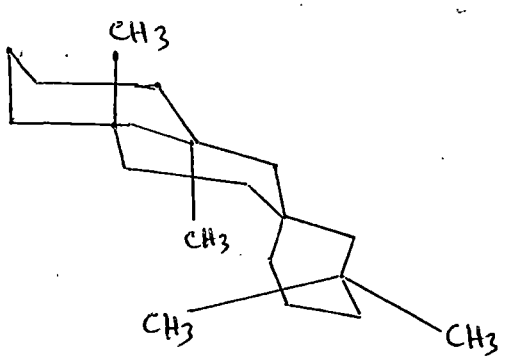
series. The terminal UV absorption of 27, 31 and 30 indicated that the double bond was trisubstituted. The resistance of hydrogenation of 30 further suggested that the ethylenic system was not disubstituted and the friedelane skeleton does not permit the existence of a tetra substituted double bond. NMR spectrum of the ketone 27 showed a singlet attributable to an olefinic proton not conjugated with the carbonyl group.

The location of the double bond introduced by the action of NBS on bromoketones 2, 3 and hydrocarbon 30 was thus restricted to position 1(10), 7 or 18. The position 1(10) and 7 were excluded by examining the dehydrobromination of 26 with silver acetate. Under these conditions they isolated a dehydrobrominated product $C_{30}H_{46}O$ which showed no UV above 200 mμ. Since there was no conjugation of carbonyl or ethylenic functions in this dienone, the original double bond could not have been located in ring A or B.

Although there had been much work on synthetic application of allylic bromination[22], comparatively little is known with regard to the action of NBS on saturated systems. It has been established that cyclohexane[23,24] and cycloheptane[24] yield the cycloalkylbromide with NBS under certain condition and that decalin gives a tetrabromo-octahydro napthalene[25] which can also be obtained from the probable intermediate 9,10-octalin. Cason and coworkers[26] have also drawn attention to the fact that NBS is not a reagent of general utility for the α-bromination of saturated esters due to selective attack on t-hydrogen atom at the other sites in the molecule.

The experiments described by these workers establish that in friedelin the tertiary α-hydrogen atom at position 4 is more reactive towards NBS than the secondary hydrogen atom at position 2 but the presence of a 2α-bromine atom effectively prevents the abstraction of 4α-hydrogen atom by its 1,3 diaxial blocking effect to approach a succinimide radical.

In absence of the activating C-3 carbonyl group or where there is a deactivation due to the steric influence of the neighbouring axial halogen, the most reactive hydrogen is the tertiary C-18 atom. An examination of an all chair form 32 of friedelane showed that a severe steric interaction must exist between the 13α- and 20α-methyl groups due to cis junction of rings D and E. This interference is removed if the terminal E ring adopted a boat configuration 33 but as a consequence an unfavourable 1:4 diaxial boat prow and stern interaction results[27]. The steric strain inherent in both conformations with cis D/E system is relieved by dissociation of the 18β-hydrogen atom, and formation of ethylenic triogonal system.



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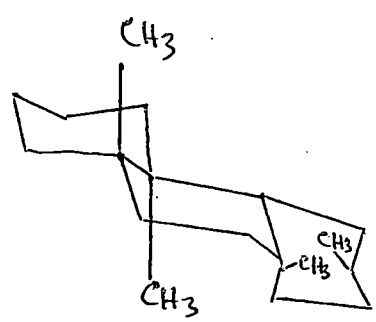


33

4. The Bromine and N-Bromosuccinimide Oxidation of saturated hydrocarbon, Friedelane[20].

Olefins may be halogenated in their allylic position by a number of reagents of which NBS is by far the most common. With this reagent an initiator is needed and this is usually a peroxide. The reaction is usually quite specific at allylic position and good yields are obtained. However, when the allylic radical intermediate is unsymmetrical, then allylic shifts can take place so that a mixture of both possible products are obtained.

$$CH_3-CH_2-CH=CH_2 + NBS \longrightarrow CH_3-\underset{\underset{Br}{|}}{CH}-CH=CH_2 + CH_3-CH=\underset{\underset{Br}{|}}{CH}-CH_2$$

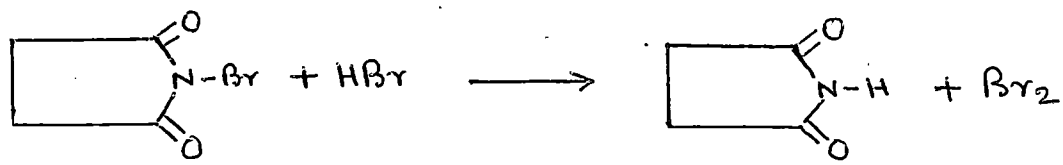
When a double bond has two different α-positions (e.g. $CH_3-CH=CH-CH_2-CH_3$), then a secondary position is substituted more readily than primary. The relative reactivity of tertiary hydrogen is not clear, though many substitutions at allylic tertiary position have been performed[22].

That the mechanism of an allylic bromination is of the free-radical type was demonstrated by Dauben and McCoy[22] who showed that the reaction was very sensitive to free radical initiators and inhibitors and indeed did not proceed at all unless at least a trace of initiater was present. Subsequent work indicated that the species which actually abstracts hydrogen from the substrate is the bromine atom. The reaction is initiated by small amounts of $Br\cdot$. Once it is formed, the main propagation steps are:

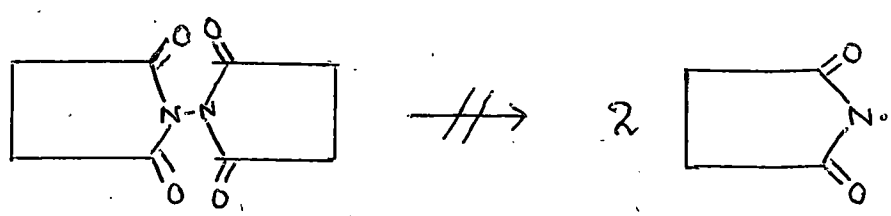
1. $Br^{\cdot} + RH \longrightarrow R^{\cdot} + HBr$

2. $R^{\cdot} + Br_2 \longrightarrow RBr + Br^{\cdot}$

The source of Br_2 is a fast ionic reaction between NBS and the HBr liberated in step 1:



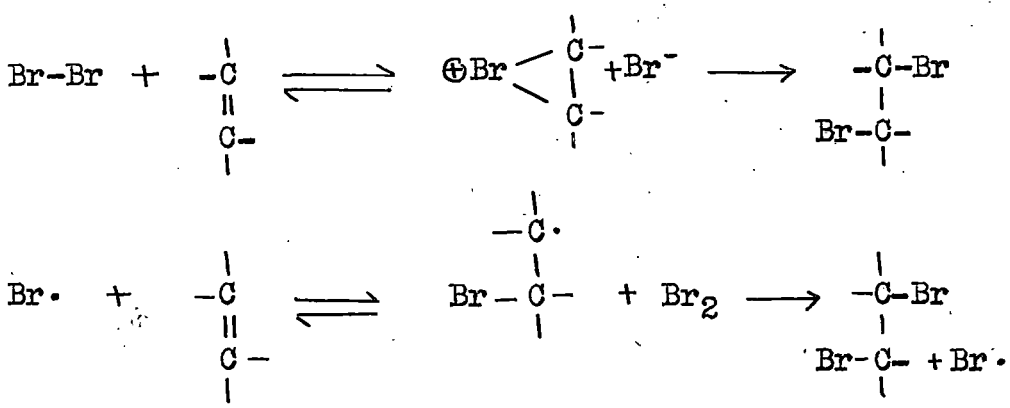
The function of NBS is therefore to provide a source of Br_2 in a low, steady-state concentration and to use up the HBr liberated in step 1[28,29]. It was previously believed that the abstracting species was the succinimide radical 33a but there is now much evidence that this species is not involved in the reaction and is probably not even formed. The main evidence is that NBS and Br_2 show similar selecti-



33a

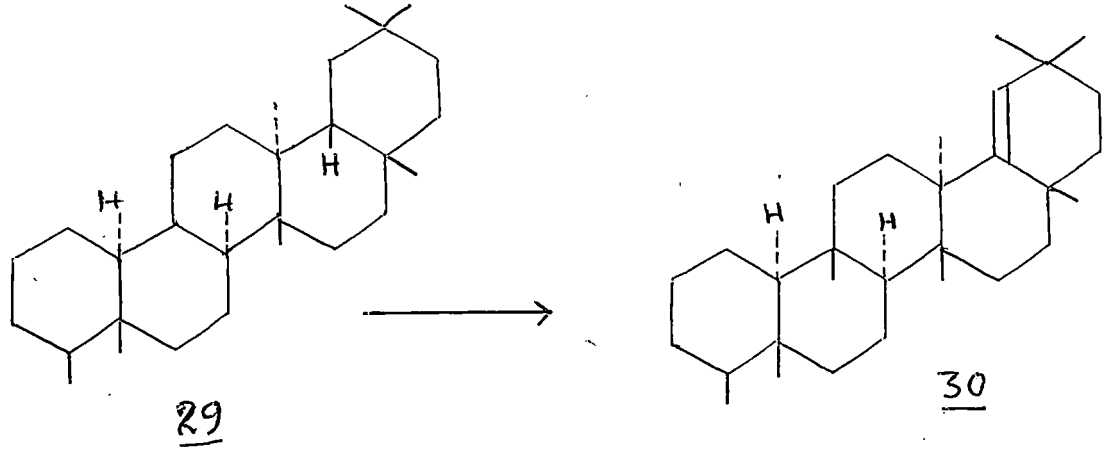
vity[30]; that the various N-bromoamides also show similar selectivity, which would not be the case if a different species was abstracting in each case[31] and that 33a has proved itself to be a much less stable species than was originally thought, since its dimer shows no tendency to dissociate[32]. The latter observation casts doubt of the ability

of NBS to dissociate. The fact that the reacting species Br_2 does not add to double bond, either by an ionic or by a free radical mechanism can be explained in the following way. Apparently the concentration is too low. In bromination of a double bond, only one atom of an attacking bromine molecule becomes attached to the substrate, whether the addition is electrophilic or free radical:



The other bromine comes from another bromine containing molecule or ion. If the concentration is sufficiently low, there will not be a high probability that the proper species will be in the vicinity once the intermediate forms and the equilibrium will lie to the left. This slows the rate of addition so that allylic substitution can successfully compete. If this is true, then it should be possible to brominate an olefin in the allylic position without competition from addition, even in the absence of NBS or a similar compound, if a very low concentration of bromine is used and if the HBr is removed as it is formed, so that it is not available to complete the addition step. This has been demonstrated by McGrath et al[33].

Stevenson et al[14,20] reported that the saturated hydrocarbon friedelane 29 was oxidised by NBS to the olefin, friedel-18-ene 30. In order to demonstrate that the function of NBS here was to provide molecular bromine, they have compared the action of bromine on 29 in carbon tetrachloride solution. A solution of bromine in carbon tetrachloride added to friedelane was decolorized and the reaction mixture when worked up in the usual way[14a], yielded friedel-18-ene 30 in comparable yield. This demonstrated that in this oxidation, the intermediacy of the succinimide radical was unessential. No unchanged friedelane was recovered by chromatographic examination. They, however, isolated an unstable bromofriedelane which readily yielded friedel-18-ene and was consequently considered by them to be an 18-bromofriedelane. The discrepancies and poor reproducibility reported[14a] in the bromination of 3-ketone, friedelin by NBS, particularly in the formation of di and tribromo derivatives at C-2 and/or C-4, may be attributed to accompanying halogenation at C-18.



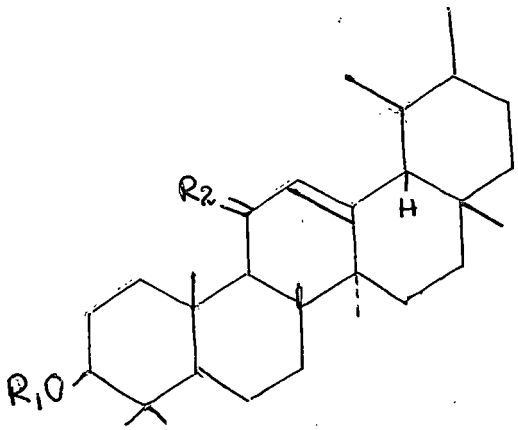
29 30

5. Allylic oxidation by N-bromosuccinimide[34].

Corsano et al[35] reported formation of 3β-acetoxy-urs-12-en-11-one 35 in 80% yield by treatment of α-amyrin acetate 34 with NBS in aqueous dioxan solution.

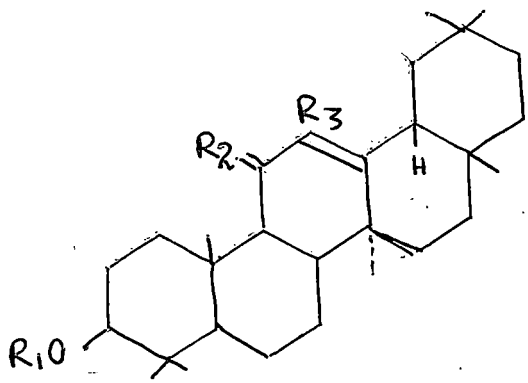
Finucane et al[34] obtained 3β-acetoxy-olean-12-en-11-one 39 from β-amyrin acetate 38 under the same conditions[35] in yields that fluctuated between 20 to 60% together with a number of by-products. They also claimed that when the reaction mixture was irradiated with visible light, αβ-unsaturated ketones were formed in near quantitative yields from a number of trisubstituted olefins containing an allylic methylene group. Finucane et al treated β-amyrin acetate 38 with NBS in aqueous dioxan in a typical ambient-light experiment as described by Corsano et al[35]. They separated the products by chromatography over silica gel and isolated starting material (Ca 50%), 3β-acetoxy-olean-12-en-11-one 39 (Ca 40%), bromo compounds (Ca 8%) and 3β-acetoxy-olean-12-en-11-α-ol 40 (Ca 2%). Oxidation of the latter 40, with CrO_3 in acetone afforded 3β-acetoxy-olean-12-en-11-one 39.

In another experiment the products were isolated by chromatography over alumina, the compounds isolated were β-amyrin acetate 38 (Ca 35%), 3β-acetoxy-olean-12-en-11-one 39 (Ca 40%), bromo-compounds (Ca 10%) and polar materials (Ca 10%). The polar fraction, on elution with methanol was acetylated and on rechromatography gave 11α-methoxy-olean-12-en-3β-yl acetate 41 together with smaller



34 R_1 = Ac, R_2 = H_2

35 R_1 = Ac, R_2 = O



38 R_1 = Ac, R_2 = H_2, R_3 = H

39 R_1 = Ac, R_2 = O, R_3 = H

40 R_1 = Ac, R_2 = α-OH, R_3 = H

41 R_1 = Ac, R_2 = α-OMe, R_3 = H

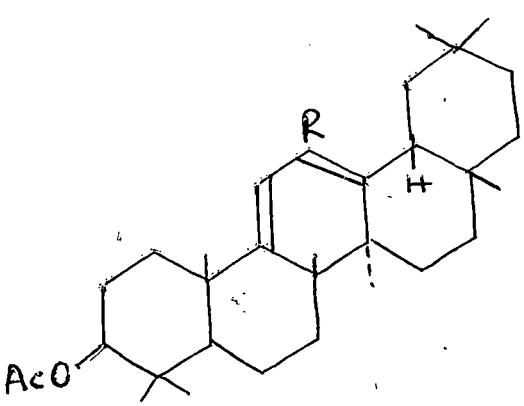
42 R_1 = Ac, R_2 = α-OAc, R_3 = H

43 R_1 = Ac, R_2 = α-OH, R_3 = Br

45 R_1 = Ac, R_2 = O, R_3 = Br

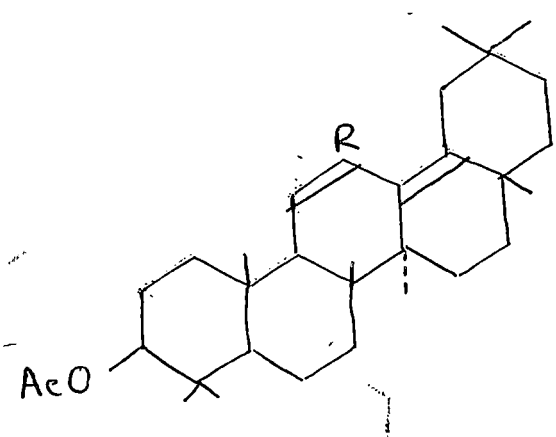
46 R_1 = Ac, R_2 = H_2, R_3 = Br

amounts of 11α-ol 40 and oleana-9(11), 12 dien-3β-yl acetate[5] 54 and a trace of 3β, 11α-diacetate[35] 42. The methoxy acetate 41 with p-toluene sulfonic acid in acetic anhydride afforded 3β-acetoxy-oleana-11(13)18-diene 56 in quantitative yield. The 11α-configuration of the methoxy group was assigned on the basis of the magnitude



54 R = H

55 R = Br



56 R = H

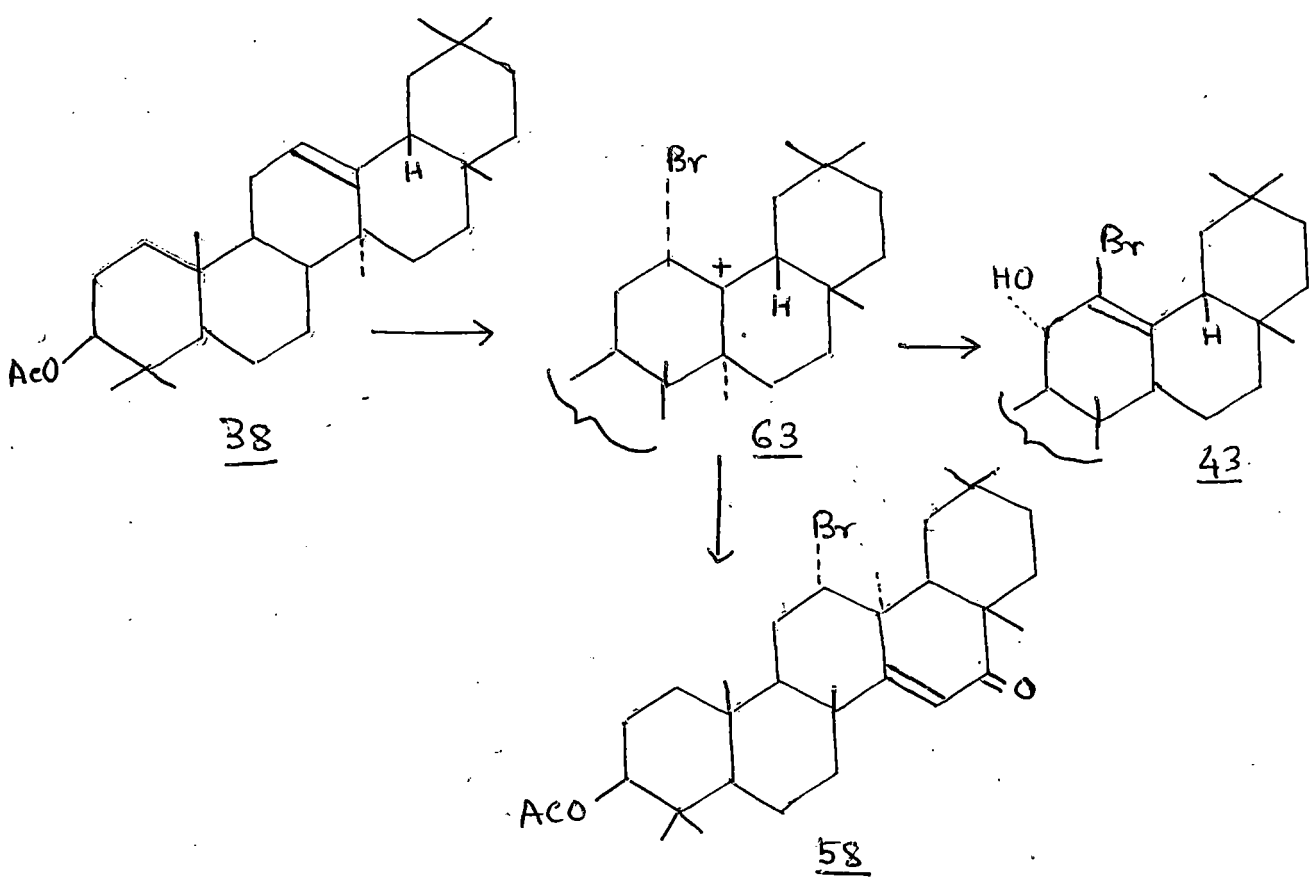
57 R = Br

of the 9α-H, 11-H coupling constant[35,36,37] 9Hz.

The fraction containing the bromo compounds was resolved by chromatography over alumina and fractional crystallisation into two components. The major product was a diol monoacetare ($C_{32}H_{51}BrO_3$, IR). Although free hydroxy group was secondary (ν 5.6, HCOH), the monoacetate resisted further acetylation. The compound gave a yellow color with trinitromethane and showed and absorption in the UV, but no olefinic proton or HCBr signal in its NMR spectrum. These data suggested that the bromo compound was 3β-acetoxy-12-bromo-olean-12-en-11-ol 43. The latter resisted attempted dehydrobromination, although prolonged treatment with lithium chloride in hot DMF resulted in dehydration with formation of 12-bromo-oleana-9(11), 12-dien-3β-yl acetate[5] 55. Acid catalysed dehydration of 12-bromo-11-ol 43 also yielded the homoannular diene 55 and not the heteroannular isomer 57. In the absence of 12-bromine atom, the heteroannular diene 56 was the sole product. Model of 57 showed a severe non-bonding interaction between the bromine atom and 19 proton, irrespective of the conformation of ring E. This interaction was absent in 55. Mild oxidation of 43 gave 3β-acetoxy-12-bromo-olean-12-en-11-one[5] 45. This confirmed the presence of 11-hydroxy group in 43. The large negative difference on molecular rotation (-266°) between the bromo alcohol 43 and 12-bromo-olean-12-en-3β-yl-acetate[5] 46 suggested the 11α-configuration for the hydroxyl group, which is supported by the

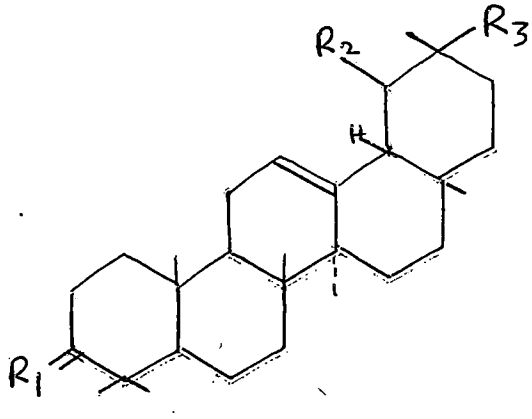
magnitude of 9α-H, 11-H coupling constant (7.8 Hz) for the 11α-ol.

The minor component of the mixture of bromo compounds was an α,β-unsaturated ketone ($C_{32}H_{49}O_3Br$) 58, λ_{max} 244 mμ. The NMR spectrum of 58 showed that the double bond was trisubstituted (τ 4.10, (1H, S) and that the bromine atom was secondary τ 5.3 (HC.Br) . Dehydrobromination of 58 yielded a dienone 66, in which the new double was not conjugated. It would not be brought into conjugation by treatment with base. NMR of 66 showed the presence of an isolated double bond and this double bond was disubstituted (τ 4.14, 2H, m). The structures 58 and 66 were consistent with the observed spectroscopic properties and were also mechanistically acceptable. Thus the initial

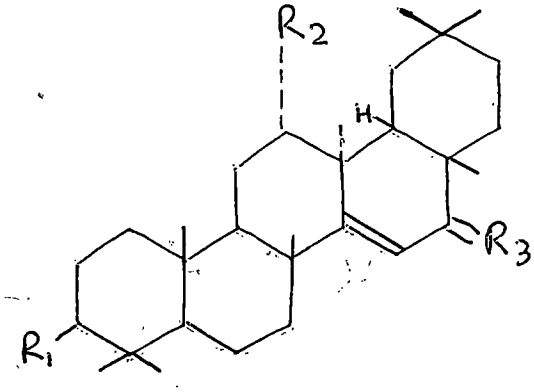


α-face attack on β-amyrin acetate 38 at C-12 would lead to a carbonium ion 63. Elimination of a proton from C-12, followed by allylic hydroxylation would then lead to 43. Alternatively migration of 14α-methyl group to C-13, elimination of a proton from C-15, and subsequent allylic oxidation would give the 12α-bromo-16-one 58.

In the NMR spectrum of the bromoketone 58 HCBr signal overlapped the 3α-H multiplet. However in the spectrum of 12α-bromotaraxer-14-en-16-one 60 which was obtained from 50 in a reaction similar to that described for β-amyrin acetate, the HCBr signal was a triplet (J, 8.1 Hz). The result was compatible with the proposed



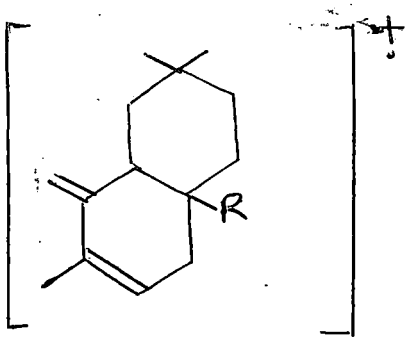
50 $R_1 = H_2$, $R_2 = H$, $R_3 = Me$
51 $R_1 = H_2$, $R_2 = Me$, $R_3 = H$



58 $R_1 = OAc$, $R_2 = Br$, $R_3 = O$
59 $R_1 = OAc$, $R_2 = H$, $R_3 = H_2$
60 $R_1 = H$, $R_2 = Br$, $R_3 = O$
61 $R_1 = OAc$, $R_2 = H$, $R_3 = H$
62 $R_1 = OAc$, $R_2 = H$, $R_3 = \beta\text{-}OH, H$

12 β-H configuration in the ring C-boat of taraxerene skeleton when the effect of the substituent on the vicinal coupling constant was considered[38].

The most characteristics feature of the mass spectra of taraxer-14-enes are the D/E ring fragments 47 and the retro-Diels Alder fragments 48[39]. The presence of an 11, 12-double bond in dehydrobromination product 66 was expected to prevent formation of 47. The mass spectrum of the dienone 66 showed no fragment corresponding to D/E



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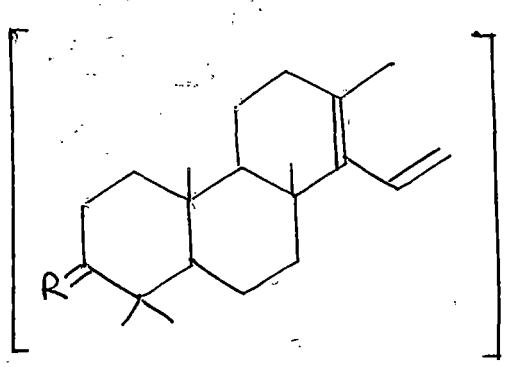

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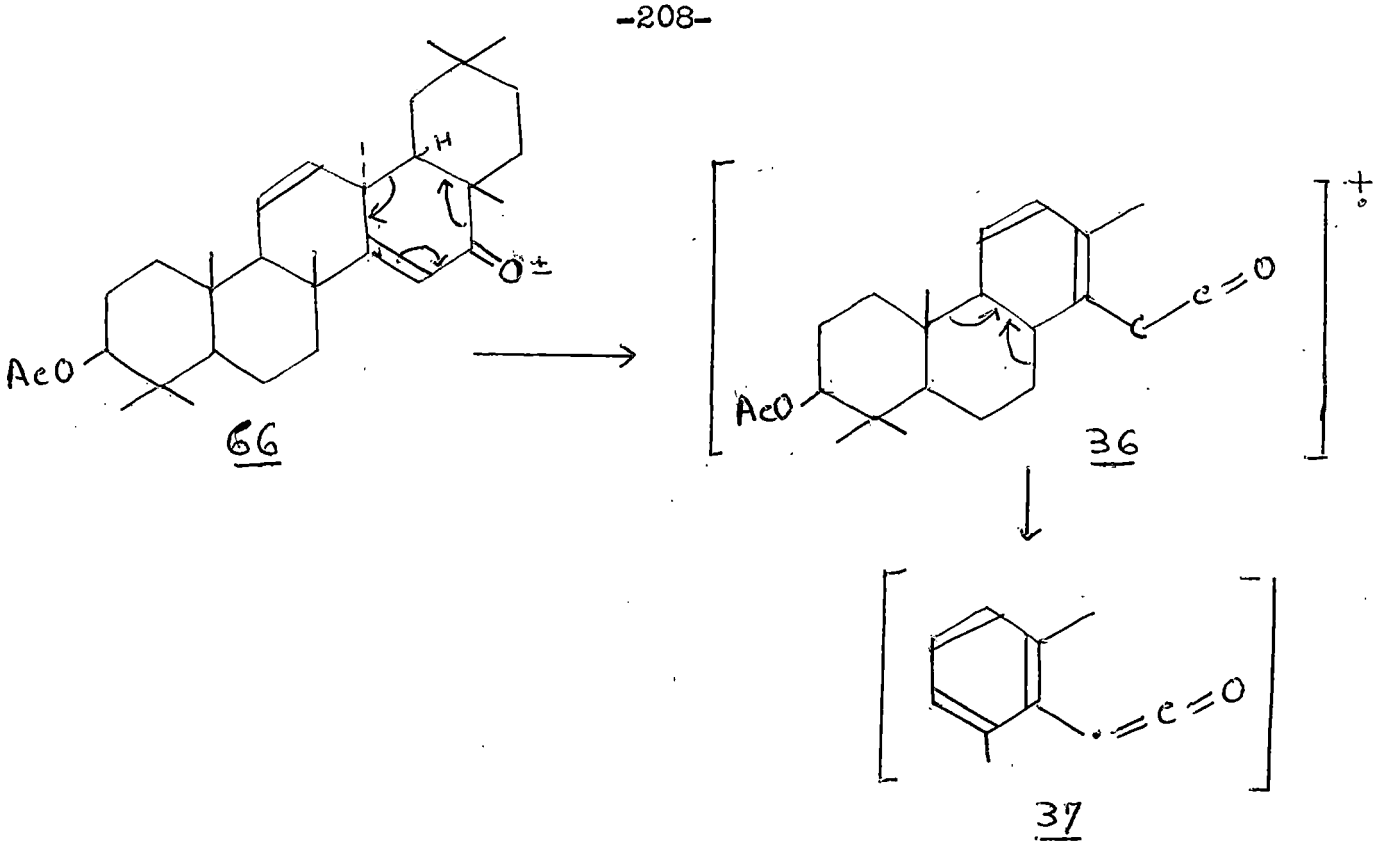
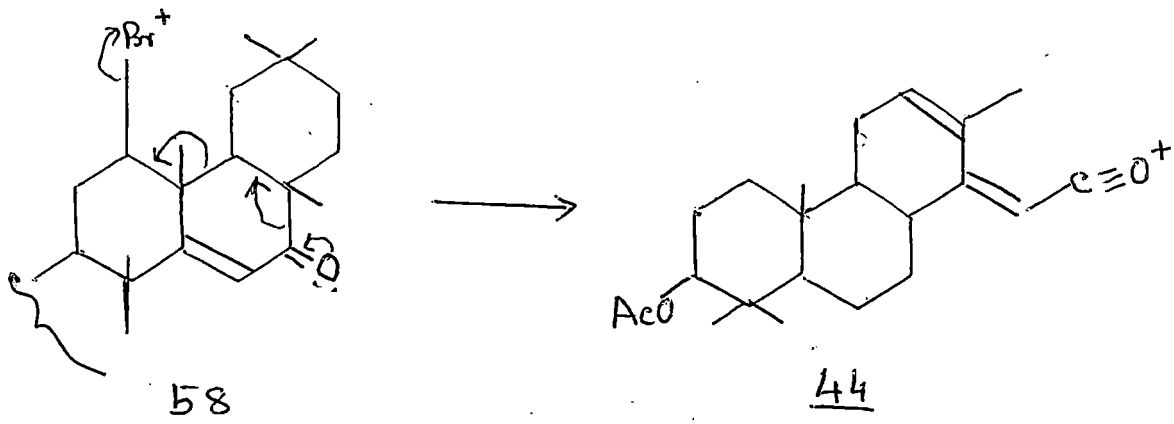
ring species 47 and only two abundant ions viz m/e 356 ($C_{22}H_{32}O_3$), 36, and 148 were prominent. High resolution measurements confirmed the constitution of m/e 356 fragment and showed that the species of m/e 148, of equal abundance was $C_{10}H_{10}O$ to which might be plausibly ascribed the aromatic ketene structure 37.



66 36 37

In the spectrum of the bromoketone 58 a D/E ring fragment was absent and the expected even electron species 44 was the most abundant ion.



58 44

Thomson et al also carried out oxidation of taraxeryl acetate 59 by the method of Corsano et al[35] and obtained two major products to

which they assigned 16-oxo taraxeryl acetate structure 61 (Ca 30%) and 16β-hydroxy taraxeryl acetate structure 62 (Ca 30%). Treatment of 62 with chromic acid in acetone gave the unsaturated ketone 61. They suggested a 16-β-configuration for the hydroxyl group from the study of the Drieding model. The 15-H signal in the NMR spectrum of taraxeryl acetate 59 appeared as a quartet (J_1-4, J_2-8 Hz) whereas in 62 as a doublet (J 4Hz). A drieding model of tarax-14-ene showed 15-H, 16-H dihedral angles to be Ca90° (16α-H) and Ca 30° (16β-H), thus the expected[40] vicinal coupling constants are very small and Ca 8Hz respectively. The increased magnitude of the smaller coupling constant may be attributed to efficient overlap of the π-orbital with the axial 16α-H bond. The appearance of the 17β-methyl signal as a doublet (J1 Hz) in the NMR spectrum was also taken as the confirmation for the α-axial configuration for the 16-H atom.

These authors reported that when solutions of α-34 or β-38 amyrin acetate in aqueous dioxan containing NBS were irradiated with visible light the corresponding 11-ketones 35 or 39 were formed in essential quantitative yield. Similar high yields of αβ-unsaturated ketones were claimed from olean-12-ene 50, urs-12-ene 51, and taraxeryl acetate 59.

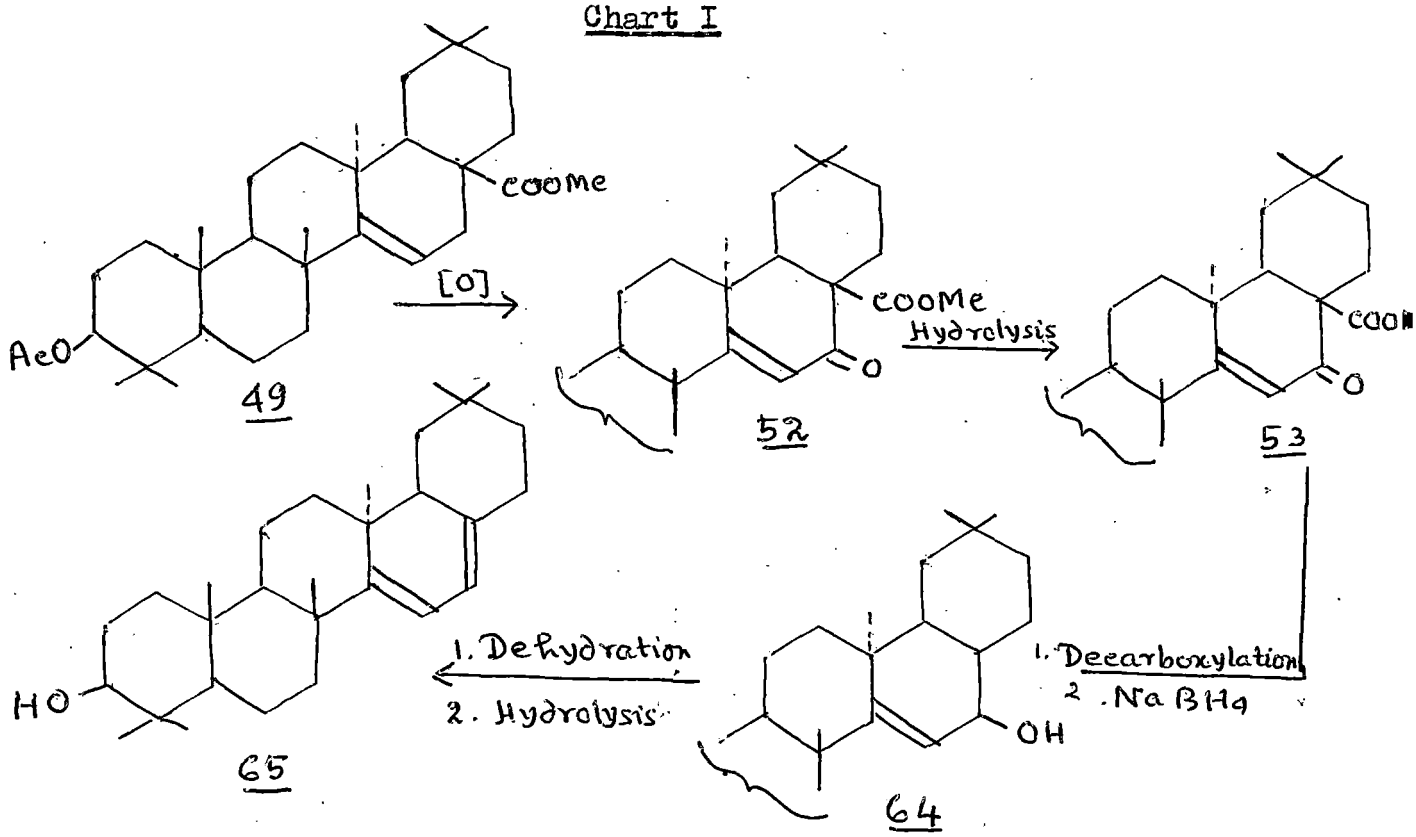
CHAPTER - II

Allylic Oxidation and bromination studies with NBS on taraxeryl acetate:

Section A: Introduction

In connection with the structure elucidation of another triterpenoid we required the compound 3β-hydroxy-28-nor-olean-14, 16-diene 65. We had planned to prepare the desired compound according to the sequence shown below. (Chart I), starting from acetyl methyl aleuritolate[41] 49. Allylic oxidation of 49 according to the method of Finucane and co-workers[34,42] would be expected to give the 16-oxo derivative 52, which on hydrolysis would furnish the β-keto acid 53. The latter on decarboxylation followed by sodium borohydride reduction would give 64, which on dehydration would afford the intended product 65.

Chart I



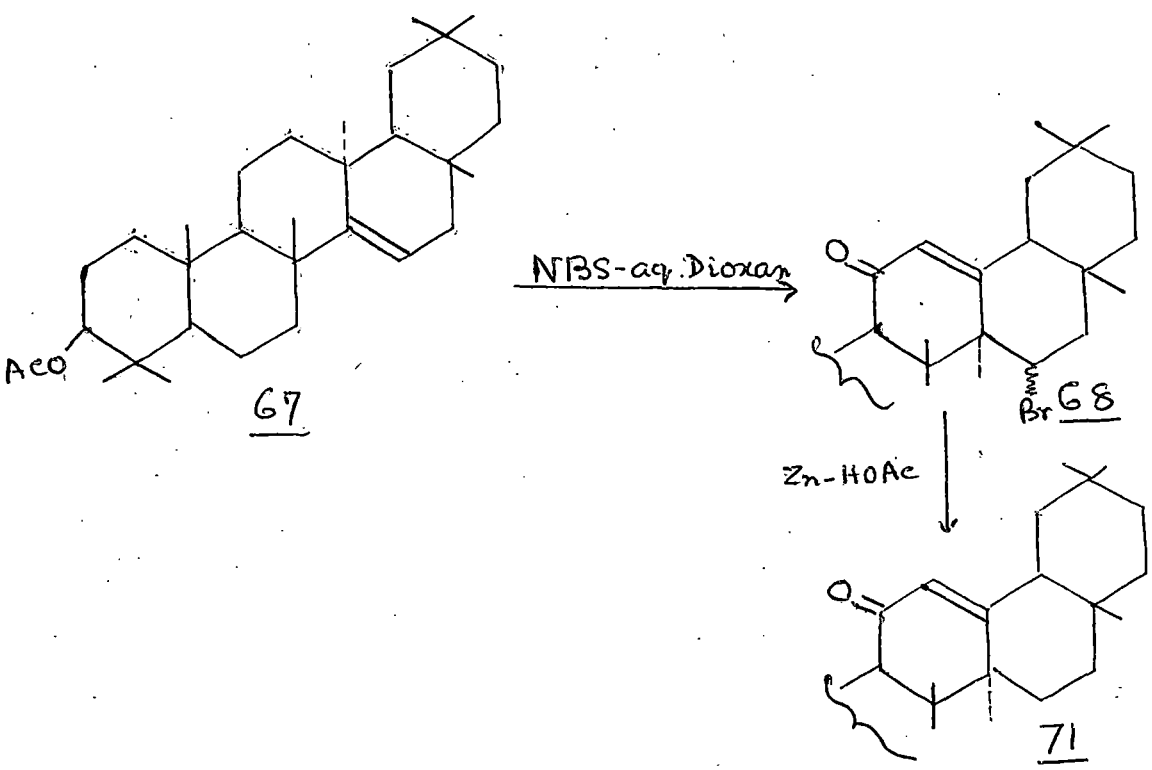
Finucane and Thomson[34,42] recently described a method for allylic oxidation of taraxeryl acetate, β-amyrin acetate etc. using NBS-$CaCO_3$ in aqueous dioxan in presence of visible light and claimed the isolation of the corresponding αβ-unsaturated ketones in high yield. Being encouraged by their results we also undertook a plan for the synthesis of 16-oxygenated β-amyrin derivatives starting from taraxeryl acetate. We planned to prepare 16-oxo taraxeryl acetate by following their procedure and then to reduce the 16-oxo compound to the 16-hydroxy derivative by a suitable method. Acid isomerisation of the latter was expected to give 16-hydroxy β-amyrin derivatives. In view of our intended plan discussed above, we first of all undertook the oxidation study on taraxeryl acetate. The results of our oxidation, was, however, widely different from those recorded by Finucane and Thomson[34,42] and are described in Section B.

Similarly with the expectation of obtaining a 16-bromo taraxeryl acetate we extended our studies on allylic bromination of taraxeryl acetate using NBS in dry carbon tetrachloride in presence of light and benzoyl peroxide as the initiator. The results obtained during these studies were interesting and is also described in Section B.

Section:B : Allylic oxidation and bromination studies with NBS on taraxeryl acetate:

Taraxeryl acetate 67 on oxidation[34] with NBS in aqueous dioxan for 5.5 hr in presence of $CaCO_3$ in visible light gave a mixture of compounds which on TLC examination showed the presence of at least

three compounds (3 distinct spots). Three compounds were separated by chromatography over alumina followed by crystallisation. The first solid 68, $C_{32}H_{49}O_3Br$, m.p. 238-40°, $(\alpha)_D$ 88.07°, λ_{max} 249.5 mμ (ε, 11,000), ν_{max}^{nujol} 1250, 1650 and 1750 cm^{-1}, indicating the presence of an αβ-unsaturated ketone. The compound also showed positive test for bromine and a single spot on a chromatoplate. In order to establish its structure, 68 was treated with Zn-dust in acetic acid and a halogen free compound 71, m.p. 265-70°, $(\alpha)_D$ 48.8°, λ_{max} 252.5 mμ (ε, 11,000) was isolated and was found to be identical with an authentic sample of β-amyrenonyl acetate[43] (m.m.p. and IR comparison) prepared

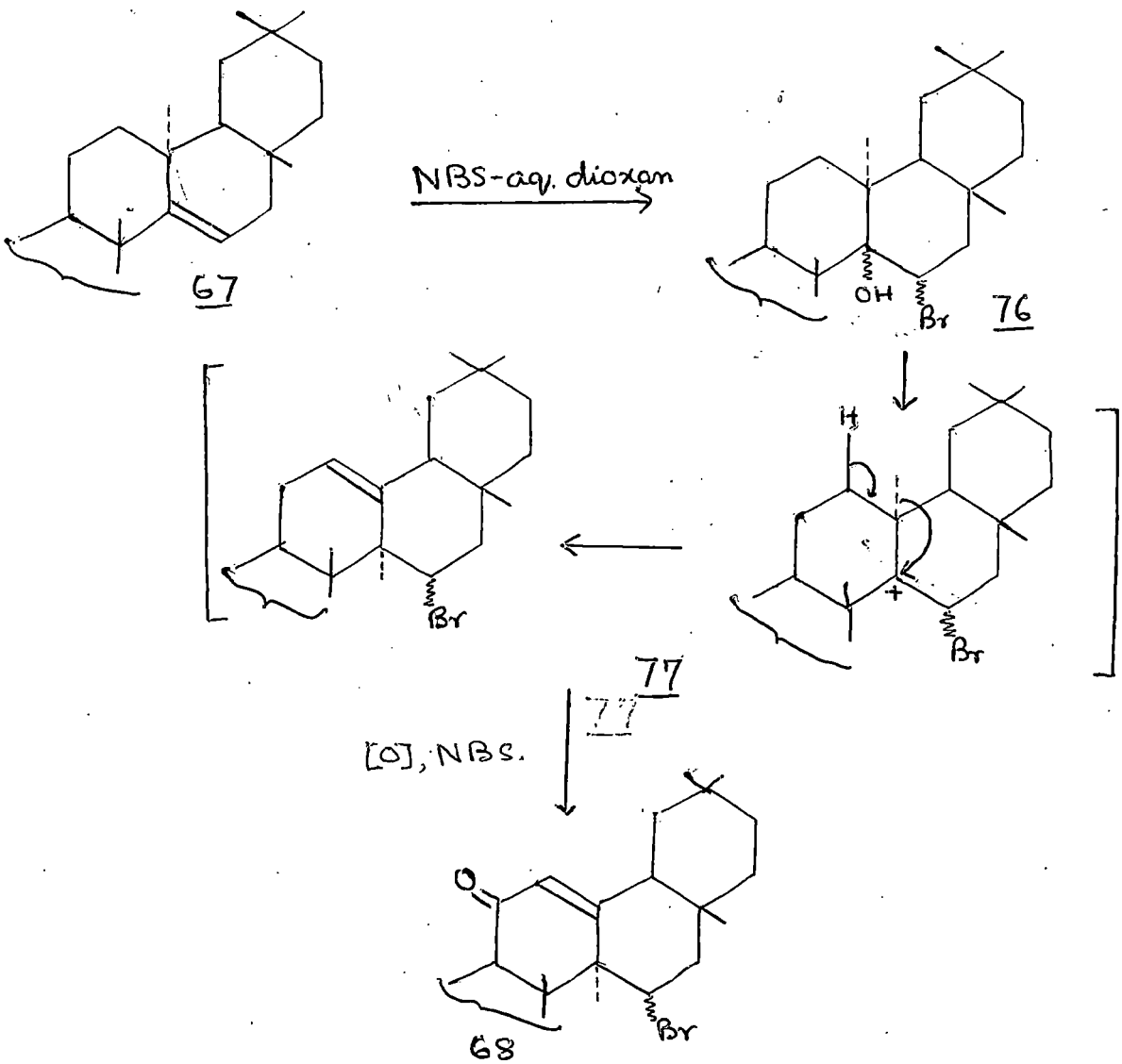


67 68 71

by oxidation of β-amyrin acetate with CrO_3 in acetic acid at 130°. This result indicated that the bromo compound was probably 11-keto-

15-bromo-β-amyrin which on treatment with Zn-acetic acid would give 71 by the removal of the bromine atom. The formation of 68 from taraxeryl acetate could be rationalised by the following mechanism (Chart II).

Chart II



The bromohydrin 76 is first formed which could easily isomerise to the β-amyrin derivative 77 through the carbonium ion intermediate

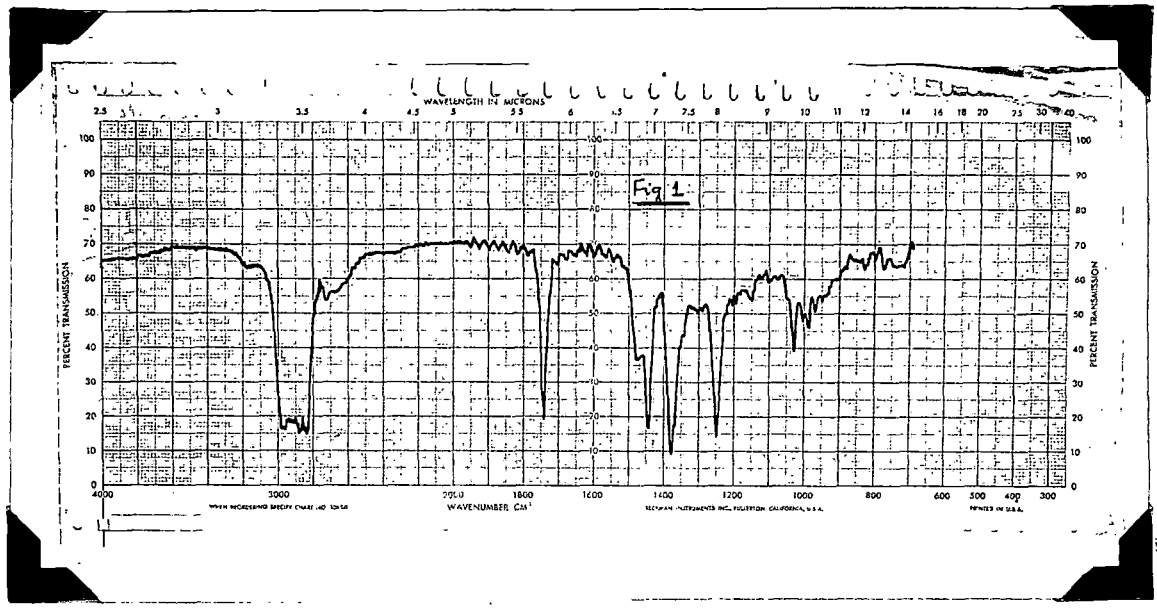


Fig. 1. IR spectrum of 15-bromo-β-amyrin acetate 72

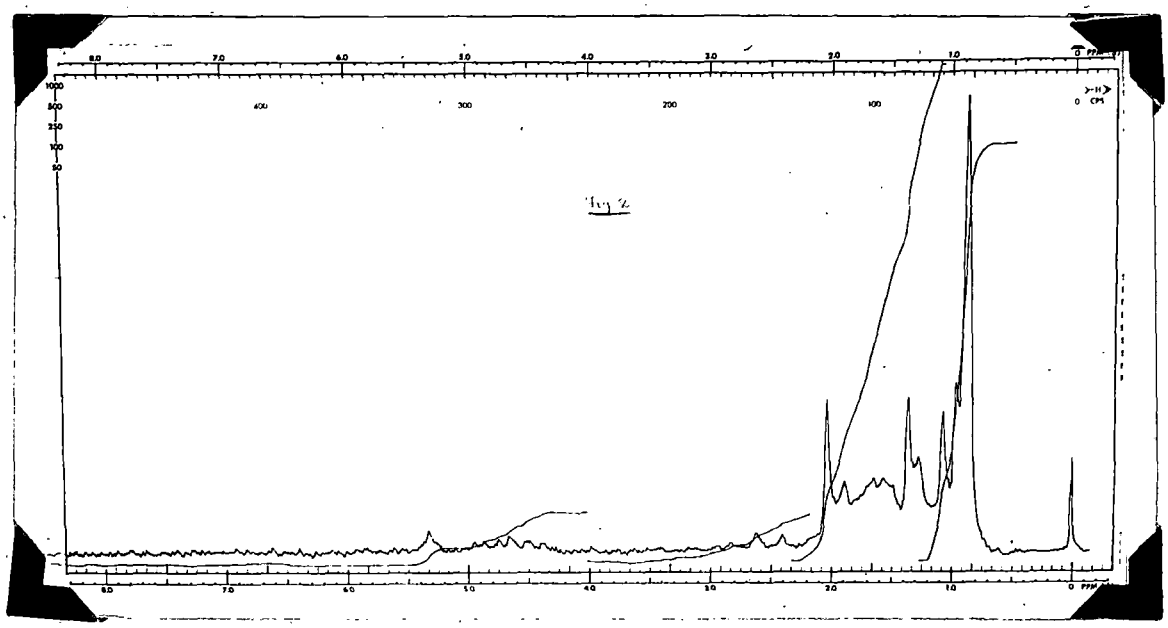
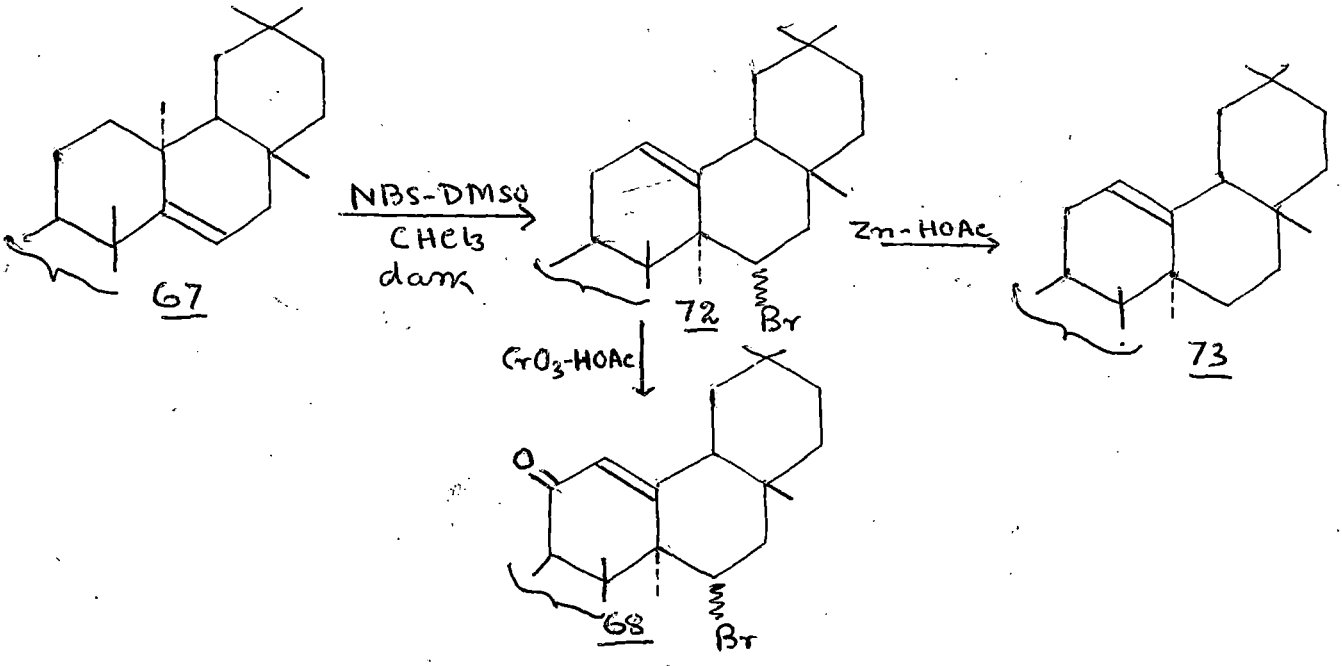


Fig.2. NMR spectrum of 15-bromo-β-amyrin acetate 72

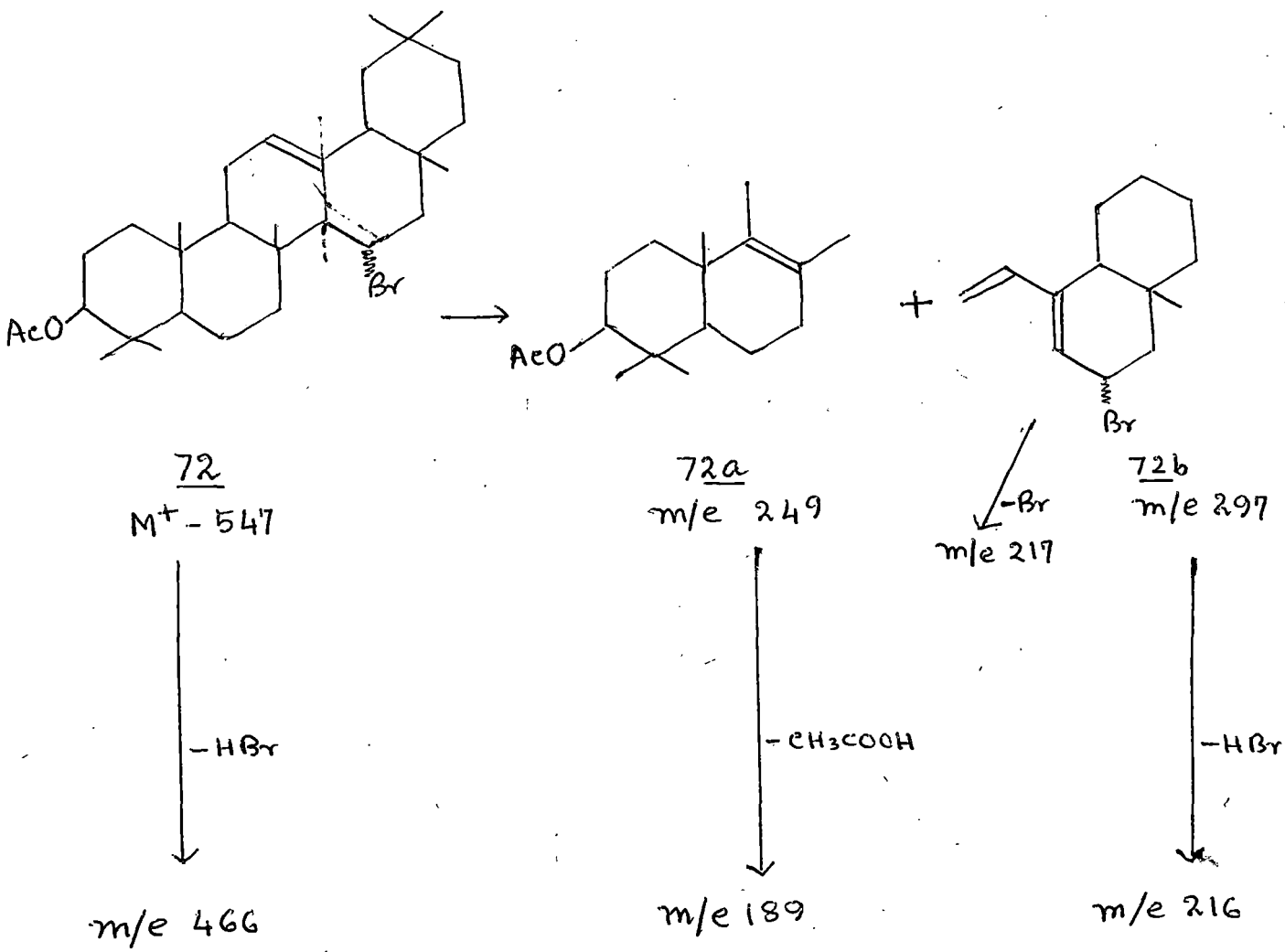
followed by methyl migration. The intermediate 77 then gets oxidised to the $\alpha\beta$-unsaturated ketone 68.

In order to prove our contention we looked for a method for the preparation of the 15-bromo compound 77 which on oxidation by established method would give 68. We tried to prepare the bromohydrin at the 14-15 double bond of taraxeryl acetate by the method of Dalton[44] using NBS in DMSO solvent[45]. Treatment of taraxeryl acetate with aqueous dimethyl sulfoxide in chloroform and NBS in dark afforded a solid 72, $C_{32}H_{51}O_2Br$, m.p. 180-2°, $(\alpha)_D$ 47.37°. The compound did not show any UV absorption between 220-330 mμ. IR spectrum of the compound (Fig.1) showed peaks at 1720 and 1250 ($-O.CO.CH_3$) cm^{-1}. NMR spectrum (Fig. 2) showed a multiplet centered at 5.3 ppm (vinyl proton) and a multiplet at 4.30 ppm for one proton attached to a carbon containing bromine and also peaks at 2.09 ppm ($-O.COCH_3$) and at 4.53 ppm for proton attached to the carbon bearing acetoxy group. All these physical evidences coupled with mass fragmentation pattern confirmed the structure depicted in 72 . The compound showed a mass



peak M^+548 (isotope) (Fig. 3) and peaks at 249 (72a) and 297 (72b) arising by retro-Diels-Alder Cleavage of β-amyrin skeleton[39]. The ion 72b gives a prominent peak at m/e 217 by the loss of bromine atom and at m/e 216 by the loss of HBr. There is also peak at m/e 189 arising by the elimination of the acetic acid from 72a. There was also a prominent peak at 466 (M^+ -HBr). The other fragmentation pattern is similar to those shown by β-amyrin acetate. These fragments are shown below (Chart III).

Chart III



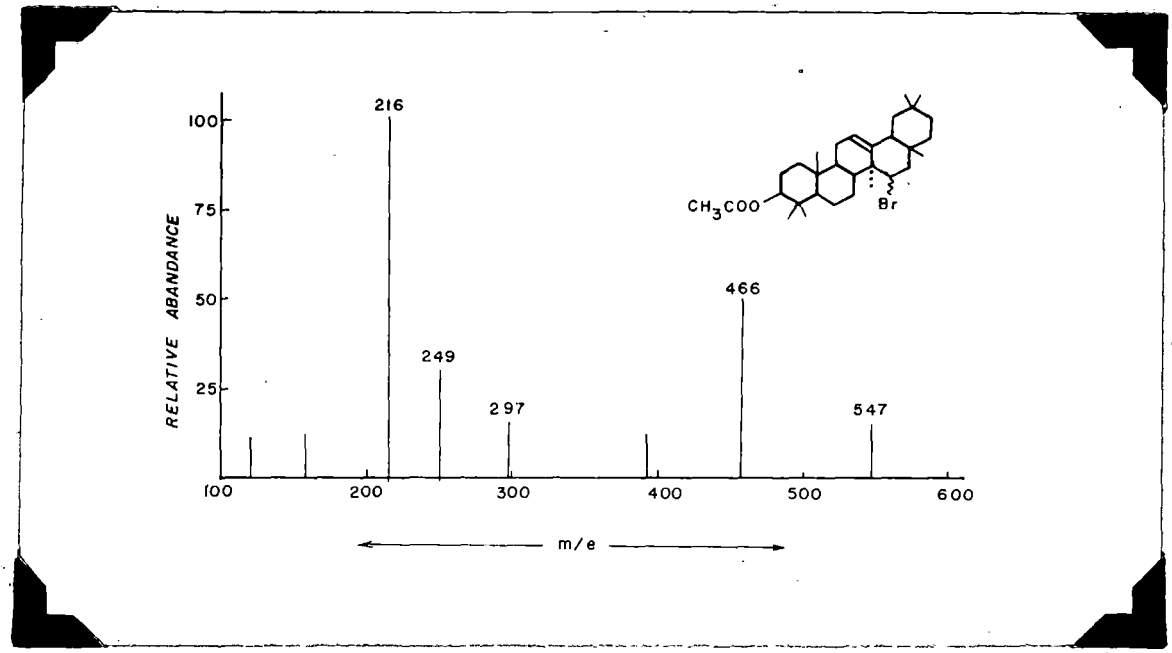


Fig. 3 Mass spectrum of 15 bromo-β-amyrin acetate 72

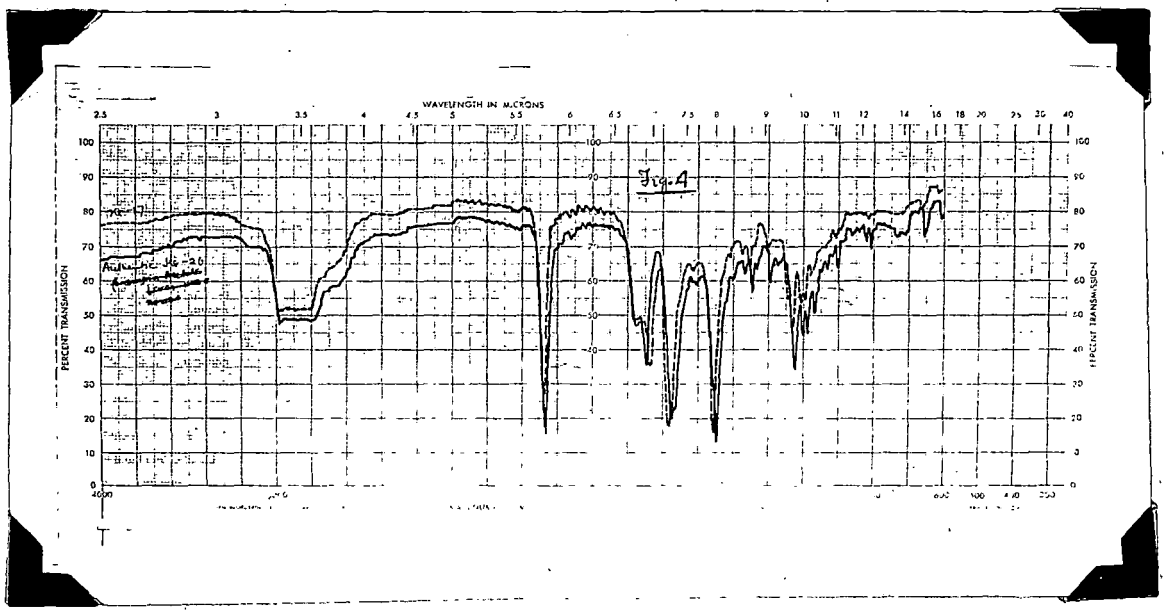
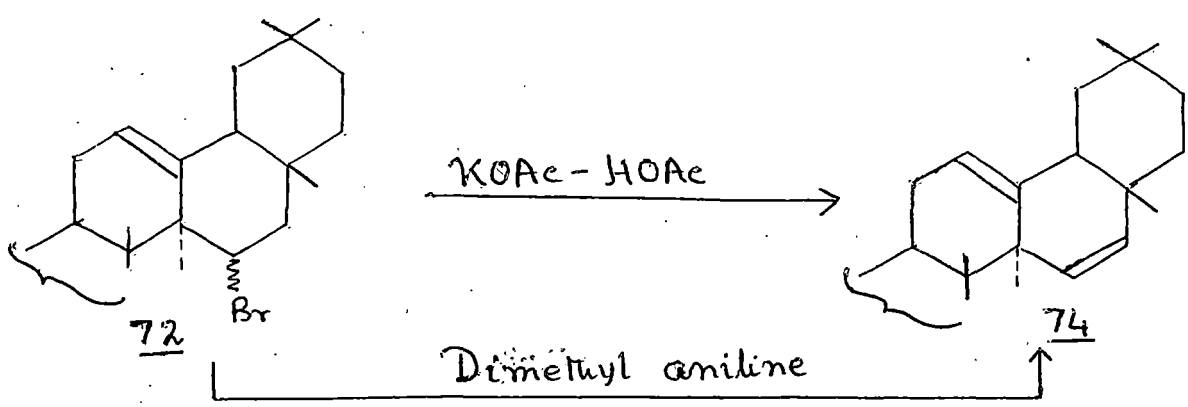


Fig. 4 IR comparison of
β-amyrin acetate 73 obtained from (broken line) 72
authentic β-amyrin acetate (solid line)

The compound 72 on treatment with zinc-acetic acid yielded a crystalline solid 73, m.p. 230-31°, $(\alpha)_D$ 85.1°, identical with an authentic sample of β-amyrin acetate (m.m.p. and IR comparison Fig. 4). The bromine atom at 15 position would be expected to have the same stereochemistry as in the product from NBS-aq. diaxan oxidation method. Compound 72 on oxidation with chromium trioxide-acetic acid[43] gave 68, m.p. 238-40° identical with the product obtained from NBS-aq. dioxan oxidation method (m.m.p and IR comparison Fig. 5). Thus the structure 72 predicted is established beyond doubt.

Solvolysis of compound 72 with K-acetate in acetic acid at 130° for four hours gave a product $C_{32}H_{50}O_2$, m.p. 199-200°, $(\alpha)_D$ 41.86°, no absorption in the UV region 220-300 mμ. NMR spectrum (Fig. 6). of this compound showed signals at 5.2 to 5.6 ppm for three vinyl protons accounting for one trisubstituted and one disubstituted double bond. From the foregoing evidences and the analytical data we assign structure 74 to this compound.



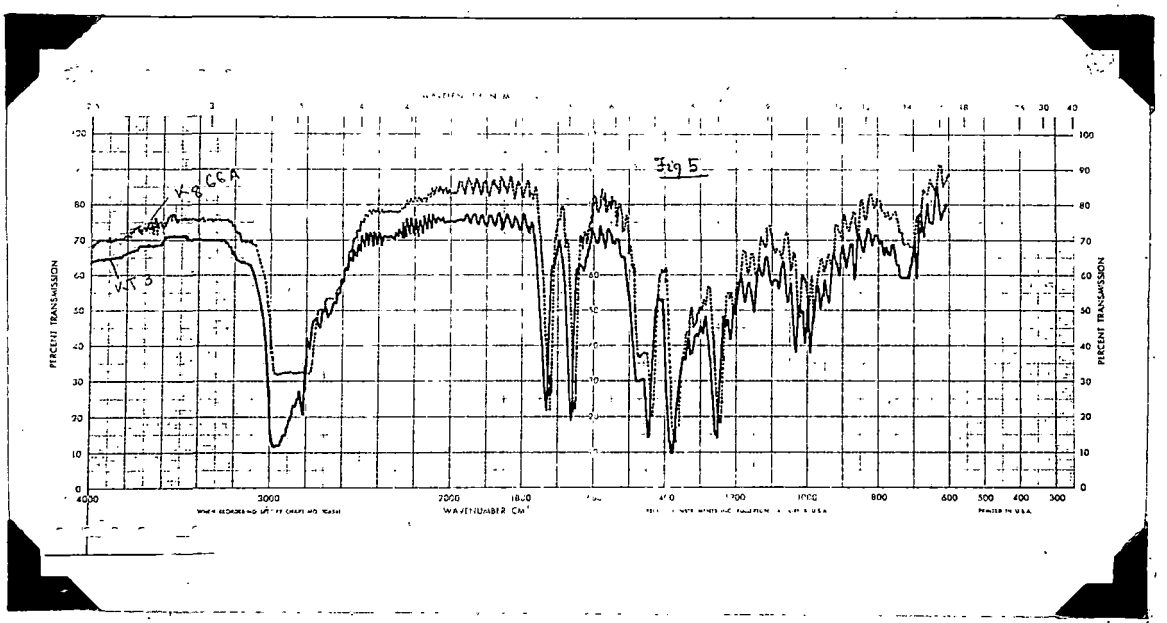


Fig. 5 IR comparison of

15 bromo β -amyrenonyl acetate 68 from taraxeryl acetate

15 bromo β -amyrenonyl acetate 68 from CrO_3-HOAc oxidation of 72

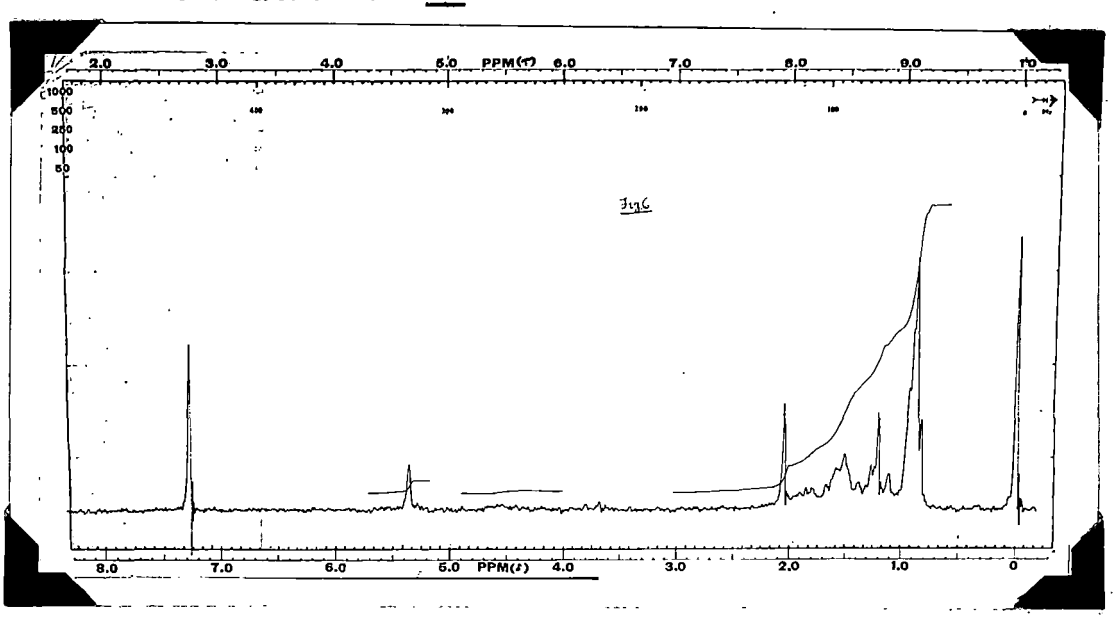
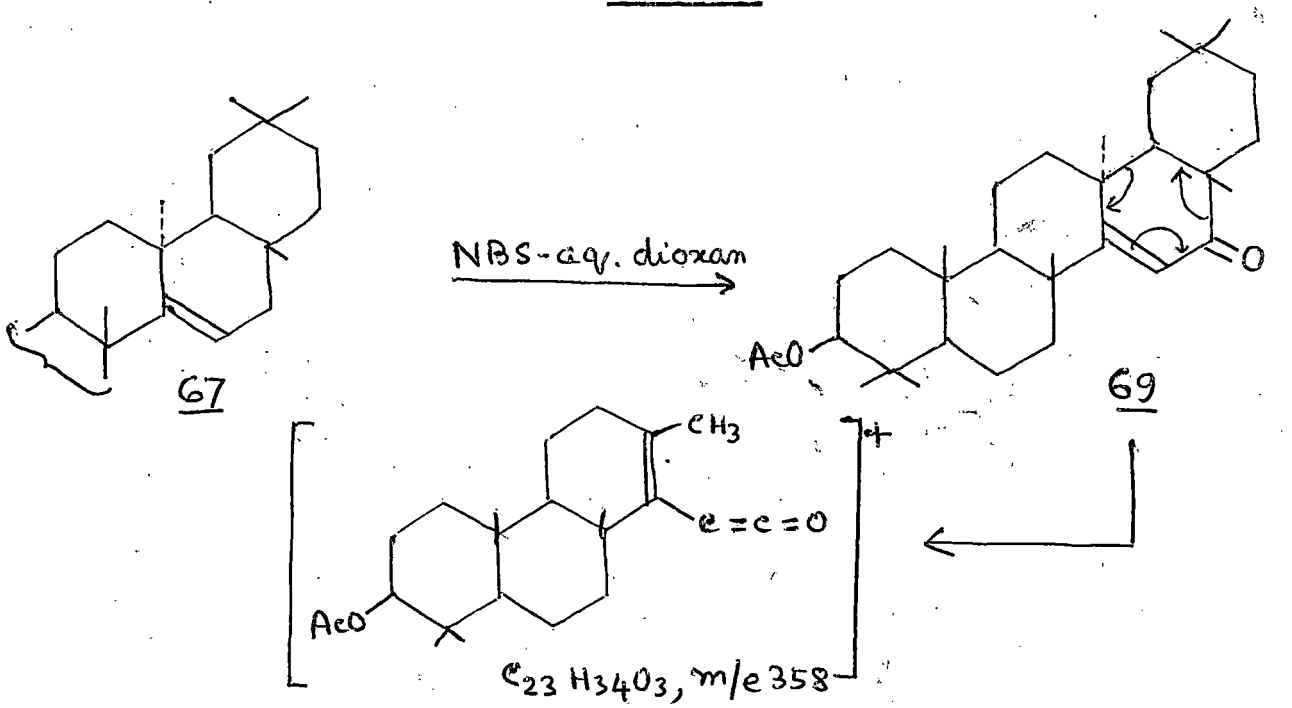


Fig. 6 NMR spectrum of olean-12, 15-dien 3β -yl acetate 74

Compound 72 on refluxing with dimethyl aniline for 4 hours also gave a compound, m.p. 199-200° which was found to be identical with 74 (m.m.p. and IR comparison Fig. 7).

The second solid 69, $C_{32}H_{50}O_3$, m.p. 280-82°, $(\alpha)_D$ - 38.71° isolated from the reaction was devoid of bromine. The compound showed UV maximum at 245 mμ (ε ,10,500), IR peaks at 1730, 1680, 1250 cm^{-1} (Fig. 8) indicating the presence of αβ-unsaturated ketone. NMR spectrum of 69 (Fig 9) showed a peak at 5.85 ppm for the vinyl proton. This low field singlet for the vinyl proton may be ascribed to the presence of the electronegative oxygen atom at C-16. In addition to this the compound also showed peaks at 2.10 (-O.CO.CH_3) and 4.5 (-C<u>H</u>-O-CO-CH_3) ppm. The mass spectrum of the compound (Fig. 10) showed mass peak at 482. Moreover, an abundant base peak at 358 was observed. The appearance of this peak may be explained by assuming that it arises from a 16-oxo taraxeryl acetate system by the following genesis. (Chart IV).

Chart IV



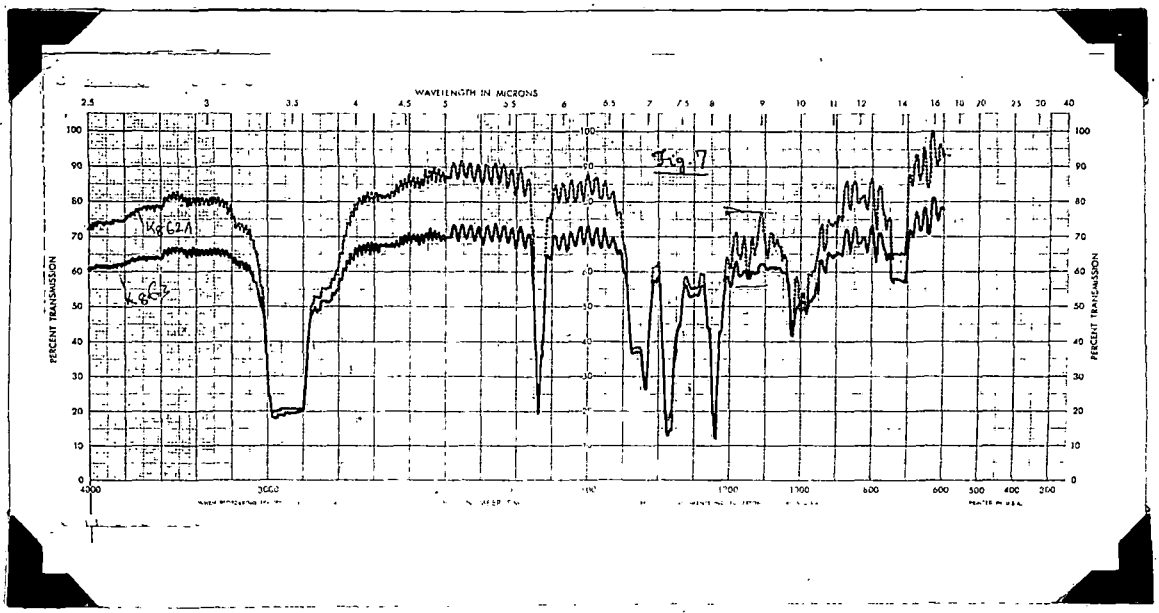


Fig. 7. IR comparison of

Olean-12,15-dien-3β-yl acetate obtained ✗ from solvolysis of 72 (dotted line)

Olean 12,15-dien-3β-yl acetate obtained from dimethyl aniline treatment of 72 (solid line)

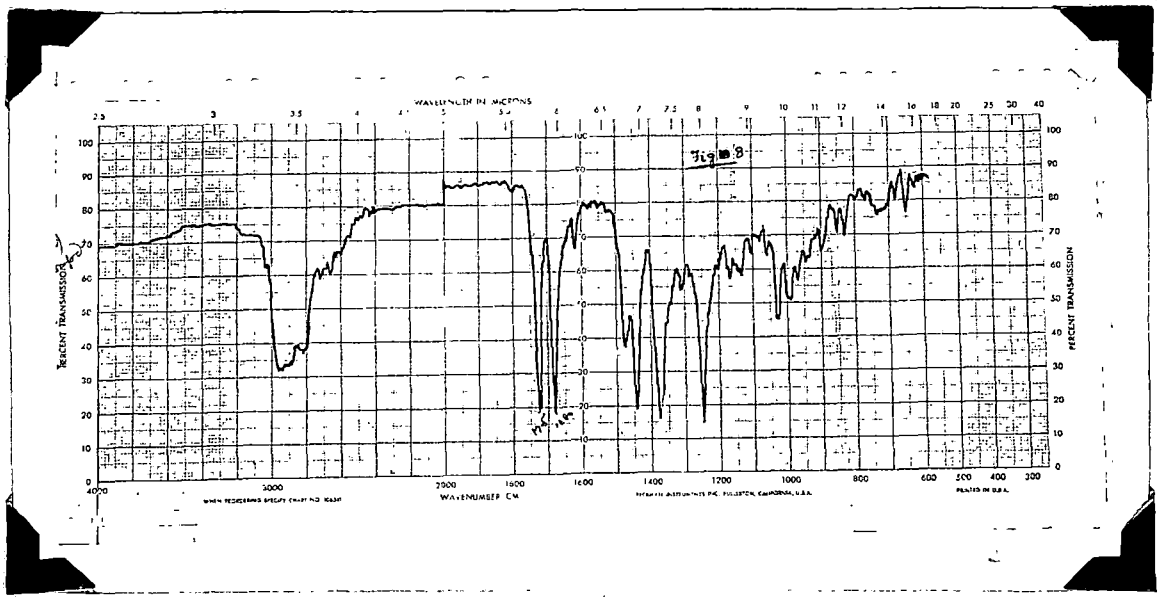


Fig.8.IR spectrum of 16-oxo taraxeryl acetate 69

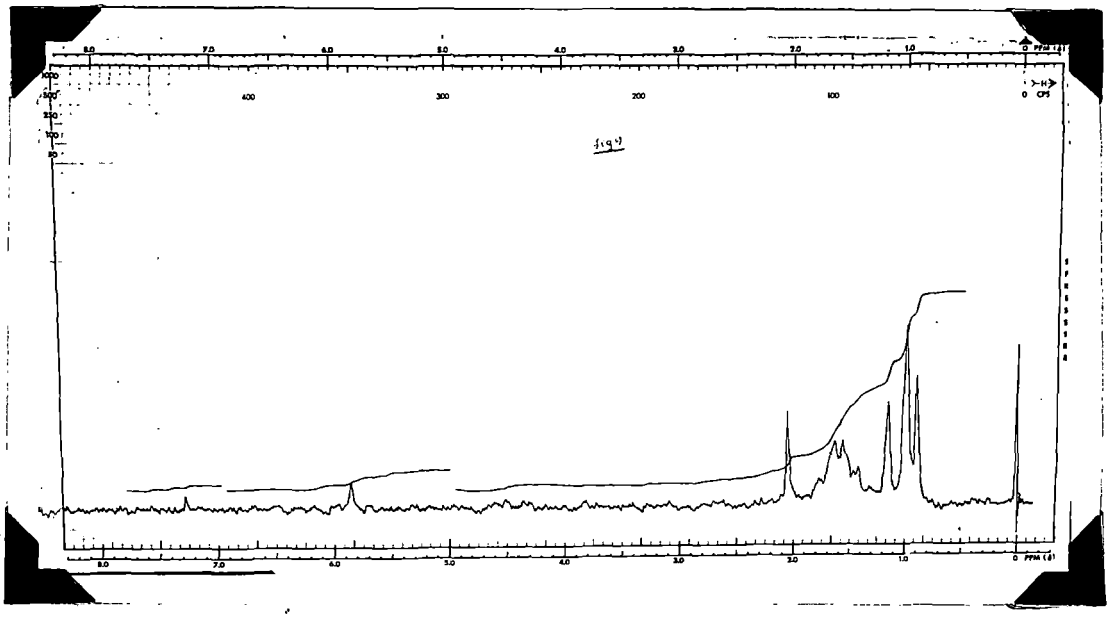


Fig. 9. NMR spectrum of 16-oxo taraxeryl acetate 69

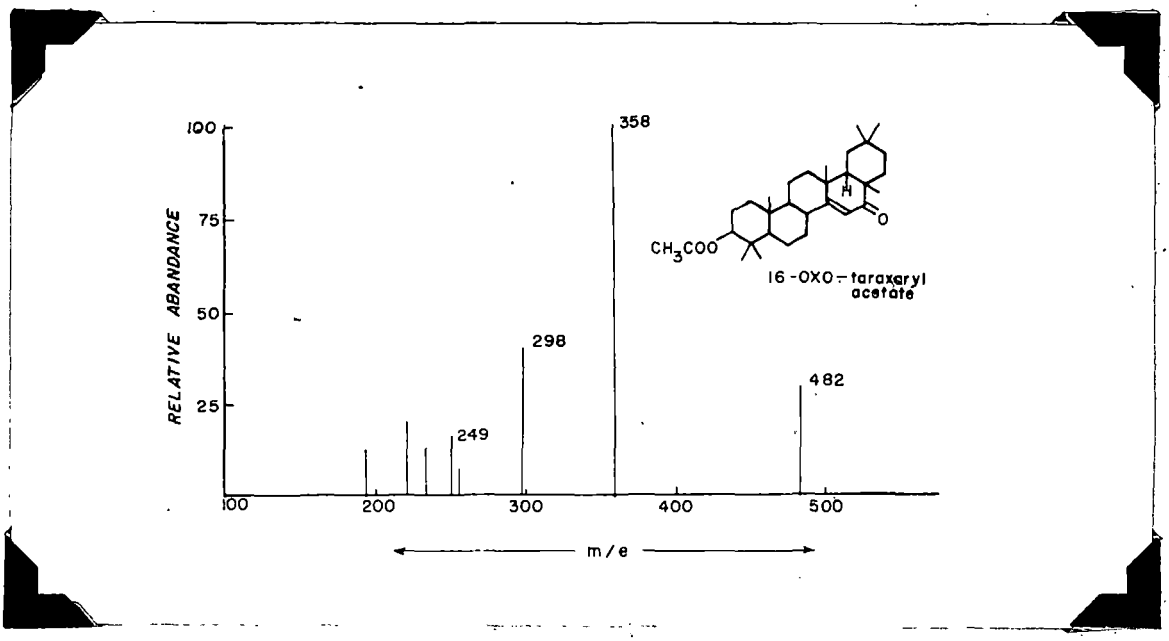


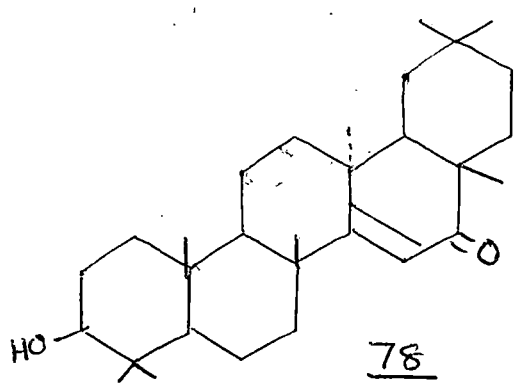
Fig. 10. Mass spectrum of 16-oxo taraxeryl acetate 69.

All the foregoing evidences led us to conclude that the product 69 was 16-oxo taraxeryl acetate although our melting point was widely different from that recorded by Finucane and Thomson[42] (m.p. 251-52° d^{42}, $(\alpha)_D$ 92°). We procured a sample of 16-oxo taraxeryl acetate, m.p. 259-60° d from Dr. Thomson which gave a positive bromine test and was different from our sample. In our hands the yield of the product never exceeded ten percent.

Further, we prepared 11-oxo-β-amyrin acetate[43] 71 from β-amyrin acetate 73 by treatment of the latter with chromium trioxide-acetic acid. The m.p., rotation and IR comparison clearly indicated that compound 69 was different from 11-oxo-β-amyrin acetate (IR comparison is shown in Fig. 11).

All these facts led us to conclude beyond doubt that our product 69, m.p. 280-2° was the correct 16-oxo taraxeryl acetate.

In order to prepare 16-hydroxy taraxeryl acetate, we attempted reduction of 69 with sodium borohydride in tetrahydrofuran and also in methanol. In both the cases, the starting material was recovered. Reduction of the carbonyl group at 16-position was also unsuccessful by Meerwein-Pondorff reduction procedure and produced 16-oxo taraxerol 78 m.p.292-3°, ν_{max}^{KBr} 3360, 1680 cm^{-1}. The same compound 78



78

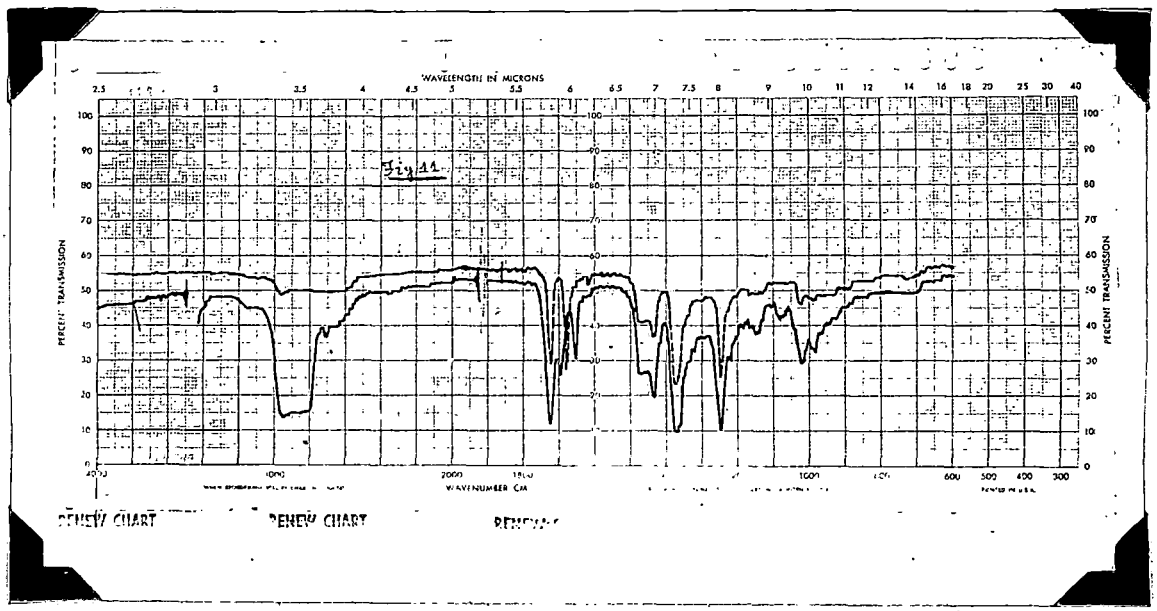


Fig. 11. IR spectra of

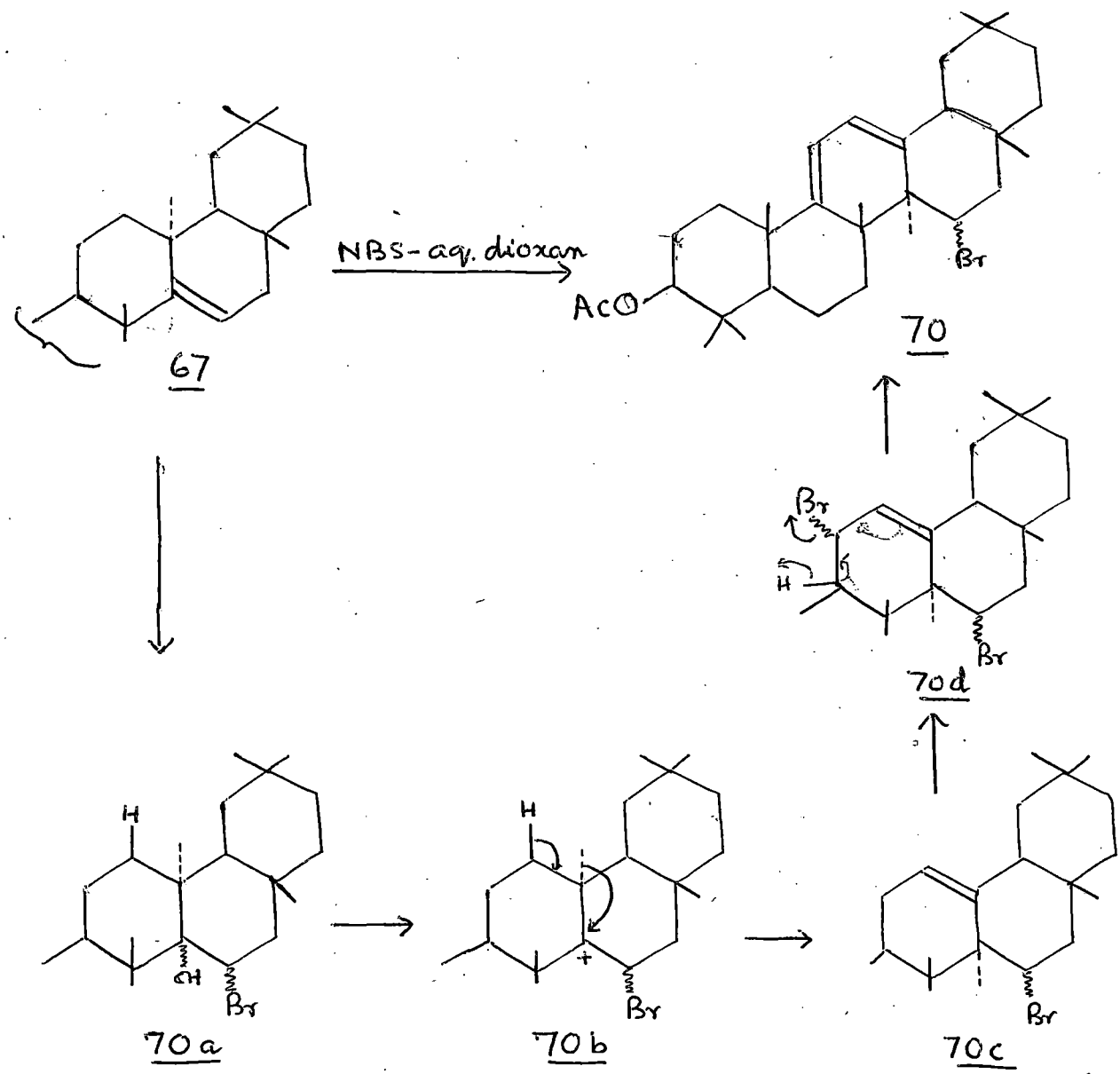
16-oxo taraxeryl acetate 69 (solid line)

11-oxo-β-amyrin acetate 71 (dotted line)

only could be isolated during reduction of 69 with lithium aluminium hydride in tetrahydrofuran solution. Acetylation of 78 with acetic anhydride pyridine gave back 16-oxo taraxeryl acetate (m.m.p. and IR comparison).

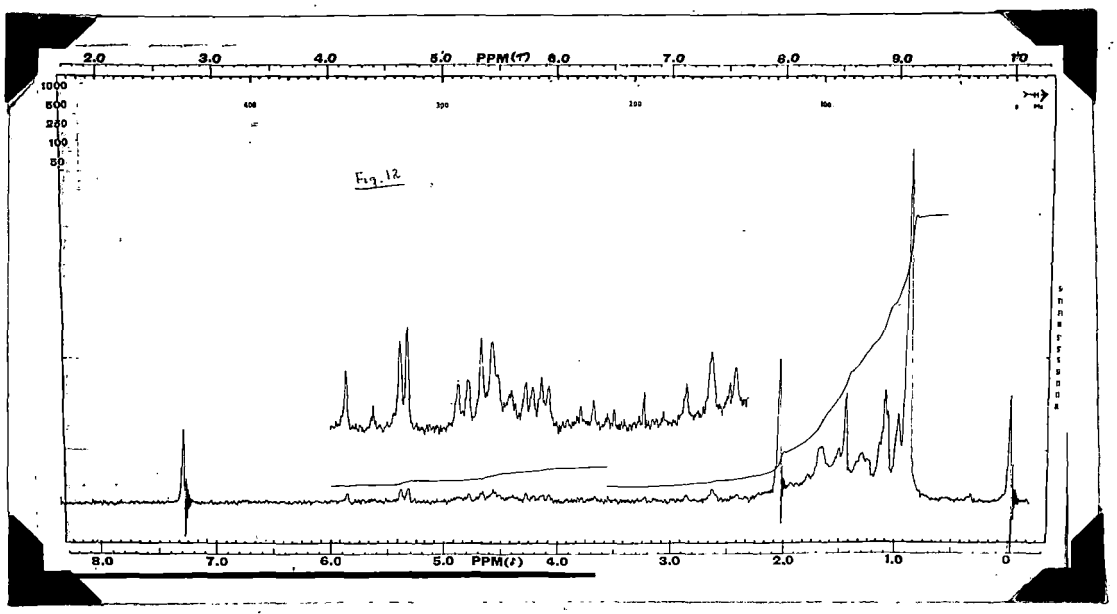
The third product, $C_{32}H_{49}O_2Br$, 70, obtained during chromatography of the mixture of products had m.p. 176-8°, $(\alpha)_D$ 249.12°, λ_{max} 276 mμ (ε, 6000) indicative of a homoannular diene. Examination of the NMR spectrum (Fig. 12) of the compound 70 showed signal at 5.34 ppm for one proton and a one proton signal at 5.85 ppm. These signals may be attributed to the protons in a homoannular diene system in which both the double bonds are trisubstituted. In addition to this the spectrum gave a sharp singlet at 2.08 ppm (-O-CO-CH_3) and a multiplet centered at 4.65 ppm for a proton attached to the carbon bearing the acetoxyl group and a multiplet centered at 4.18 ppm for the proton attached to the carbon bearing the bromine atom. On the basis of the above data the compound is assigned structure 70.

The mechanism for the formation of 70 from 67 can be explained in the following way. Most probably bromohydrin 70a is first formed which isomerises to the β-amyrin derivative 70c through the carbonium ion intermediate 70b followed by methyl migration. The intermediate 70c is further converted to the bromo compound 70d by allylic bromination which on further elimination gives 70.



67 70 70d 70a 70b 70c

The mass spectrum of the compound 70 showed a mass peak at M^+ 546 (weak), but a prominent peak was observed at 465 (m/e- HBr). Other fragments were similar to those of 15-ξ-bromo-9(11), 12-oleandiene. (Fig. 13)



Fir. 12 NMR spectrum of 15-bromo-9(11), 12 Olean-diene70

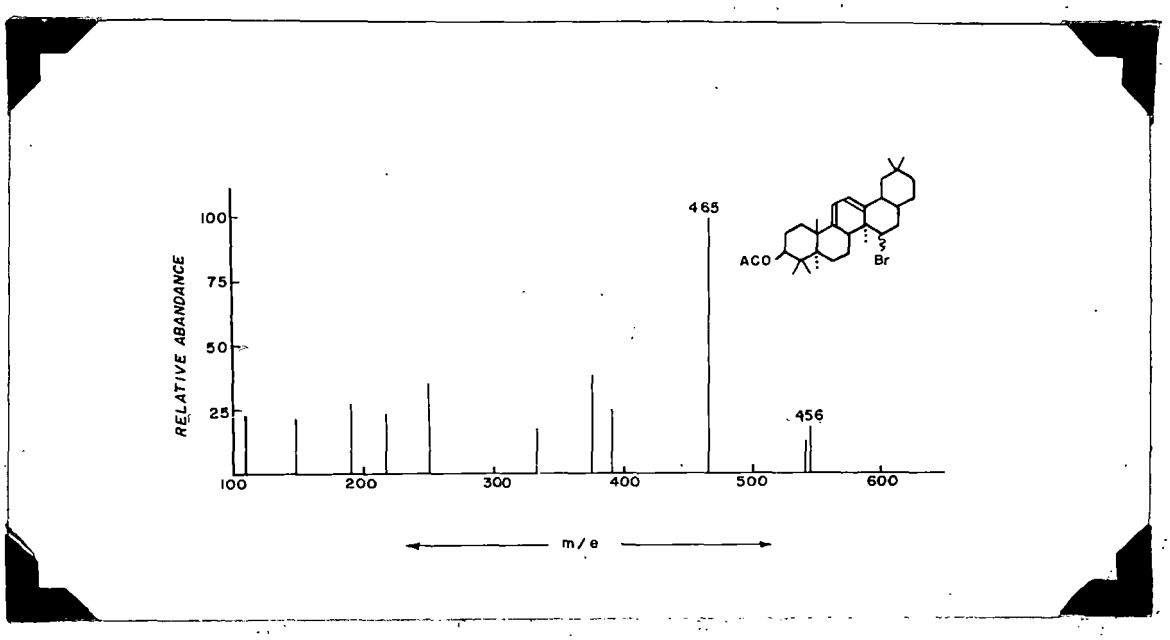


Fig. 13 Mass spectrum of 15-bromo-9(11), 12 olean-diene70

With a view to preparing 16-bromo taraxeryl acetate we studied the allylic bromination of taraxeryl acetate 67 with NBS in dry carbon tetrachloride in presence of light. The results obtained are discussed in the following lines.

When taraxeryl acetate 67 was refluxed with 2 mole equivalents of NBS in carbon tetrachloride using visible light for 3 hours, a product, $C_{32}H_{47}O_2Br$, m.p. 240°, $(\alpha)_D$ 267.53° was obtained. It showed a single spot in a chromatoplate and also gave positive test for bromine. UV spectrum showed a peak at λ_{max} 279 mμ (ϵ, 6000), indicating the presence of a homoannular diene system. IR spectrum (Fig. 14) of the compound showed peaks at 1725, 1250 (-O-CO-CH_3) and 840 (trisubstituted double bond) cm^{-1}. NMR spectrum (Fig. 15) showed the presence of three vinyl protons between 5.63 - 5.88 ppm. This can be explained by assuming that two of the vinyl protons arise due to the presence of a homoannular diene in ring C and the third vinyl proton is present in ring D. In addition to this it also showed a sharp singlet at 2.08 (-O-CO-CH_3), and a multiplet centered at 4.65 for a proton attached to the carbon bearing the acetoxyl group.

The bromine atom in ring D was resistant to reactions (i) Zn-HOAc (ii) H_2/Pd-C (iii) H_2/PtO_2 (iv) Li_2CO_3-LiBr (v) anhydrous KOAc-HOAc (vi) $C_6H_5N(CH_3)_2$. This inertness of the bromine atom in ring D can only be explained if it is assumed that the bromine atom is attached in ring D as a vinyl bromide. In conformity with the above facts we assign structure 75 to this compound. The mass spectrum of the

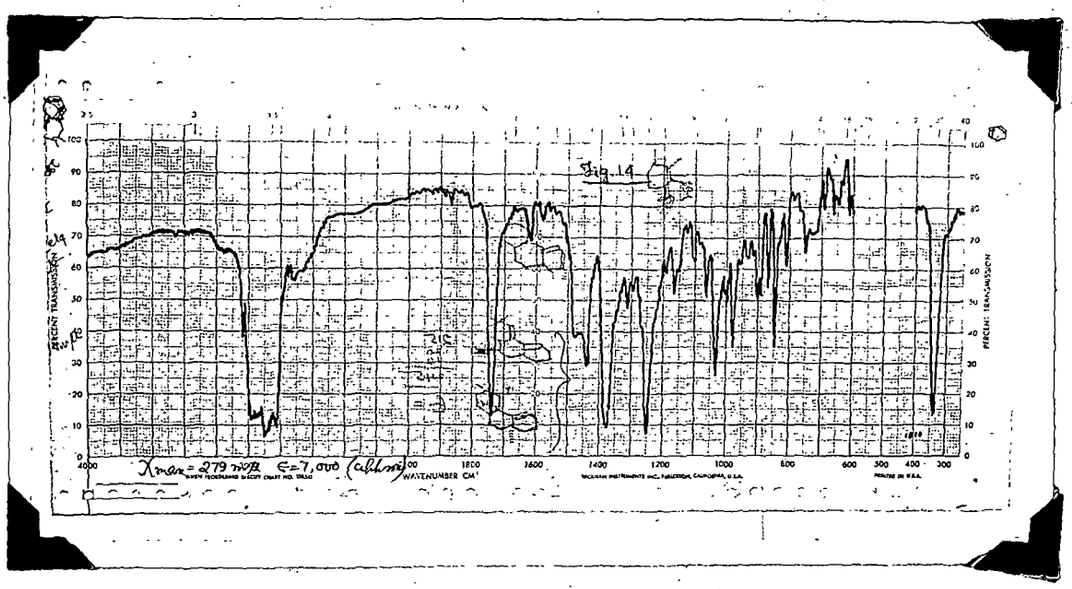


Fig. 14 IR spectrum of olean 9(11), 12,15 trien-3β -yl acetate 75

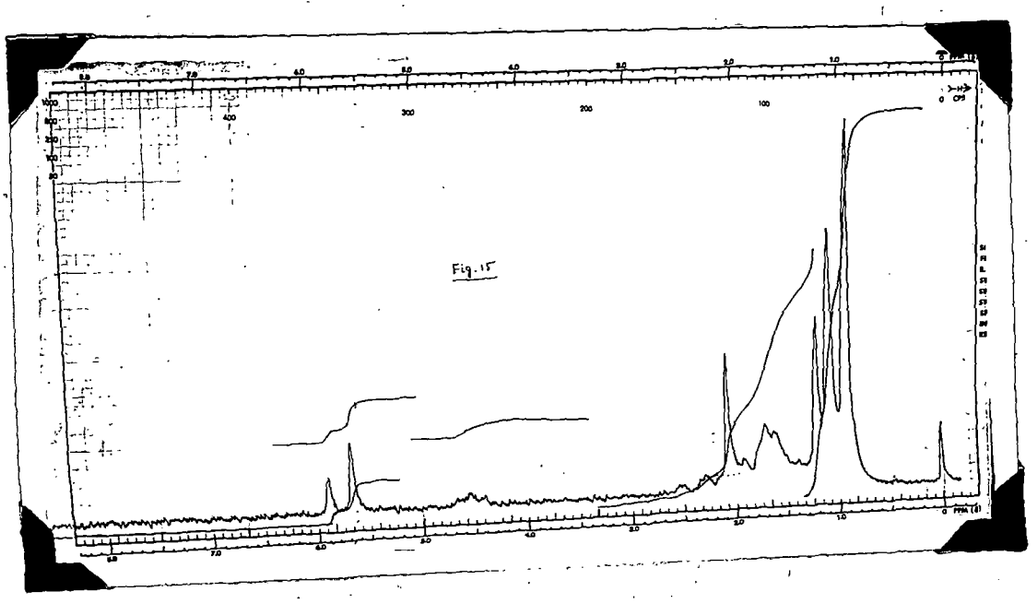
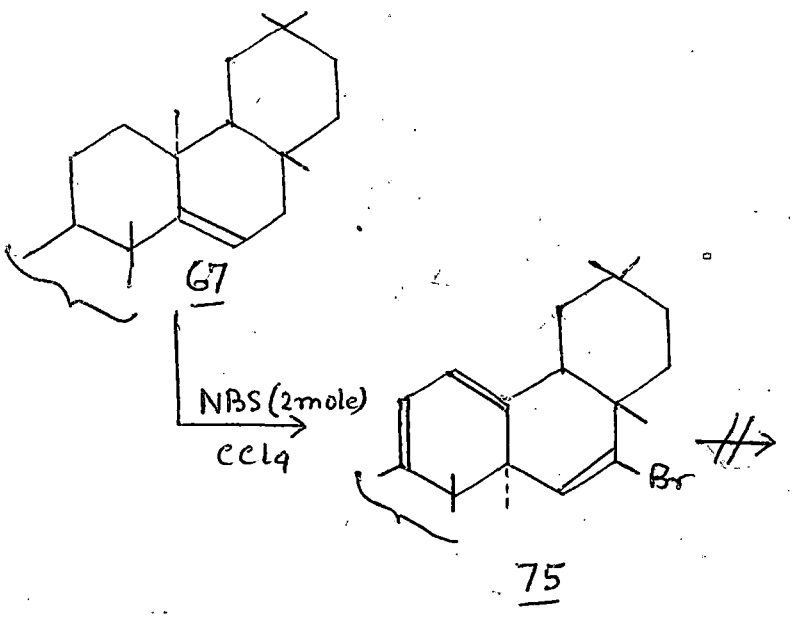


Fig. 15 NMR spectrum of olean, 9(11), 12,15 trien-3β-yl-acetate 75

compound 75 showed the mass peak (strong) at M^+ 542, (544 isotope). The intensity of the peak due to m/e 542 was greater compared to the molecular ion peak at m/e 543 showing that an allylic proton is probably lost initially from the molecular ion to give a peak at m/e 542. (Fig. 16) In another run of the same experiment we isolated the same compound 75 and its mass spectrum (Fig. 17) however, showed a weak peak around the region M 620 - 25 (it was not possible to count) in addition to peaks shown in Figure 16, which indicated that a small contaminant of a dibromo compound was present. This observation gave us a clue to the mechanism of the formation of allylic monobromo compound 75.



Most probably at first allylic 16-dibromo compound 75a is formed which immediately undergoes allylic rearrangement as shown in Chart V below

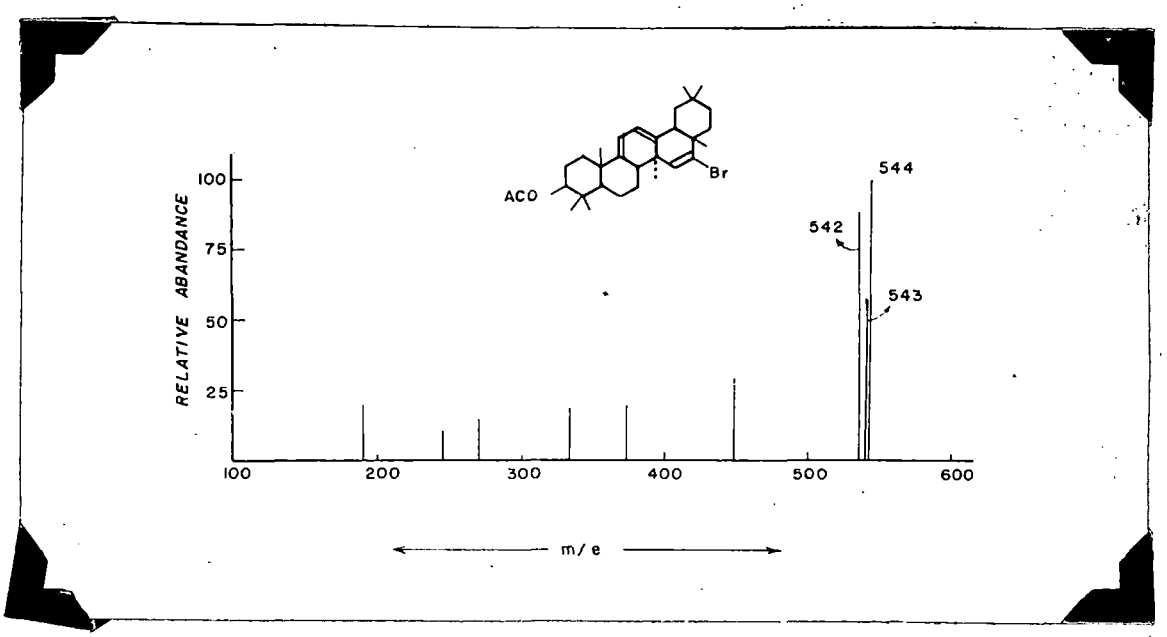


Fig. 16. Mass spectrum of olean-9(11), 12,15-trien 3 β-yl acetate 75

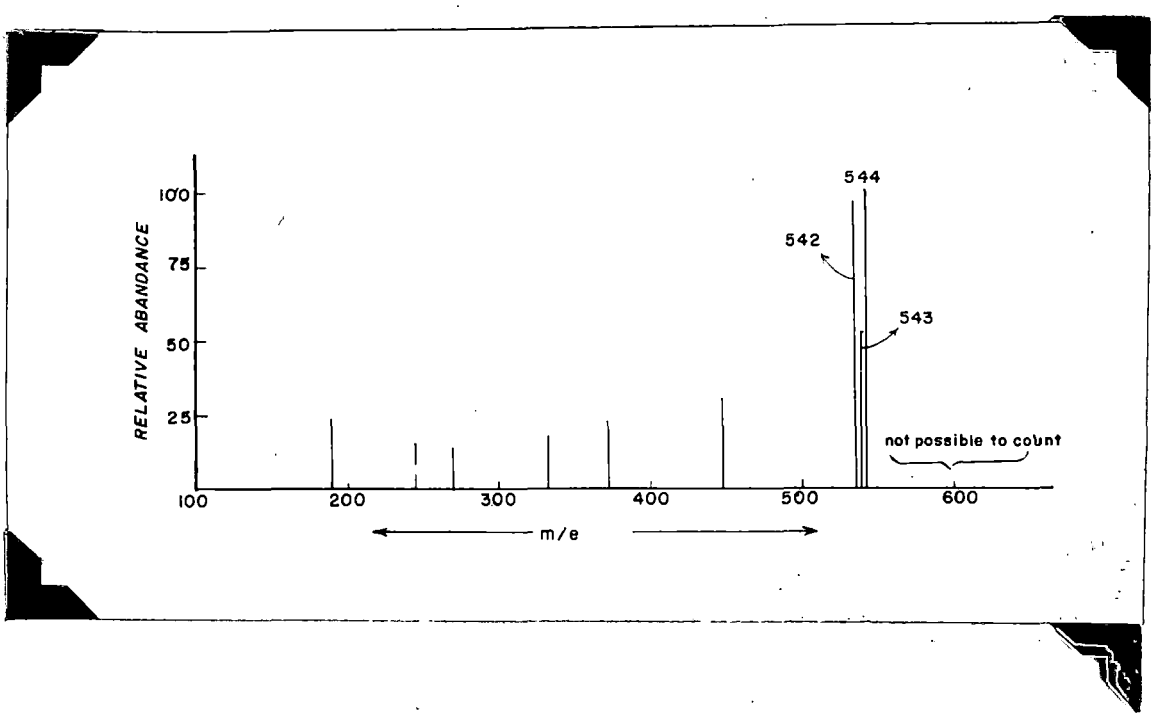
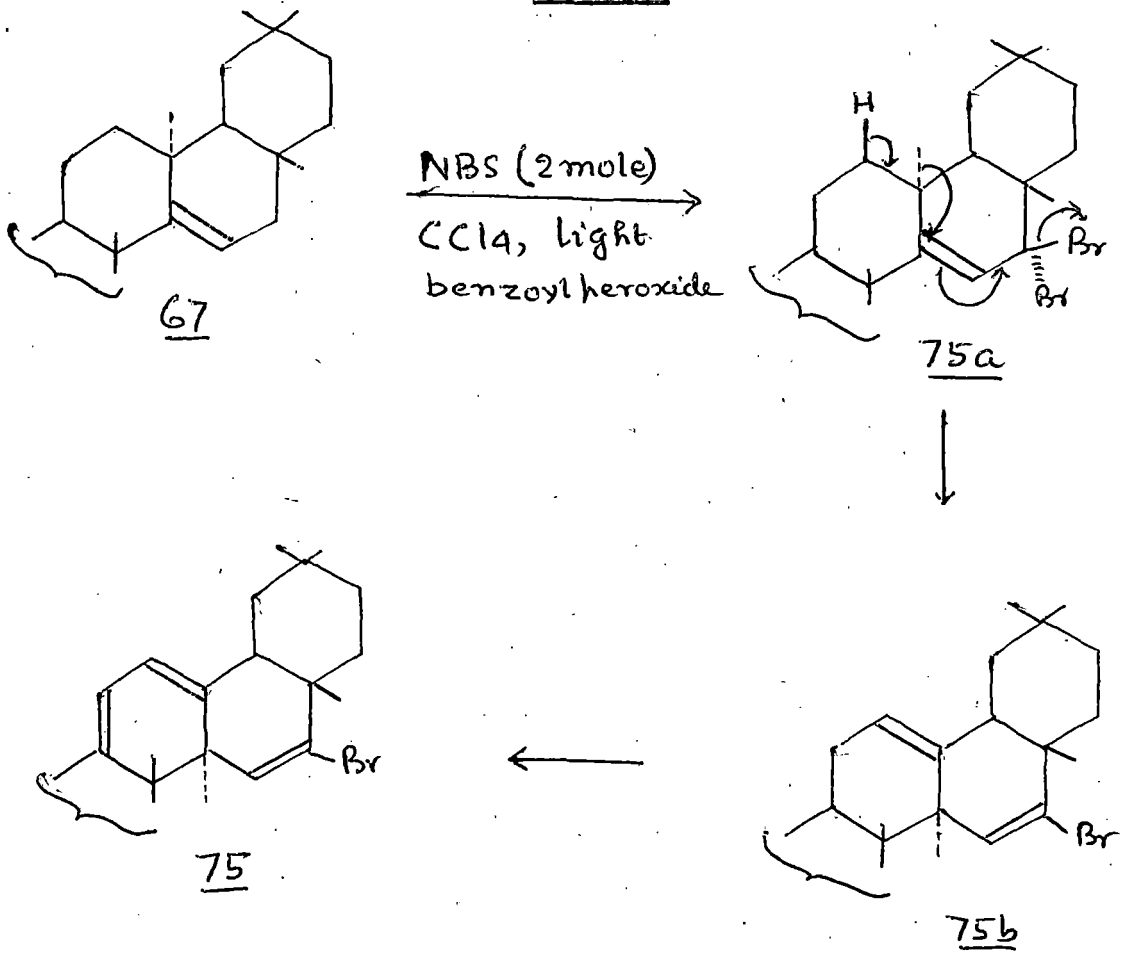


Fig. 17. Mass spectrum of 75 (second run)

to give predominantly the monobromo compound 75b which on allylic bromination at C-11 and subsequent elimination of HBr gives 75

Chart V

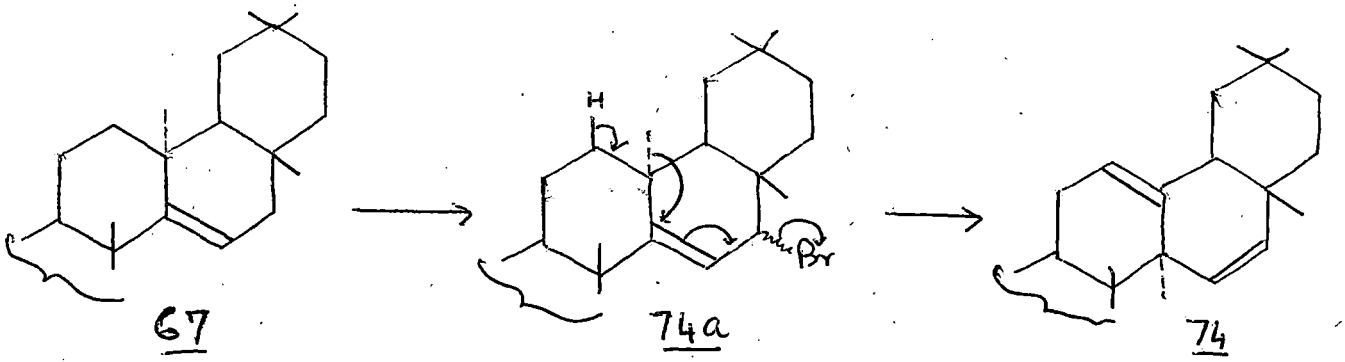


67 75a 75b 75

When the same reaction was carried out with 1 mole equivalent of NBS, it afforded a halogen free product, $C_{32}H_{50}O_2$, m.p. 196-9°, $(\alpha)_D$ 41.86° and was found to be identical with 74 by m.m.p. and IR comparison. The formation of this compound from taraxeryl acetate can also be explained by the same mechanism as depicted in Chart VI.

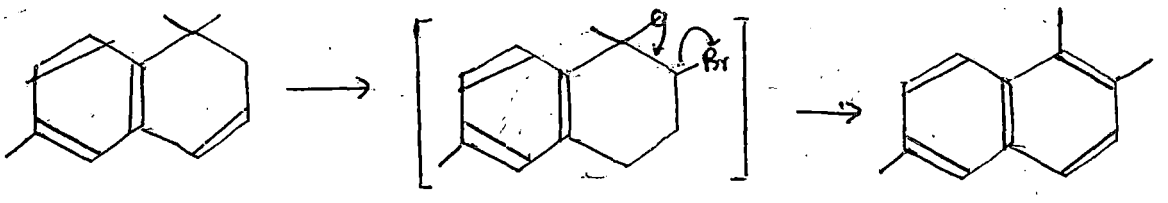
Most probably allylic monobromo compound 74a is first formed which undergoes allylic rearrangement followed by methyl migration as shown below:

Chart-VI



67 74a 74

Barnes and co-workers[46] have shown that treatment of 1,1,6-trimethyl-1-2-dihydronapthalene 75 with NBS gave an allylic bromide which aromatized to 1,2,6-trimethyl napthalene 76 by silver ion or heat (temperature of refluxing carbon tetrachloride).



75 76

CHAPTER - III

EXPERIMENTAL

Melting points are uncorrected. Petroleum ether used through out the experiments had b.p. 60-80°. All optical rotations were determined in chloroform solution unless otherwise stated. NMR spectra were determined on Varian-60 spectrometers using chloroform-d solution containing tetramethyl silane as reference. UV absorption were taken in a Zeiss VSU 1 spectrophotometer in 95% ethanol solution. TLC was done on chromatoplate of silica Gel (E.Merck) and the spots were developed with sulphuric acid-acetic anhydride (9:1) mixture.

Oxidation of taraxeryl acetate 67 with N-Bromosuccinimide: Isolation of 15-bromo β-amyrenonyl acetate 68, 16-oxo taraxeryl acetate 69 , 15-bromo-9(11), 12-olean diene 70 :

To a solution of taraxeryl acetate 67 (200 mg) in dioxan (400 ml) containing water (20 ml) and calcium carbonate (1.0 gm) was added NBS (1.2 gm) and the mixture was stirred for 5.5 hr. at room temperature in presence of visible light (100 watt bulb). A few drops of triethanol amine was added to discharge the yellow color. The reaction mixture was then diluted with water (200 ml) and extracted with ether. The ethereal layer was then washed with water and dried over anhydrous Na_2SO_4. Removal of ether gave a solid (180 mg).

TLC of the latter showed three distinct spots (using benzene as solvent, R_f 0.93, 0.62, 0.46) indicating a mixture of at least three compounds. The residue (180 mg) was dissolved in benzene (2 ml) and poured on a column of alumina (12 gm deactivated with 0.5 ml of 10% aqueous acetic acid) developed with petroleum ether. The following solvents were used for elution.

Table I

Eluent	Fractions 50 ml each	Residue on evaporation	Melting point
Petroleum ether	1-5	Solid (150 mg)	236-7°
Petroleum ether: benzene (4:1)	6-10	Solid (20 mg)	279-80°
Petroleum ether: benzene (4:1)	11-13	Solid (10 mg)	175-6°

Elution with more polar solvents did not give any solid material.

Examination of fractions 1-5: Isolation of 15-bromo-β amyrenonyl acetate 68 :

The fractions 1-5 (Table I) were combined (150 mg) and crystallised from a mixture of chloroform and methanol. After three crystallisations it gave crystals 68, (130 mg), m.p. 238-40° $(\alpha)_D$ 88.8°. Compound 68 showed positive copper-wire test for bromine. TLC

showed a single round spot (R_f = 0.93 in benzene).

Found: C, 68.14; H, 8.68%

Calculated for $C_{32}H_{49}O_3Br$: C, 68.44; H, 8.73%

UV : λ_{max} 249.5 m (ϵ, 11,000)

IR : ν_{max}^{nujol} 1750, 1650, 1250 cm^{-1}

Examination of fractions 6-10: Isolation of 16-oxo taraxeryl acetate 69 :

The fractions 6-10 (Table I) were combined (20 mg) and crystallised from a mixture of chloroform and methanol to give crystals 69, (15 mg) m.p. 280-82°, $(\alpha)_D$ -38.71°, negative copper-wire test for bromine. TLC showed a single spot (R_f 0.62 in benzene).

Found: C, 79.62; H, 10.44%

Calculated for $C_{32}H_{50}O_3$: C, 79.29; H, 10.81%

UV : λ_{max} 245 mμ (ϵ, 10,500)

IR : ν_{max}^{nujol} 1730, 1680, 1250 cm^{-1} (Fig. 8)

NMR spectrum (60 Mc): Peaks at 5.85 (vinyl proton), 2.10 (-O-CO-CH_3), 4.5 (-CH - O-CO-CH_3) ppm (Fig. 9)

Mass spectrum: m/e 358 (base peak), 298, 249 and 482 (M^+) (Fig. 10)

Examination of fractions 11-13: Isolation of 15-bromo-9(11), 12-olean diene 70 :

Fractions 11-13 (Table I) were combined (10 mg) and crystallised from methanol to furnish crystals of 70 (8 mg), m.p. 176-8°,

$(\alpha)_D$ 249.12°. The compound showed a single round spot on a chromatoplate (R_f = 0.45 in benzene) and gave a positive copper wire test for bromine.

Found: C, 70.47; H, 8.84%

Calculated for $C_{32}H_{49}O_2Br$: C, 70.46; H, 8.99%

UV : λ_{max} 276 mμ (ε, 6000)

NMR spectrum (60 Mc): Peaks at 5.34, 5.85 (two vinyl proton), 2.08 (singlet, -O-CO-CH_3), 4.65 (multiplet, HC-O-CO-CH_3), 4.18 ppm (multiplet, H-C-Br), (Fig. 12)

Mass spectrum : 465, 546 (M^+) (Fig. 13).

Zinc dust-acetic acid treatment of 68 : Formation of β-amyrenonyl acetate 71 :

To a solution of 15-bromo β-amyrenonyl acetate 68 (200 mg) in acetic acid (26 ml), was added zinc dust (12 mg) and the mixture refluxed for 3 hours. The reaction mixture was filtered, diluted with water and extracted with ether. The ether layerwas washed with water till neutral and then dried (Na_2SO_4). Removal of ether gave a solid residue (175 mg) which on crystallisation from chloroform-methanol furnished solid 71, m.p. 265-70°, $(\alpha)_D$ 48.08. This was found to be identical with an authentic sample of β-amyrenonyl acetate (m.m.p. and IR comparison).

Found: C, 79.60; H, 10.32%

Calculated for $C_{32}H_{50}O_3$: C, 79.62; H, 10.44%

UV : λ_{max} 252.5 mμ (ε, 11,000).

Preparation of 15-bromo- β-amyrin acetate 72 : NBS - DMSO treatment of taraxeryl acetate 67 :

To a solution of taraxeryl acetate 67 (700 mg) dissolved in chloroform (46.5 ml) and dimethyl sulfoxide (23.25 ml) was added NBS (700 mg) in portions and kept overnight. Next day, the reaction mixture was filtered. The filtrate was extracted with chloroform (150 ml), washed with water and dried (Na_2SO_4). On removal of the solvent under reduced pressure, a yellowish solid (500 mg) was obtained. The solid was dissolved in benzene (5 ml) and poured on a column of alumina (25 gm deactivated with 1.0 ml of 10% aqueous acetic acid) developed with petroleum ether. The following solvents were used for elution.

Table II

Eluent	Fractions 50 ml each	Residue on evaporation	Melting point
Petroleum ether	1-5	Solid (475 mg)	178-80°

Elution with more polar solvents did not give any solid.

Fractions 1-5 (Table II) were collected and on crystallisation from a mixture of chloroform-methanol afforded crystals of 15-bromo β - amyrin acetate 72, m.p. 180-2°, $(\alpha)_D$ 47.37°. It gave a single spot

on a chromatoplate (R_f = 0.8) in petroleum ether: benzene (3:2) and also showed positive copper-wire test for bromine.

Found: C, 70.09; H, 8.9%

Calculated for $C_{32}H_{51}O_2Br$: C, 70.20; H, 9.3%

UV : No absorption in the region 220-300 mμ

IR : ν_{max}^{nujol} 1720 and 1250 cm^{-1} (Fig. 1)

NMR spectrum (60 Mc) : Peaks at 5.3 (multiplet, vinyl proton), 4.30 (multiplet, HCBr), 2.09 (-O-CO-CH_3) and 4.53 (H-C-O-CO-CH_3) ppm. (Fig. 2)

Mass spectrum: Peaks at m/e 189, 216, 217, 249, 297, 466 (M^+ -HBr), 547 (M^+). (Fig. 3)

Zinc-acetic acid treatment of 72: Isolation of β-amyrin acetate 73:

To 15-bromo-β-amyrin acetate 72 (100 mg) in acetic acid was added zinc-dust (10 mg) and the mixture was refluxed for 5 hours. The reaction mixture was filtered and then diluted with water. The product which precipitated out was filtered and washed thoroughly with water. The crude product (75 mg) was dissolved in benzene (3 ml) and poured on a column of alumina (12 gm deactivated with 0.5 ml of 10% aqueous acetic acid) developed with petroleum ether. The following solvents were used for elution.

Table III

Eluent	Fractions 50 ml each	Residue on evaporation	Melting point
Petroleum ether	1-4	Solid (60 mg)	226-28°

Elution with more polar solvents did not give any solid material.

Fractions 1-4 (Table III) were combined (60 mg) and after repeated crystallisation from acetone afforded crystals of β-amyrin acetate 73, m.p. 227-30°, $(\alpha)_D$ 85.1°. The solid was found to be identical with an authentic sample of β-amyrin acetate (m.m.p and I.R. comparison, Fig. 4).

Found: C, 81.89; H, 11.08%

Calculated for $C_{32}H_{52}O_2$: C, 81.99; H, 11.18%

Chromium trioxide-acetic acid oxidation of 15-bromo-β-amyrin acetate 72 : Preparation of 15-bromo β -amyrenonyl acetate 68 :

Chromium trioxide (300 mg) dissolved in acetic acid (7 ml) was added to a solution of 15-bromo β-amyrin acetate 72 (400 mg) in acetic acid (26.6 ml). The mixture was refluxed for one hour at 130-32°. The reaction mixture was then cooled, diluted with water and extracted with ether. The ethereal layer after being washed with

water till neutral was dried (Na_2SO_4). The solvent was removed and a solid residue (300 mg) was obtained. The solid was dissolved in benzene (2 ml) and poured on a column of alumina (25 gm deactivated with 1 ml of 10% aqueous acetic acid) developed with petroleum ether. The chromatogram was eluted with the following solvents.

Table IV

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1-7	Solid (275 mg) m.p. 236-38°

Further elution with more polar solvents did not give any material.

Fractions 1-7 (Table IV) were collected and combined, which on crystallisation from methanol gave crystals of 68, m.p. 238-40°, $(\alpha)_D$ 88.08°. This was found to be identical with 15-bromo β-amyrenonyl acetate obtained during aq. dioxan- NBS treatment of taraxeryl acetate (from fraction 1-5, Table I) m.m.p. and IR comparison, Fig.5.

Found: C, 68.24; H, 8.63%

Calculated for $C_{32}H_{49}O_3Br$: C, 68.44; H, 8.73%

UV : λ_{max} 249 mμ (ε, 11,000).

Solvolysis of 15-bromo β-amyrenonyl acetate 72: Formation of Olean-12, 15-dien-3β-yl acetate 74 :

To the acetate 72 (200 mg) dissolved in acetic acid (27 ml) was added anhydrous potassium acetate (500 mg) and the mixture was refluxed for 5 hr. It was then cooled, diluted with water and extracted with ether. The ether solution after being washed with water till neutral, was dried (Na_2SO_4). On removal of the sovent, a solid residue (150 mg) was obtained. The solid was dissolved in benzene (2 ml) and poured on a column of alumina (12 gm deactivated with 0.5 ml of 10% aqueous acetic acid) developed with petroleum ether. The following solvents were used for elution.

Table V

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum ether	1-2	Oil (trace)
Petroleum ether	3-7	Solid (120 mg) m.p. 195-7°

Elution with more polar solvents did not give any solid.

Fractions 3-7 (Table V) were combined and on crystallisation from methanol afforded crystals of 74, m.p. 199-200°, $(\alpha)_D$ 41.86°. It showed a single spot on a chromatoplate (R_f = 0.43 in benzene) and negative copper wire test for bromine.

Found : C, 81.96; H, 10.81%

Calculated for $C_{32}H_{50}O_2$: C, 82.35; H, 10.80%

UV : No absorption in the region 220-300 mμ.

NMR spectrum (60 Mc): Peaks at 5.2 to 5.6 (three vinyl Protons), 2.08 (-O-CO-CH_3), 4.50 (H-C-O-CO-CH_3) ppm (Fig. 6)

Dimethyl aniline treatment of 15-bromo-β-amyrin acetate 72 : Isolation of olean-12, 15-dien-3β-yl acetate 74 :

15-bromo β-amyrin acetate 72 (200 mg) was refluxed with dimethyl aniline (30 ml) for 6 hours. The reaction mixture was diluted with water, acidified with 6N hydrochloric acid (20 ml) and extracted with ether. The ethereal layer was washed with water till neutral and dried (Na_2SO_4). On removal of the solvent, a solid (175 mg) was obtained. The latter was dissolved in benzene (3 ml) and poured on a column of alumina (15 gm, deactivated with 6 ml of 10% aqueous acetic acid) developed with petroleum ether. The following solvents were used for elution.

Table VI

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1-6	Solid (150 mg) m.p. 196-7°

Elution with more polar solvents did not give any solid material.

Fractions 1-6 (Table VI) were combined and crystallised from chloroform-methanol to give crystals of 74, m.p. 199-200°, $(\alpha)_D$ 41.86°. The solid was found to be identical (m.m.p. and IR, Fig. 7) with a sample of olean-12, 15-dien-3β-yl acetate 74 obtained from solvolysis of 15-bromo-β-amyrin acetate with anhydrous potassium acetate and acetic acid.

Found : C, 82.57; H, 10.82%

Calculated for $C_{32}H_{50}O_2$: C, 82.35; H, 10.80%

Preparation of β-amyrenonyl acetate 71 from β-amyrin acetate 73 :

β-amyrin acetate (200 mg) dissolved in acetic acid (25 ml) was added to a solution of chromium trioxide (330 mg) in acetic acid (7 ml, 80%). The mixture was refluxed for one hour. On cooling

it was diluted with water, filtered and washed with water till neutral. The solid was dissolved in benzene (3 ml) and poured on a column of alumina (12 gm deactivated with 0.5 ml of 10% aqueous acetic acid). Following solvents were used for elution.

Table VII

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1-2	Oil (trace)
Petroleum ether	3-8	Solid (150 mg) m.p. 265-67°

Elution with more polar solvents did not give any solid material.

Fractions 3-8 (Table VII) were combined and on crystallisation from methanol gave 71, m.p. 268-70°, $(\alpha)_D$ 48.8°.

Found: C, 79.55; H, 10.30%

Calculated for $C_{32}H_{50}O_3$: C, 79.62; H, 10.40%

UV : λ_{max} 252.5 mμ (ϵ, 11,000).

Attempted sodium borohydride reduction of 16-oxo-taraxeryl acetate 69 in tetrahydrofuran (THF) :

To a solution of 16-oxo-taraxeryl acetate 69 (200 mg) in dry tetrahydro furan (50 ml) was added sodium-borohydride (200 mg) and

the mixture was stirred for 6 hours at room temperature. A portion of the solvent was removed by distillation, cooled and acidified with dilute hydrochloric acid and then extracted with ether. The ethereal layer was washed with water till neutral and then dried (Na_2SO_4). Removal of the ether gave a solid which on crystallisation from methanol gave crystals m.p. 280-82°. The solid was found to be identical with the starting material 16-oxo-taraxeryl acetate (m.m.p.).

Attempted reduction of 16-oxo-taraxeryl acetate 69 with sodium borohydride in methanol:

To compound 69 (200 mg) dissolved in methanol (50 ml) was added sodium borohydride (200 mg) and the reaction mixture was stirred for 4 hours at room temperature. After working up in the usual way it gave a solid, m.p. 279-81°, identified as the original 16-oxo-taraxeryl acetate by m.m.p.

Meerwein-Pondorff reduction of 16-oxo-taraxeryl acetate 69:

A mixture of 16-oxo-taraxeryl acetate 69 (250 mg), aluminium isopropoxide (325 mg) in dry isopropanol (6 ml) was distilled slowly with the addition of isopropanol to maintain constant volume. After 6 hours, the distillate no longer contained acetone. The solution was concentrated to a small volume. The reaction mixture was then diluted with water followed by 10% sulphuric acid (20 ml) and then extracted with ether. The solid product obtained after removal of ether was dissolved in benzene (2 ml) and poured on a column of

alumina (12 gm deactivated with 0.5 ml of 10% aqueous acetic acid) developed with petroleum ether. The following solvents were used as eluent.

Table VIII

Eluent	Fractions 50 ml each	Residue
Petroleum ether	1-2	trace of oil
Petroleum ether : benzene (4:1)	3-7	Solid m.p. 290-2° 200 mg

Further elution with more polar solvents did not give any solid material.

The solid from fraction 3-7 (Table VIII) were combined which after crystallisation from methanol afforded 16-oxo-taraxerol 78 m.p. 292-3°.

Found: C, 81.20; H, 11.08%

Calculated for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38%

IR : ν_{max}^{KBr} 3360, 1680 cm^{-1}.

Lithium aluminium hydride reduction of 16-oxo-taraxeryl acetate 69: Formation of 16-oxo-taraxerol 78 :

To the ketone 69 (200 mg) dissolved in dry tetrahydro furan

was added lithium aluminium hydride (25 mg) and the mixture was refluxed for 10 hours on water bath. The reaction mixture was then cooled and to this was added dropwise a cold saturated solution of sodium sulphate (15 ml). After this addition the mixture was extracted with ether, washed to neutral with water and dried (Na_2SO_4). Removal of ether gave a solid (190 mg) which was chromatographed over alumina. A column of alumina (10 gm deactivated with 0.4 ml of 10% aqueous acetic acid) was developed with petroleum ether and the above residue dissolved in benzene (4 ml) was added to it. The following solvents were used for elution (Table IX).

Table IX

Eluent	Fractions 50 ml each	Residue
Petroleum ether	1-2	Nil
Petroleum ether: benzene (4:1)	3-6	Crystalline solid 180 mg m.p. 290-2°.

Elution with more polar solvents did not yield any material.

Fractions 3-6 (Table IX) were combined and the solid (180 mg) was crystallised from chloroform and methanol mixture when pure crystals of 78 m.p. 292-3°, was obtained. This was found to be identical with

16-oxo-taraxerol obtained by Meerwein-Pondorff reduction of 69 (m.m.p. and I.R. comparison).

Acetylation of 16-oxo-taraxerol 78 : Preparation of 16-oxo-taraxeryl acetate 69 :

The compound 78 (200 mg) was acetylated with pyridine (2 ml) and acetic anhydride (2 ml) in the usual way. The solid obtained, after crystallisation from methanol gave crystals of 69 m.p. 280-2°, found to be identical with 16-oxo-taraxeryl acetate 3 (m.m.p.).

Treatment of taraxeryl acetate 67 with NBS (2 moles) in carbon tetrachloride: Isolation of 16-bromo-olean-9(11),12,15-trien-3 β-yl acetate 75 :

To a solution of taraxeryl acetate (1 gm) dissolved in dry carbon-tetrachloride (185 ml), was added NBS (700 mg) and the reaction mixture was refluxed for 3 hours in presence of visible light (100 watt bulb). As the reaction proceeded, insoluble succinimide, which was at the bottom, floated on the surface. The reaction mixture was then cooled and filtered. The filtrate was extracted with chloroform (200 ml), washed with water and then dried (Na_2SO_4). On removal of the solvent under reduced pressure a solid (800 mg) was obtained which was chromatographed on a column of alumina (25 gm deactivated with 1 ml of 10% aqueous acetic acid). The chromatogram was developed with petroleum ether and the solid (800 mg) dissolved in benzene (4 ml) was placed on the column. It was eluted with the following solvents (Table X).

Table X

Eluent	Fractions 50 ml each	Residue
Petroleum ether	1-9	Solid m.p. 238-39° (775 mg)

Elution with more polar solvents did not give any material.

Fractions 1-9 (Table X) were combined (775 mg) and after several crystallisation from methanol furnished 75, m.p. 240°, $(\alpha)_D$ 267.53°. It showed a single spot on a chromatoplate (R_f 0.84) in petroleum ether: benzene (3:2) and showed positive copper wire test for bromine.

Found: C, 70.40; H, 8.37%

Calculated for $C_{32}H_{47}O_2Br$: C, 70.70; H, 8.67%

UV : λ_{max} 279 mμ (ε , 6000)

IR : ν_{max}^{nujol} 1725, 1250, 840 cm^{-1} (Fig. 14)

NMR spectrum (60 Mc) : Peaks between 5.68 to 5.88 (three vinyl protons), 2.10 (-O-CO-CH_3) and 4.5 (-CHOCOCH$_3$) ppm (Fig. 15)

Mass spectrum: m/e, 544 (isotope), 543, 542 (M^+) (Fig. 16)

In another experiment this product showed a very weak peak between 620-25 (it was not possible to count Fig. 17) due to a small contaminant of a dibromide, other peaks were similar to as shown in Fig. 16.

Attempted zinc-acetic acid treatment of 16-bromo-olean-9(11),12,15-trien-3β-yl acetate 75:

To a solution of 75 (200 mg) in glacial acetic acid (40 c.c) was added zinc-dust (40 mg) and the reaction mixture was refluxed for 8 hours. After working up in the usual way it afforded a solid which on crystallisation from methanol gave crystals m.p. 239-40°. This solid was found to be identical with the starting material 75 (m.m.p).

Attempted dehalogenation of 75 with palladium-on-charcoal catalyst :

To the 16-bromo-olean-9(11),12,15-trien-3 β-yl-acetate 75 (200 mg) dissolved in ethyl acetate (40 ml) was added 10% palladium-on-charcoal catalyst (100 mg) and the mixture was stirred at room temperature in an atmosphere of hydrogen. No absorption of hydrogen took place even after eight hours. It was then filtered and the filtrate was evaporated to dryness. The residue m.p. 238-39° (190 mg), on crystallisation from methanol gave crystals, m.p. 240° and was found to be unchanged 75 (m.m.p.).

Attempted dehalogenation of 75 with Adam's catalyst:

A solution of compound 75 (200 mg) in ethyl acetate (40 ml) was stirred in presence of Adams catalyst (20 mg) at room temperature and pressure. During a period of ten hours the mixture did not show any absorption of hydrogen. On working up the reaction mixture in the usual manner, a residue (200 mg) was obtained. The latter on crystallisation from methanol afforded crystals, m.p. 240°, and was found to be identical with the starting material 75 .

Attempted dehalogenation of 75 with lithium carbonate and lithium bromide:

A mixture of compound 75 (200 mg) in dimethyl formamide (30 ml), lithium carbonate (200 mg) and lithium bromide (270 mg) was stirred in an atmosphere of nitrogen at 100° for 3 hours. The product was then taken up in chloroform and the chloroform layer was washed with 40% acetic acid (50 ml), then with water till neutral and then dried (Na_2SO_4). Evaporation of the solvent gave a residue (175 mg), which on crystallisation from methanol furnished a solid, m.p. 239-40°, which did not depress the melting point when mixed with the starting material 75 .

Attempted solvolysis of 75 with anhydrous potassium acetate and acetic acid: (SN1)

16-bromo compound 75 (200 mg) was refluxed with anhydrous potassium acetate (150 mg) and acetic acid (20 ml) for 4 hours. The product was then diluted with water and the precipitate was filtered,

and washed thoroughly with water. The crude product (150 mg) on crystallisation from methanol furnished a solid, m.p. 240°, identical with the starting material (m.m.p.).

Attempted dehydrohalogenation of 75 with dimethyl aniline:

Compound 75 (200 mg) was refluxed for 10 hours with freshly distilled dimethyl aniline (50 ml). The solvent was removed by distillation under reduced pressure, and the residue was diluted with water and extracted with ether. The ethereal layer was washed with 6N hydrochloric acid (10 ml) followed by water till neutral and then dried (Na_2SO_4). On removal of solvent a solid residue (175 mg) was obtained which on crystallisation afforded a solid, m.p. 240°, identical with 16-bromo-olean-9(11),12,15-trien-3β-yl acetate 75 (m.m.p).

Formation of olean-12,15-dien-3β-yl acetate 74: Treatment of taraxeryl acetate 67 with NBS (1 mole) in carbon tetrachloride :

To a solution of taraxeryl acetate 67 (300 mg), dissolved in dry carbon tetrachloride (100 ml), was added NBS (116 mg) and benzoyl peroxide(a few crystals) and the reaction mixture was refluxed for six hours in presence of visible light (600 watt bulb). The reaction mixture was cooled and filtered and the filtrate was extracted with chloroform. The chloroform layer was washed with water and dried (Na_2SO_4). On evaporation of solvent, a yellow solid (250 mg) was obtained. The latter was taken up in ether, washed with 5% cold sodium

hydroxide solution, followed by water till neutral and dried over anhydrous sodium sulphate. On removal of ether, a solid residue was obtained which after several crystallisations gave solid 74 m.p. 196-9°, $(\alpha)_D$ 41.86°. It showed a single spot on a chromatoplate (R_f = 0.43 in benzene) and showed negative copper wire test for bromine. This product 74 was found to be identical with the product obtained by solvolysis of 15-bromo-β-amyrin acetate 72 (m.m.p. and I.R).

Found: C, 82.00; H, 10.70%

Calculated for $C_{32}H_{50}O_2$: C, 82.35; H, 10.80%

UV : No absorption above 220 mμ.

R E F E R E N C E S

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