CHAPTER - II

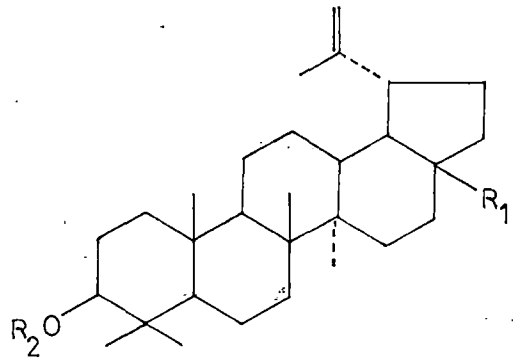
A Short Review on the Triterpene Lactones of Lupane Series.

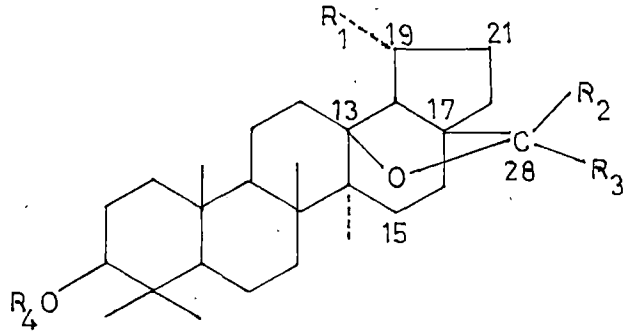
The Structure of the Lactone derived from mercuric acetate oxidation of betulinic acid 62a

(i) The C-28, 13β lactone structure 63a proposed initially:

The successful introduction of an additional unsaturation in some unsaturated steroids by Hg(II) acetate provided the stimulus for application of this dehydrogenation reaction in triterpenes. Beidebach[51] first applied this reaction on α-amyrin, β-amyrin and lupene derivatives. Of these, only triterpenes of the lup-20(29)-ene series underwent this reaction. Betulin and lupeol gave dehydro compounds of unknown structure. Since their esters also underwent dehydrogenation but not their dihydroderivatives, it was concluded that this dehydrogenation was associated with the presence of olefinic bond.

Allison and co-workers[52] carried out Hg(II) acetate oxidation on acetyl betulinic acid 62b and obtained a γ-lactone assigned as 63a, IR ν_{max} 1792 cm^{-1}. This on hydrogenation gave a dihydrolactone 63b which was also obtained by Hg(II) acetate oxidation of betulin 62c followed by hydrogenation, acetylation and oxidation. Betulin 62c afforded a cyclic ether assigned as 63c, I.R. ν_{max} 1630, 836 (vinylidene group). NMR of the corresponding acetate 63d exhibited peaks at 63 and 89 cps ($C-CH_2O-$).





62a R_1 = COOH, R_2 = H

62b R_1 = COOH, R_2 = Ac

62c R_1 = CH_2OH, R_2 = H

62d R_1 = $COOCH_3$, R_2 = Ac

62e R_1 = CH_2OAc, R_2 = Ac

62f R_1 = CH_3, R_2 = Ac

63a R_1 = isopropenyl, R_2, R_3 = O
R_4 = Ac

63b R_1 = isopropyl, R_2, R_3 = O,
R_4 = Ac

63c R_1 = isopropenyl, R_2 = R_3 = H
R_4 = H

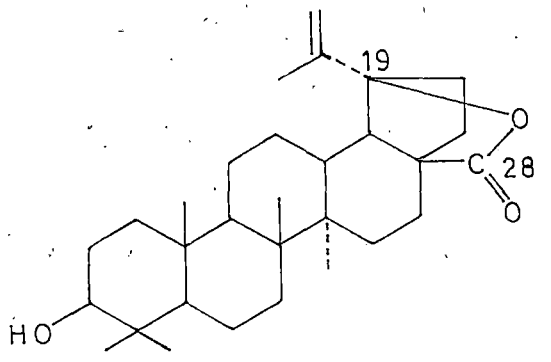
63d R_1 = isopropenyl, R_2 = R_3 = H,
R_4 = Ac

63e R_1 = isopropenyl, R_2, R_3 = O,
R_4 = H

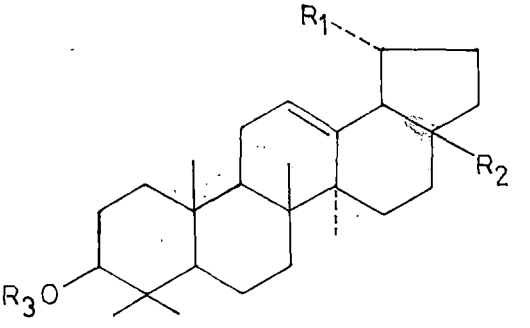
63f R_1 = isopropyl, R_2, R_3 = O,
R_4 = H

Hydrogenation of 63d followed by oxidation gave the lactone 63b. The authors advanced the following arguments in favour of their structure 63b for the lactone. The termination of lactone could be at 14, 15, 19 or 21 position. Lithium aluminium hydride reduction product of 63b on acetylation afforded a diacetate and not a triacetate, thus excluding positions 15 and 21 for the lactone termination and it was thought that one of the

hydroxyl groups was tertiary in nature. The smooth dehydration of the diacetate with $POCl_3$ - pyridine confirmed the tertiary nature of the third hydroxyl group and the product obtained in this reaction was assigned structure 65f, λ_{max} 206 nm (ϵ=7960), ν_{max} 1650 and 3050 cm^{-1}. Consequently, the lactone termination in 63b and the ether linkage in 63d must be at the same point, either at C-13 or C-19. The lactone 63e, the hydroxy derivative of the lactone 63a, was found to be different from the cactus lactone thurberogenin which was previously assigned the C-28, C-19 lactone structure 64 by Djerassi et al[53] in 1955. Non identity of thurberogenin with their lactone led Allison and co-workers in 1961 to assign structure 63a for the acetoxy lactone and 63e for the corresponding hydroxy lactone,



64 thurberogenin old structure advanced in 1955



65a R_1 = isopropenyl, R_2 = CO_2CH_3, R_3 = Ac

65b R_1 = isopropyl, R_2 = $COOCH_3$, R_3 = Ac

65c R_1 = isopropyl, R_2 = COOH, R_3 = H

65d R_1 = isopropenyl, R_2 = CH_2OAc, R_3 = Ac

65f R_1 = isopropenyl, R_2 = CH_3, R_3 = Ac.

65f R_1 = isopropyl, R_2 = CH_2OAc, R_3 = Ac

Allison and co-workers also observed that 3β-acetoxy methyl betulinate 62d on similar oxidation with Hg(II) acetate gave a diene λ_{max} 206 nm (ϵ 7100), ν_{max} 3078, 1634, 901 (C = CH_2), 1730, 1250 cm^{-1} (-OCOCH_3) which was then (in 1961) given structure 65a. The corresponding hydrogenated product 65b, λ_{max} 205 nm (ϵ 5300), ν_{max} 876 cm^{-1}, on hydrolysis with sodium ethoxide afforded the acid 65c. The latter on treatment with hydrogen chloride in chloroform gave the lactone 63f identical with the lactone obtained from mercuric acetate reaction of 62b followed by hydrogenation and hydrolysis.

Betulin diacetate 62e on Hg (II) acetate oxidation gave a diene assigned at that time (in 1961) as structure 65d, which was also prepared from the ester 65a and the lactone 63a. Reduction of the ester 65a with LAH gave a diol which on acetylation furnished the same dienyl acetate 65d. Similar reduction of the lactone 63a followed by acetylation gave an acetoxy alcohol which on $POCl_3$-pyridine dehydration furnished the dienyl acetate 65d.

(ii) Revised formulation of the lactone as C-28, 19β-lactone 66:

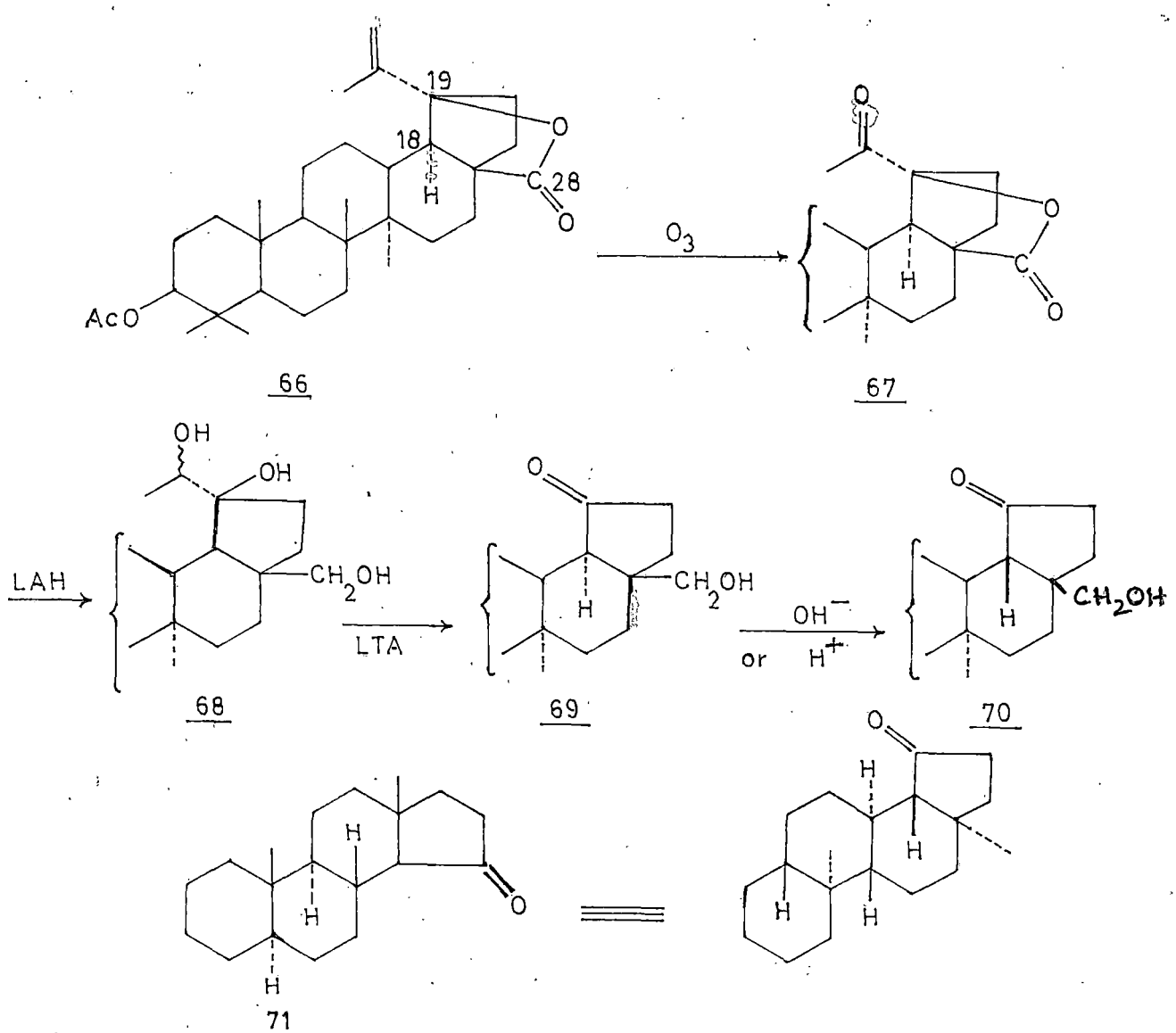
The structural assignment of the lactone as C-28, 13-lactone derived from Hg(II) acetate oxidation of acetyl betulinic acid by Allison et al[52] rested partly on its non-identity with the acetate of thurberogenin which was previously assigned C-28, 19-lactone 64. Later Djerassi et al[54] revised the structure of thurberogenin as C-28, 21-lactone 85a and this invalidated

the structural assignment of the mercuric acetate oxidation product. Three groups of workers[55,57,62] almost simultaneously but independently put forward both spectral and chemical degradative results in support of the revised formulation of this lactone as C28-19βlactone 66.

Baddeley and co-workers[55] carried out ozonolysis of the lactone derived from acetyl betulinic acid 62b by Hg(II) acetate, in methanol-chloroform at -10° to give a nor ketone 67. The norketone 67 was reduced by LAH to the tetrol 68 which on lead tetra-acetate treatment afforded the trisnorketone 69 whose IR absorption at 1738 and 1410 cm^{-1} (Nujol) was that expected for a cyclopentanone with methylene adjacent to the carbonyl group. The C-18 Hα stereochemistry of 69 was deduced from the negative cotton effect in the ORD curve ($[\phi]_{312}$ -5340°, $[\phi]_{273}$ + 6200°; amplitude -115) and from the minimum in the CD curve ($[\theta]_{296}$ - 8220°). The 18α(H)-trisnorketone 69 was isomerized by heating with 0.1N methanolic KOH or in AcOH to ketone 70 epimeric at C-18. The ORD curves of the trisnorketones 69 and 70 have nearly the same amplitudes as are near reflection of, those of androstan-15-one 71 and its 14β(H)-isomer, respectively whose ring systems are essentially enantiomeric with the B/C/D/E rings in the two trisnorketones.

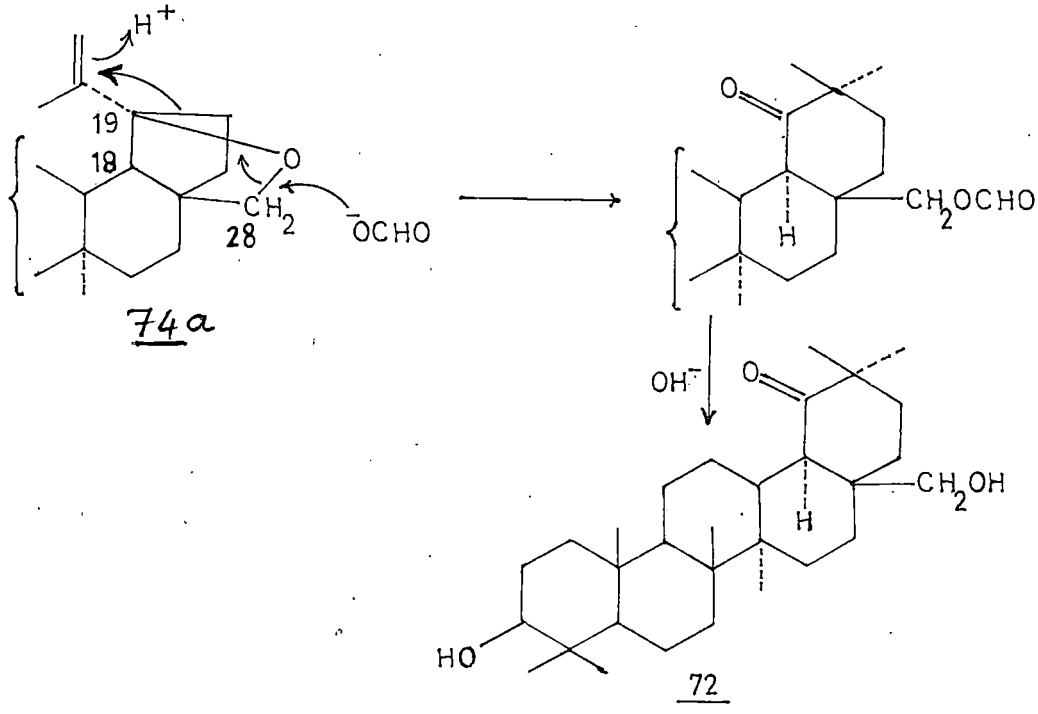
Based on this evidence they advanced the structure 66 for the lactone from acetyl betulin acid 62b. Clearly there can be no ambiguity in the stereochemistry at C-19 in this lactone and this structure also explains the recovery of the

norketone 67 unchanged after treatment with alkali (i.e. with re-acetylation of the 3β-hydroxyl group where necessary) including the method of Khastgir and Bose[56]. The whole sequence may be illustrated in the scheme V.

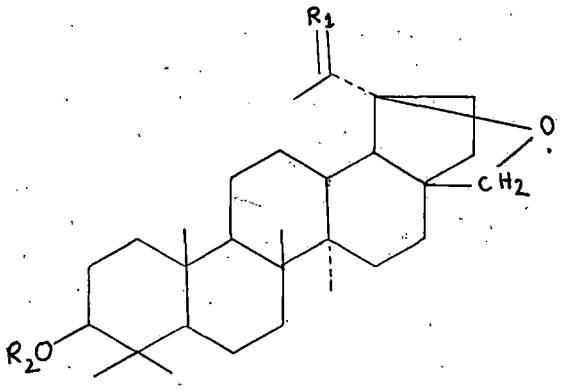


Scheme V

Vystrcil and Blecha[57] almost simultaneously published the correct structure of mercuric acetate oxidation product of betulin 62c. They showed that the primary product of oxidation of betulin 62c by mercuric acetate had the structure 74a. They advanced the following evidence in support of their conclusion. The oxidation product of betulin, 74a readily underwent acid-catalysed isomerisation on treatment with 85% formic acid at elevated temperature and yielded a mixture which on alkaline hydrolysis afforded a uniform product identified as the known 3β, 28-dihydroxy-19 oxo-18 α H - oleanane 72[58]. The isomerization is initiated similarly to betulin[59] but the transiently formed electron deficiency at C-19 is compensated by oxygen at the same position bound in an oxo group which is further made possible by the simultaneous attack of the formate nucleophile at C-28. They also demonstrated that under the same acid-catalysed condition the 20(29)-lupen-28 → 19β olide system failed to undergo isomerisation.

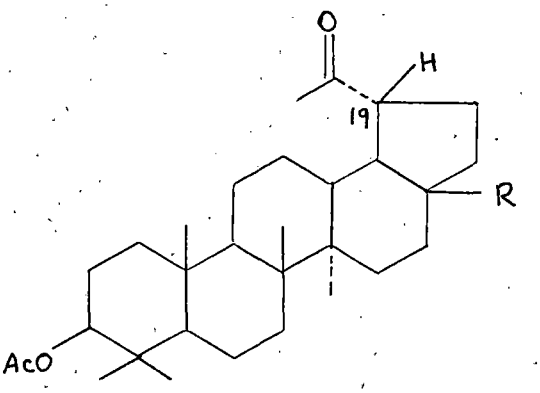


The positive cotton effect of 3β, 28-diacetoxy-30-norlupan-20-one **73a** $[\phi]_{308}$ + 1284°, $[\phi]_{272}$ - 3925°, a = 52 (dioxan) is changed to negative value in 3β-acetoxy-19β, 28-epoxy-30 norlupan-20-one **74b** $[\phi]_{307}$ - 1883°, $[\phi]_{270}$ + 203°, a = -21 (dioxan) which is the familiar effect of α-substitution[60] of methyl ketones with restricted rotation. Whereas the ^{1}H NMR spectrum of the diacetyl norketone **73a** contains a clear signal at 19β-H (τ 7.38, multiplet) it is completely absent in the spectrum of the acetyl epoxy norketone **74b**. Furthermore, by the condensation of **74b** with benzaldehyde, the benzal derivatives **75** $C_{38}H_{52}O_4$ m.p. 276-277°, $[\alpha]_D$ + 34, λ_{max} 294 nm (log ϵ 4.3) was obtained. This on subsequent sodium borohydride reduction affords a mixture of isomeric alcohols **76** $C_{38}H_{54}O_4$, λ_{max} 252 nm (log ϵ 4.3), ν_{max} 3600, 3520, 1083 cm^{-1} (OH) which cannot be dehydrated to the phenyl butadiene system C_6H_5 - CH = CH-CH = $\overset{1}{C}$. Had the epoxide bridge been terminated differently from 19β, the above mentioned dehydration reaction would be expected by analogy with the literature report[61].



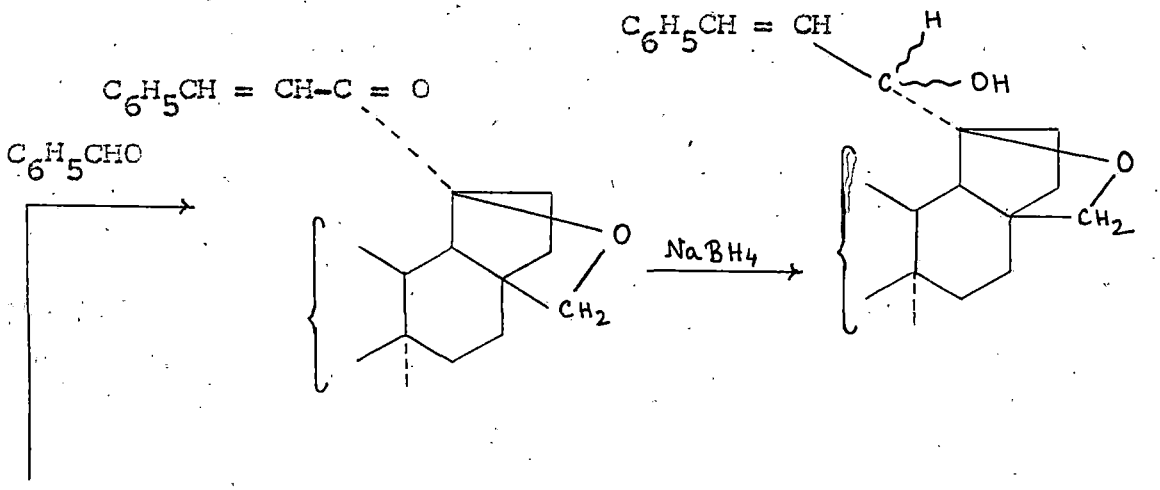
74a $R_1 = CH_2$, $R_2 = H$

74b $R_1 = O$, $R_2 = Ac$



73a $R = CH_2OAc$

73b $R = CO_2CH_3$

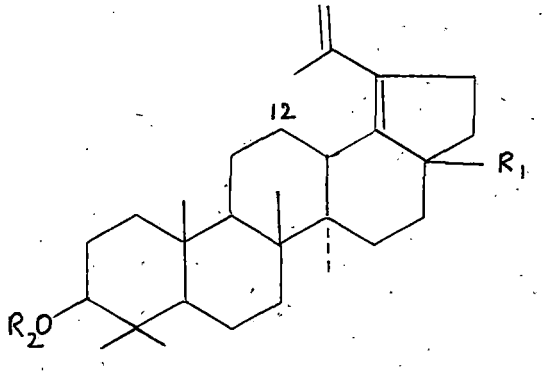


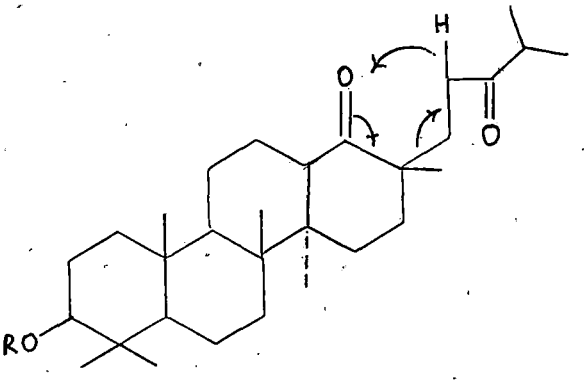
74b 75 76

At the time when the correct structures of the lactone[55] and ether[57] were advanced McLean and co-workers[62], the original workers, also came forward with their revised formulation on the Hg(II) acetate oxidation products of lupane series. They demonstrated that the new double bond introduced into lupeol acetate 62f, betulin diacetate 62e and methyl betulinate 62d by dehydrogenation with Hg(II) acetate should be placed in

conjugation with the side-chain double bond and should be represented by 77a, 77b and 77c respectively. The anomalous absorption [λ_{max} 207 nm (ϵ,7000)] in the products 77a — 77c, they ascribed, was due to the inability of the double bonds to attain co-planarity as a result of the interaction between the C-12 hydrogen atoms and the hydrogens of the isopropenyl side chain[63]. Osmylation of dihydro dehydro lupenyl acetate 77d followed by Pb (IV) acetate cleavage afforded the dioxo-acetate 78a which in carbon disulphide showed three distinct peaks in the carbonyl region at 1698 (acyclic carbonyl), 1707 (six membered ring ketone) and 1732 (OAc) cm^{-1}.

Further evidence that the dioxo compound possessed structure 78a came from mass spectral study. 78a showed a base peak at m/z 402 ($C_{26}H_{42}O_3$), corresponding to the ion 79a formed as a result of a Mclaffenty rearrangement depicted below[64].





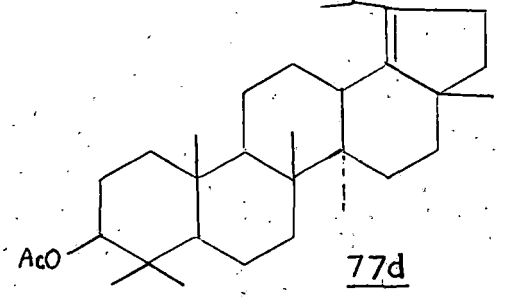
77a R_1 = CH_3, R_2 = Ac

77b R_1 = CH_2OAc, R_2 = Ac

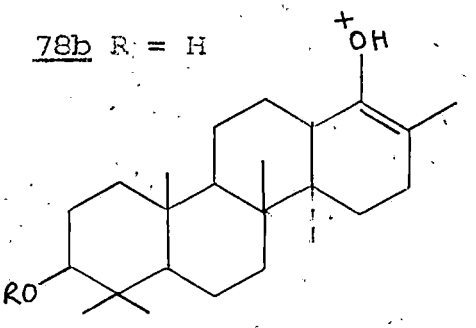
77c R_1 = $COOCH_3$, R_2 = Ac

78a R = Ac

78b R = H



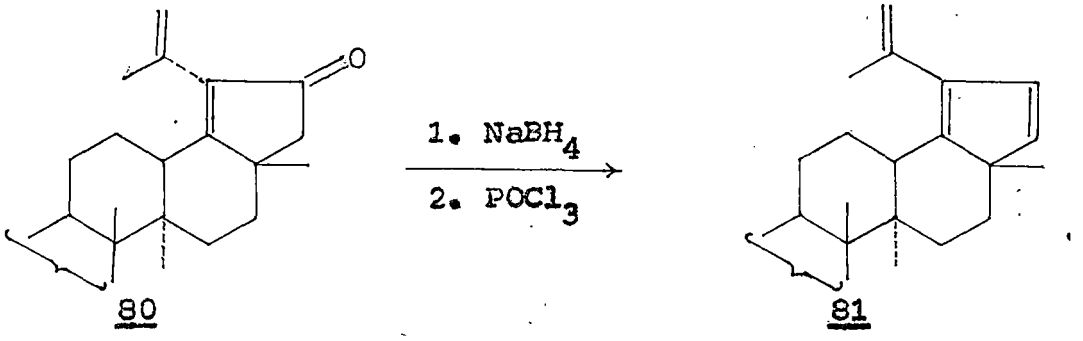
77d



79a R = Ac

79b R = H

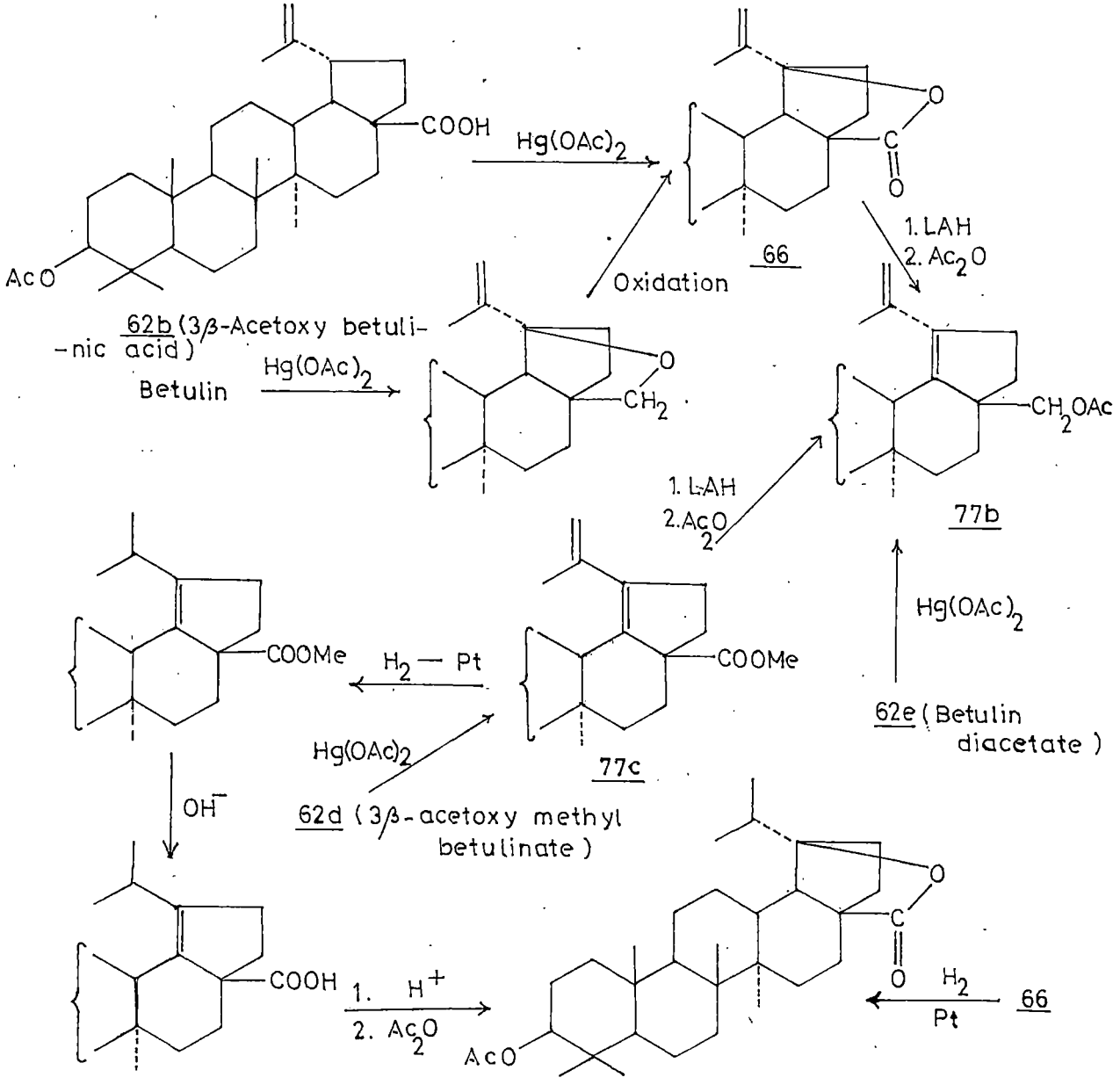
A prominent peak at m/z 360 corresponding to the ion 79b also occurred in the mass spectrum of the corresponding dioxo-alcohol 78b. Oxidation of 77a with chromic acid gave a mixture of products from which the dienone acetate 80 was isolated by chromatography.



80 81

Spectroscopic analysis of 80 indicated the presence of an isopropenyl group and a cyclopentenone system. ^1H NMR showed a sharp singlet at τ 7.75 (2H), assigned to the methylene protons adjacent to the carbonyl group suggesting that these protons were magnetically equivalent and were flanked by a quarternary carbon atom. Reduction of the dienone acetate with $NaBH_4$ followed by dehydration with $POCl_3$ afforded the trienyl acetate 81. Besides showing the presence of the isopropenyl group, the ^1H NMR spectrum of 81 exhibited a pair of doublets (J_{AB} 6.0 Hz) at τ 3.73 and 3.91 indicative of a cis - disubstituted double bond in a five membered ring and flanked by quarternary carbon atoms. Thus a combination of spectral and chemical evidence led them to revise their initial

structures 65e, 65d, 65a and enabled them to formulate correct structures for the products of Hg(II) acetate oxidation of lupeol acetate 62f, betulin diacetate 62e and methyl acetyl betulinate 62d as 77a, 77b and 77c respectively. Since the correct structure of ether[57] and lactone[55] obtained by Hg(II) acetate oxidation of betulin and betulinic acid has already been proposed, the following interconversions[62] as shown in the scheme VI further substantiated their revised formulations.



Scheme VI

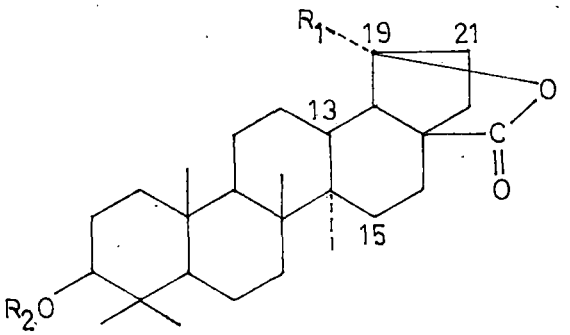
The Structure of the Cactus Triterpene Lactone Thurberogenin:

(i) The structure of thurberogenin 28, 19β-lactone [64] originally proposed:

Djerassi and co-workers isolated thurberogenin from the cactus Lemaireocerus thurberi[65]. It possesses[66] a reduceable double bond, gave an acetate which on treatment with SeO_2 is AcOH afforded an unsaturated aldehyde (λ_{max} 222 nm, log ε 4.01). These reactions eliminate completely from consideration of an α- or β amyrin skeleton but rather suggest that it belongs to the class of lupeol triterpenes. Thurberogenin acetate on ozonolysis produced formaldehyde and a norketone characterised as the oxime. The unsaturated aldehyde mentioned above on ozonolysis afforded a bis- nor acid characterized as the methyl ester. This behaviour was in complete analogy to betulin[67]. Oxidation of thurberogenin with CrO_3 - pyridine oxidation yielded thurberogenone, which showed two carbonyl bonds, ν_{max} 5.66 and 5.90μ, the latter corresponding to a six membered (or larger) ring ketone. Thurberogenone on $NaBH_4$ treatment regenerated thurberogenin indicating the hydroxy group is most likely equatorial and hence β-oriented. Dihydrothurberogenin on PCl_5 treatment and subsequent ozonolysis afforded acetone and A-nor-dihydro thurberogenone ν_{max} 5.64 (lactone) and 5.78μ (five membered ring ketone). Thus the sequence of reactions stated above proved the presence of an isopropenyl group and a six membered ring A having 3β hydroxy

4,4-dimethyl moiety, the latter is present in most of the pentacyclic triterpenes alcohols. Based on biogenetic grounds they also proposed that the carbonyl group of the lactone ring of thurberogenin and also stellatogenin[68], originates at C-17. Some support for this assumption came from co-occurrence of betulinic acid and oleanolic acid with these lactones. Stellatogenin 82 is a dihydroxy lactone and was correlated with thurberogenin by dehydrative elimination of the side-chain tertiary hydroxyl group. Four alternatives (position 13, 15, 19, 21) were considered as termination point of the five-membered lactone ring. With LAH treatment thurberogenin yielded a triol which gave a diacetate which was resistent to CrO_3 oxidation; and this led them to propose at that time the tertiary hydroxyl rather than secondary hydroxyl function (position 15 or 21) which they found to be incorrect later[54]. Of the two tertiary positions of lactone termination i.e. C-19 and C-13 they proposed the former based mainly on a base-catalysed rearrangement which they then considered E-homo rearrangement of the 30-nor-20 ketone 67, obtained by ozonolysis of thurberogenin acetate 66. They then formulated 83 as the structure of the rearranged product and the structure of thurberogenin as 64. Based on a detailed spectral analysis, they in a later communication[54] revised the formulation of this rearranged product as well as the structure of thurberogenin. The alternative structure 63e for thurberogenin was discurded by them as the derived nor-

ketone lactone 84 would be incapable of this rearrangement.



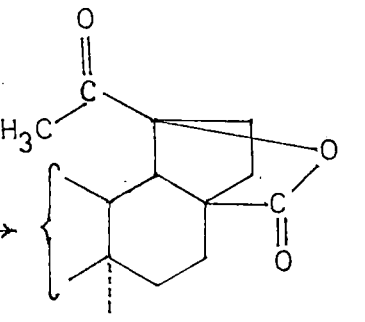
64 R_1 = C(CH3)=CH2 , R_2 = H

66 R_1 = C(CH3)=CH2 , R_2 = Ac

82 R_1 = C(CH3)(OH)CH3 , R_2 = H

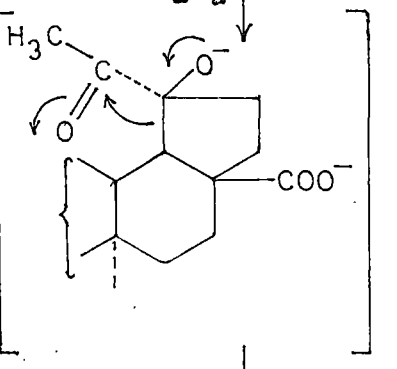
(Old structure of thurberogenin 64 and stellatogenin 82 proposed in 1955)

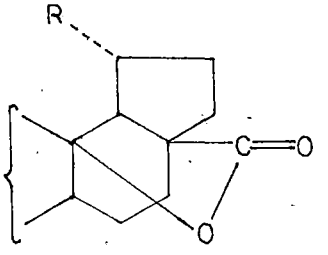
66 $\xrightarrow{O_3}$



67

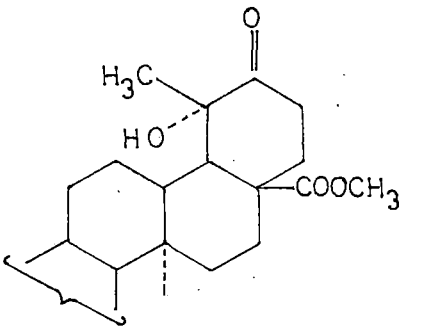
1. OH^-
2. CH_2N_2





63e R = isopropenyl

84 R = $COCH_3$

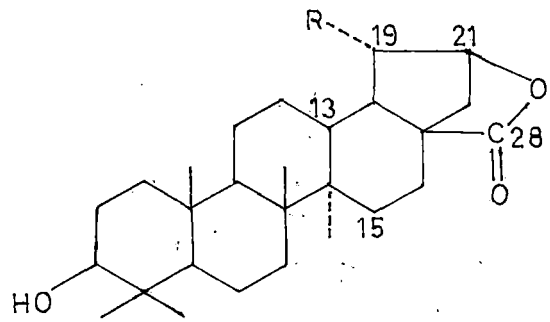


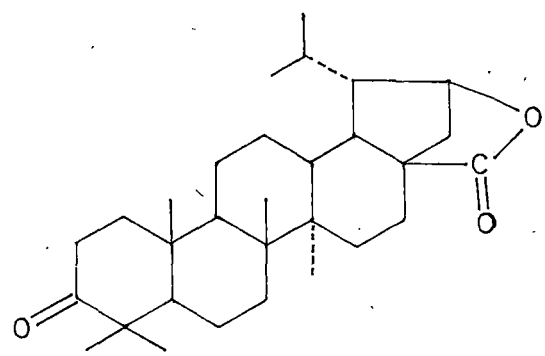
83

In order to establish the genetic relationship of thurberogenin with lupane triterpenes, Djerassi and Hodges[69] treated the 30-nor-20-ketone 67 with calcium in liquid ammonia and isolated an acidic material in low yield which on esterification with CH_2N_2 followed by acetylation afforded the known methyl 3-acetoxy-30-nor-20-keto betulinate 73b[70a]. Identity with an authentic specimen was established by mixture melting point determination, IR comparison and close similarity of the ORD curves, later they revised the structure of 83 based on the revised formulation of thurberogenin.

(ii) Revised Structure of Thurberogenin as C-28, 21 lactone 85a.

After more than a decade of the original proposition of the structure of thurberogenin by Djerassi et al[53], the same group[54] published in 1967 a communication concerning the revised structure of thurberogenin 85a. They intensively applied ^1H NMR and mass spectrometry which were not available at the time when they proposed[53] their initial structure 64 for thurberogenin.





87

85a R = isopropenyl

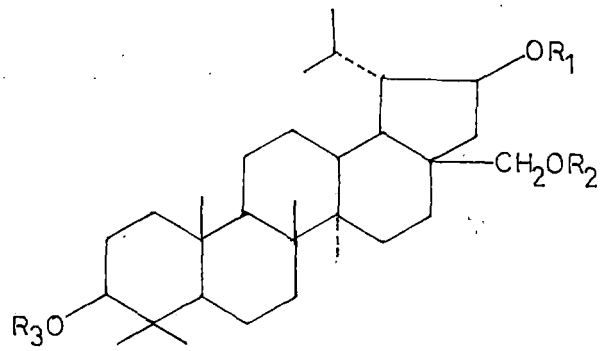
85b R = isopropyl

85c R = $-C(OH)(CH_3)_2$

86 R = $-COCH_3$

(revised structure of thurberogenin 85a and stellatogenin 85c proposed in 1967).

^{1}H NMR spectrum of dihydro thurberogenone[54] (now known to have structure 87) obtained by hydrogenation of the side chain and oxidation of the C-3 hydroxyl group, showed a complex resonance at δ4.6 (integrating for one proton) indicative of the grouping H-C-OR. Since the other two oxygens in 87 are carbonyls, this function must involve the lactonic hydroxyl group and the hydroxy group involved in the lactone formation must therefore be secondary. Further evidence for this conclusion came from mass and ^{1}H NMR analysis of the triacetate 88b obtained by LAH reduction and subsequent acetylation of dihydro thurberogenin 85b. The acetylation product 88b,



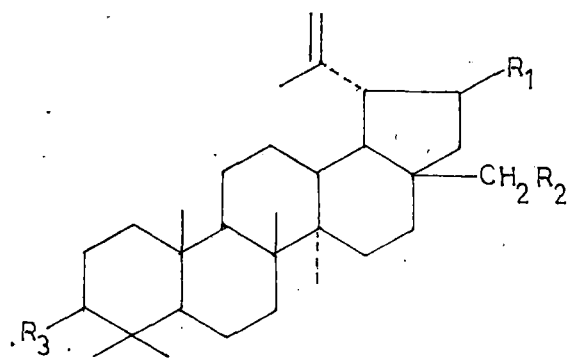
88a $R_1 = R_2 = R_3 = H$

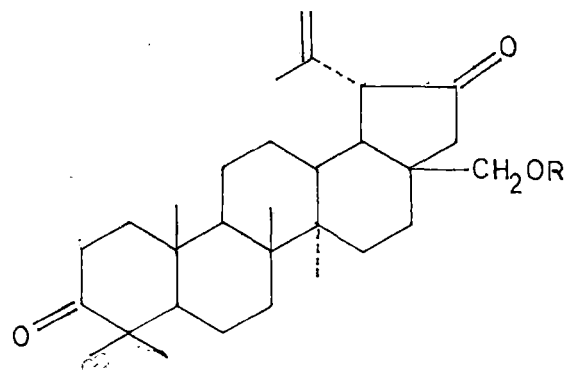
88b $R_1 = R_2 = R_3 = Ac$

originally thought to be a diacetate, demonstrated a molecular ion peak in the mass spectrum at m/z 586 and also a prominent fragment ion at 526 (M - AcOH) suggesting it to be in fact a triacetate ($C_{36}H_{58}O_6$). The presence of three acetyl groups was confirmed by ^1H NMR analysis which showed signals at δ1.96 (singlet, 3H) and 2.04 (singlet, 6H). 100 Mz spectrum resolved the complex absorption pattern in the δ 4.2 - 5.2 region integrating for four protons to an AB quartet (2H, $-CH_2OAc$). The presence of a primary alcohol (from reduction of lactonic carboxyl), a secondary alcohol (ring A) and another secondary alcohol (lactonic hydroxyl) in triol 88a has been thus confirmed.

As the lactonic hydroxyl was established as secondary, only two termini (C-15 and C-21) for the five membered lactone ring were considered by them. Acetylation of 89a under carefully controlled conditions produced a monoacetate 89b which on Jones oxidation and mild hydrolysis afforded the diketo-alcohol 90a, $C_{30}H_{46}O_3$ (M^+ 454 m/z), $\nu_{max}^{CHCl_3}$ 5.77 and 5.70μ. The higher wavelength absorption in IR spectrum was attributed

to C-3 carbonyl and the lower wavelength due to cyclopentanone. This led to the structure of the diketo alcohol as 90a, the triol 89a and its progenitor, thurberogenin 85a. A correlation with a known compound of lupane series was also achieved by them. The acetate 90b from the diketo alcohol 90a on WolffKishner reduction afforded the known compound 3-deoxybetulin 89c.





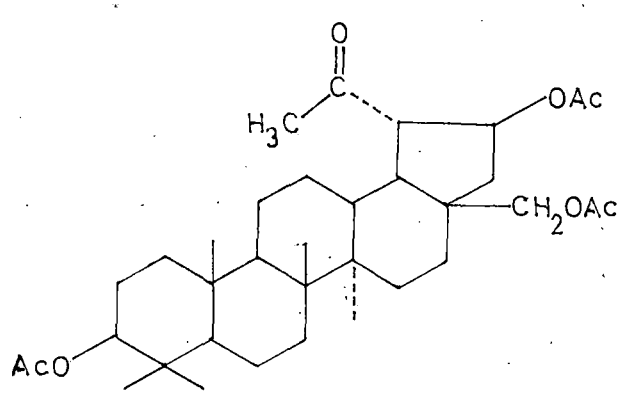
89a $R_1 = R_2 = R_3 = OH$

89b $R_1 = R_3 = OH$, $R_2 = OAc$

89c $R_1 = R_3 = H$, $R_2 = OH$

90a R = H

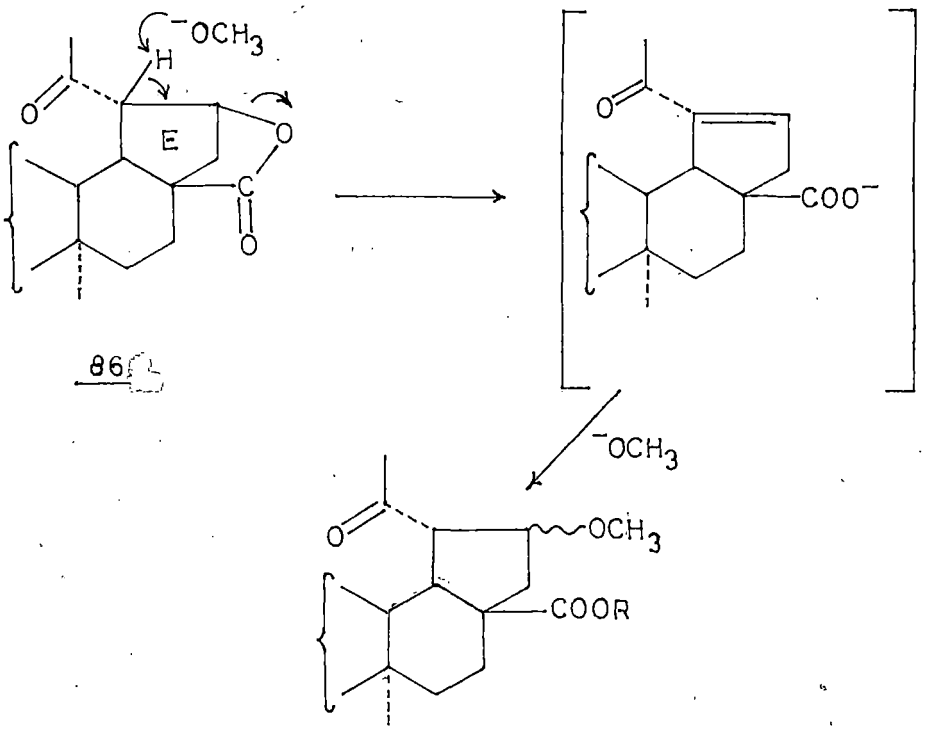
90b R = Ac



91

They compared the ORD spectra of the known 30-nor-keto ester 73b with that of 30-nor ketone 91 derived from the oxidation of the triacetate of thurberogenin triol 89a. The ORD spectra of 73b and 91 are very similar both exhibiting moderately strong positive CE. This demonstrates that the isopropenyl group at C-19 in thurberogenin 85a possesses α-stereochemistry. They made an in-depth study of the base-catalyzed rearranged product which was formulated previously as 83. The mass spectrum showed molecular ion peak M^+ at m/z 502 ($C_{31}H_{50}O_5$) and a prominent fragment ion at m/z 470 ($M-CH_3OH$). The molecular composition $C_{31}H_{50}O_5$ of the revised formulation 92a differed from that of previous one 83 by the presence of an extra CH_2 unit which were not amenable to detection by conventional elemental analysis. ^1H NMR spectrum further supported the structure 92a. The presence of an ethereal $-OCH_3$ group was confirmed by a peak δ 3.21 (3H, singlet) besides showing signals at δ 3.70 (3H, singlet, $-COOCH_3$) and δ 2.17 (3H, singlet, $-COCH_3$).

Since with their revised formulation of thurberogenin 85a the base catalysed E-homo rearrangement of the derived nor-ketolactone 86 cannot occur, they proposed[54] the following mechanism for the formation of 92a .



92a R = CH_3

Interestingly enough, the nor-ketolactone 67 derived from ozonolysis of mercuric acetate oxidation product (revised structure 66) of acetyl betulinic acid 62b possesses the necessary structural requirements for E-homo rearrangement and should undergo this ring expansion reaction under appropriate reaction condition. Indeed, Vystrcil and Blecha[70b] and Khastgir et al[70c] independently advanced the structure of the E-homo rearranged product 92b based on a combination of physical and chemical studies.

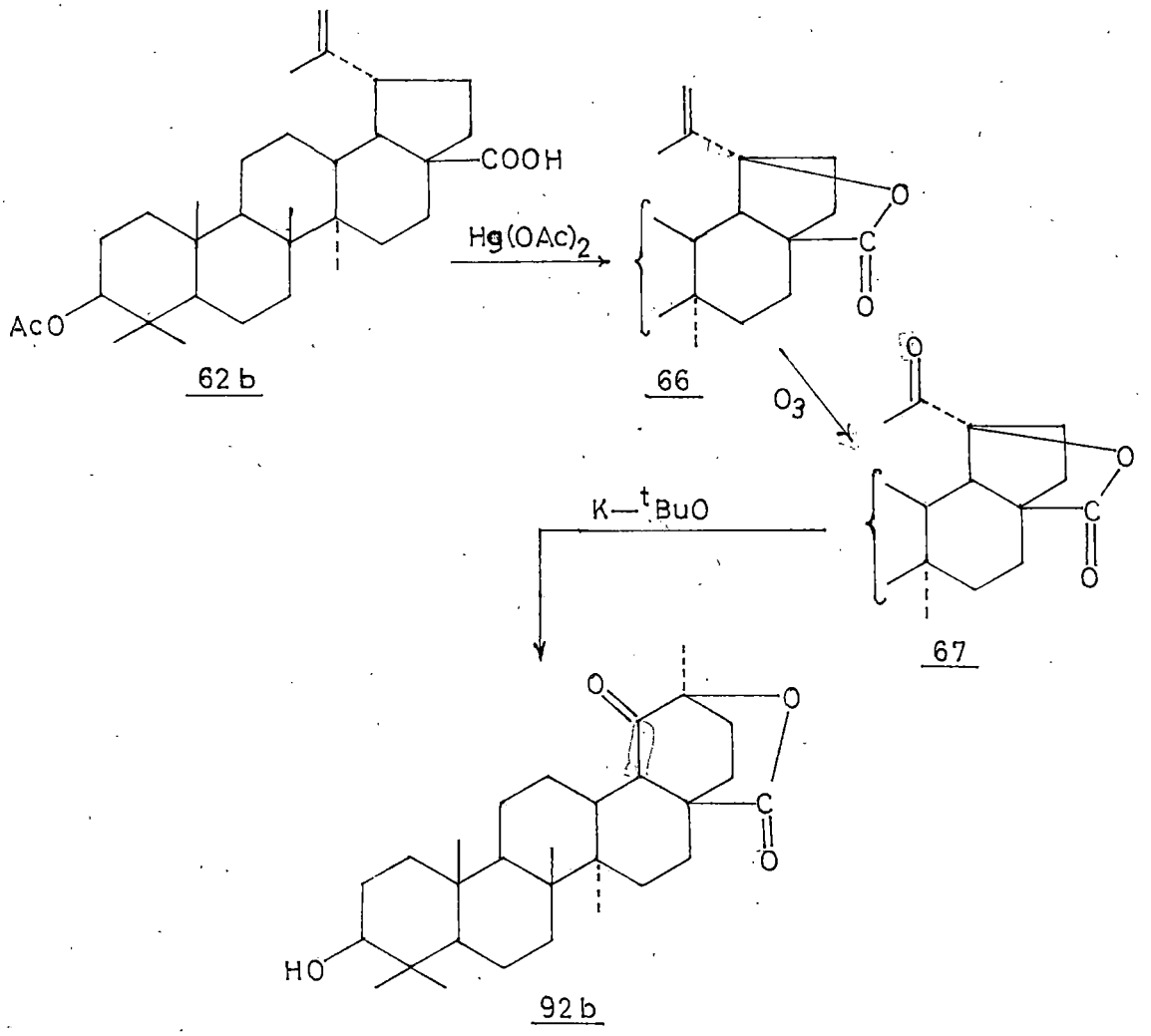
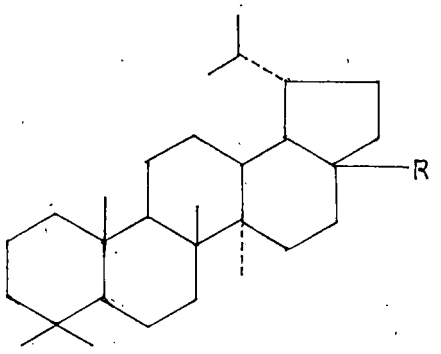
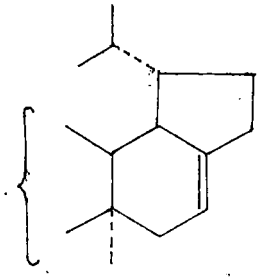


Photo-Oxidation of 28-Lupanol and its derivatives

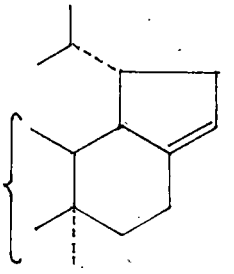
Photo-oxidation of 28-lupanol (3-deoxybetulin) 93 with Pb(IV) acetate has been carried out by Vystrcil and Protiva[71]. Besides the hydrocarbons 28-nor-16-lupene 94, 28-nor-17(22) lupene 95 from the reaction mixture, they isolated 13β, 28-epoxy lupane 96.



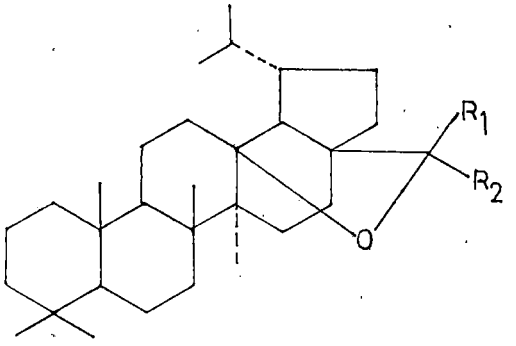
93 R = CH_2OH



94



95



96 $R_1 = R_2 = H$

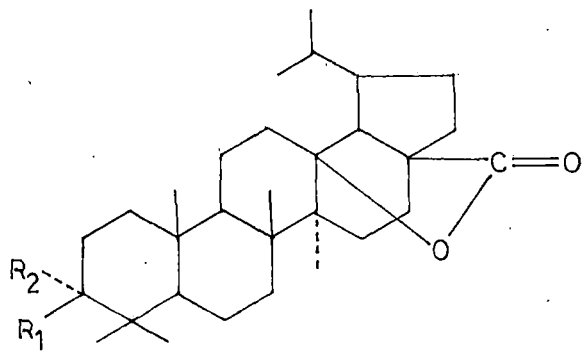
97 $R_1 + R_2 = O$

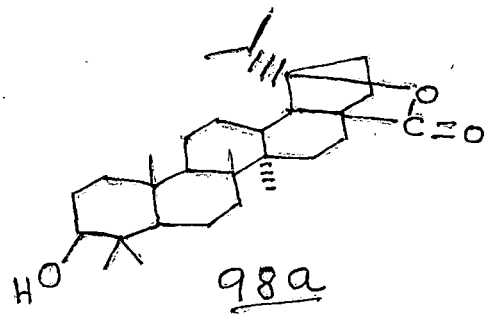
The structure of the cyclic ether 96 was proposed on the basis of spectral data and chemical conversions. A band at 1019 cm^{-1} in the IR spectrum of 96 indicated the presence of an ether linkage and ^1H NMR spectrum showed that it must

be connected with a tetra substituted carbon atom at C-13 . The confirmation of this assumption came from oxidation of the ether 96 to a lactone 97. IR band at 1765 cm^{-1} demonstrated that the lactone 97 is 5-membered. A detailed spectra analysis led them to advance the structure of the ether and lactone as 96 and 97 respectively.

The lupane triterpene lactone 3β-hydroxy-lupene-13β, 28-olide 63f from Dillenia indica.

Banerjee and co-workers[72] isolated a hydroxy lactone 63f $C_{30}H_{48}O_3$ (M.W 456 by MS) m.p. 325° (d) $[\alpha]_D$ + 63.4 from methanol extract of stem-bark of Dillenia indica (Dilleniaceae). IR showed bands at 1754 (5-membered lactone) and 3350 cm^{-1} (OH) suggestive of a hydroxy lactone. It gave an acetate 63b m.p. 319-320°. On oxidation with Jones reagent it afforded a ketone 98 m.p. 328-330°, $[\alpha]_D$ + 70°, ν_{max} 1689 and 1754 cm^{-1}. The ketone gave a positive Zimmerman's test and on reduction with $NaBH_4$ gave back the hydroxy lactone 63f which proved the β configuration of OH group at C-3. Its mass spectrum exhibited a strong peak (M-43) corresponding to the loss of isopropyl group[73]. Analysis of NMR spectrum coupled with biogenetic ground indicated the compound to be in the lupane series. It was not identical with dihydro thurberogenin 85b or the dihydrolactone 98a obtained from Hg(II) acetate oxidation of acetyl betulinic acid followed by hydrogenation and hydrolysis. 63f on reduction with LAH gave a triol 99a m.p. 280-82°, $[\alpha]_D$ + 34°.



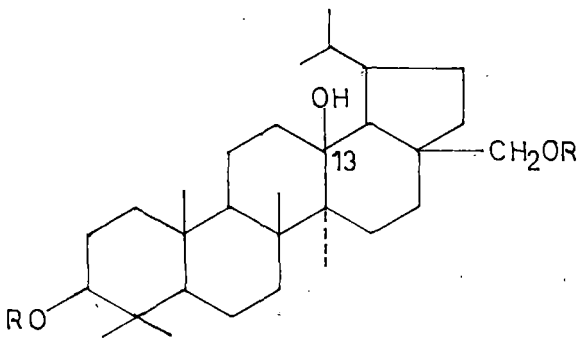


98a

63f R_1 = OH, R_2 = H

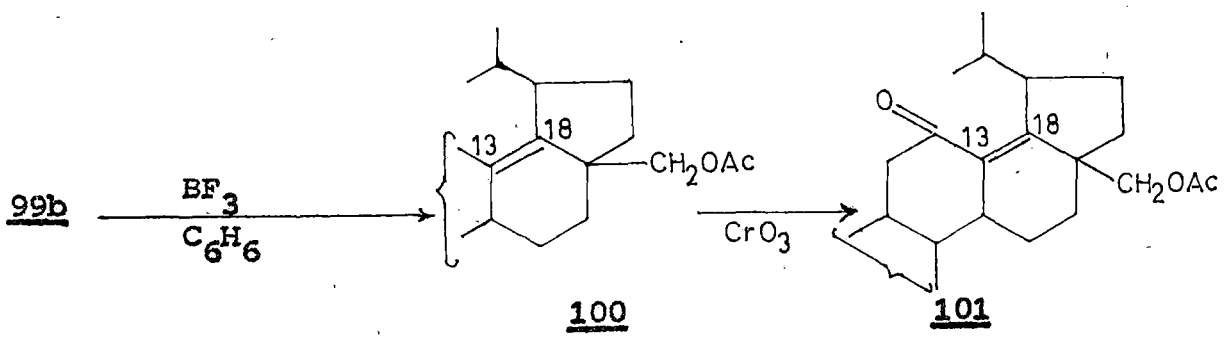
63b R_1 = OAc, R_2 = H

98 R_1, R_2 = O



99a R = H

99b R = Ac

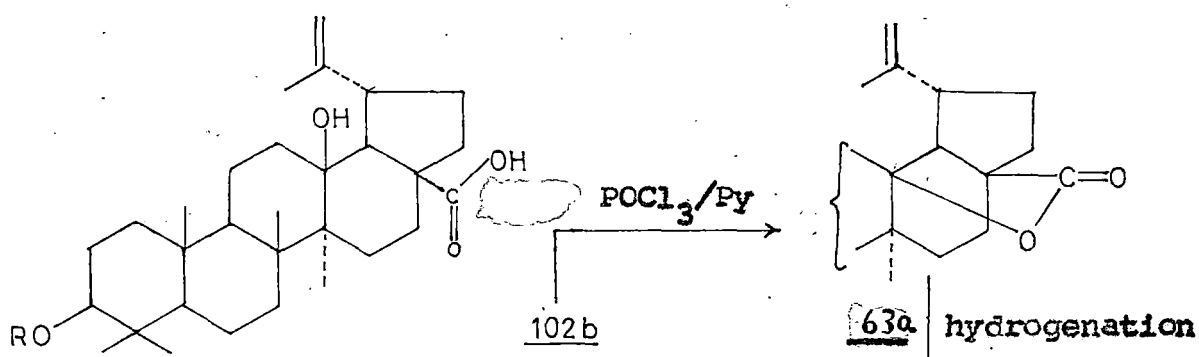


Its IR spectrum was devoid of carbonyl band and on acetylation yielded a diacetate 99b $C_{34}H_{56}O_5$ which was stable to CrO_3 oxidation indicating that the -OH group must be tertiary one i.e. at C-13. The triol diacetate 99b on treatment with BF_3 in dry benzene yielded a compound 100 m.p. 295-296°, $[\alpha]_D$ + 70.2. Its IR spectrum did not show absorption for a trisubstituted double bond. UV λ_{max} 210 (ϵ = 5650), 215 (ϵ = 4520), 220 nm (ϵ = 3650) were indicative of a tetrasubstituted double bond[74]. On oxidation with CrO_3 in AcOH it yielded a conjugated ketone 101 m.p. 290°, $[\alpha]_D$ + 65°, ν_{max} 1690 cm^{-1}, λ_{max} 242 nm (ϵ = 13520). The absence of any absorption for vinylic proton in ^1H NMR showed that the position of the double bond introduced by dehydration of triol diacetate in 100 was most likely to be at $\Delta^{13(18)}$. These considerations led them to suggest the structure of the hydroxy lactone as 3β-hydroxy-lupane-13β, 28-lactone (63f).

(63f) obtained as a transformation product :

Almost a decade after the isolation of (63f), Anjaneyulu et al[75] isolated a new dihydroxy lupane triterpene carboxylic acid, terminic acid 102a from the n-hexane extract of the heart wood of Terminalia arjuna. A combination of chemical and specta evidence has been presented by them to establish the structure of this lupane dihydroxy carboxylic acid as 3β, 13β-dihydroxy lup-20(29)-en-28 oic acid 102a. When 3-acetyl terminic acid 102b was treated with $POCl_3$ - pyridine a γ-lactone (63a), ν_{max} 1785 cm^{-1}, $C_{32}H_{48}O_4$ m.p. 296-298°, $[\alpha]_D$ + 16° was obtained.

Its ^{1}H NMR spectrum showed signals at δ 4.6 and 4.7 (2H, brs, methylene group), δ 1.66 (3H, s, vinylic methyl) indicating that the five membered ring E is intact.



102a R = H

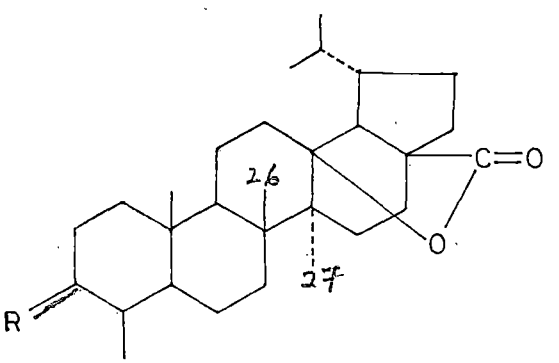
102b R = Ac

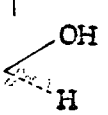
Incidentally, this γ-lactone 63a on hydrogenation furnished a dihydrolactone whose physical constants and spectral properties are identical with 63b, the acetylated product of the natural lactone 63.

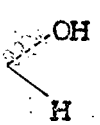
Structure of 24-nor-3β hydroxy lupan - 13β, 28 lactone 103a and 24-nor-3α hydroxy lupan-13β, 28lactone 103b.

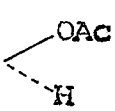
Bhandari and Rastogi[76] have isolated 24-nor-3β hydroxy lupan-13β, 28 lactone (Caltholide) 103a and its 3α isomer (epicaltholide) 103b from ethanol extract of Caltha palustris

(Ranunculaceae). Caltholide **103a** m.p. >310°, $C_{29}H_{46}O_3$ (M^+ at m/z 442.3506) gave a positive test for triterpenes. The IR spectrum showed absorption for a γ-lactone (1755 cm^{-1}) and a hydroxyl group (3350 cm^{-1}). Its ^1H NMR spectrum exhibited signals for six methyl groups in the region δ 0.78-1.11 and a carbinolic proton at δ 3.7. The absence of any additional peak around δ 4 indicated that the γ-lactone terminated at a tertiary centre.

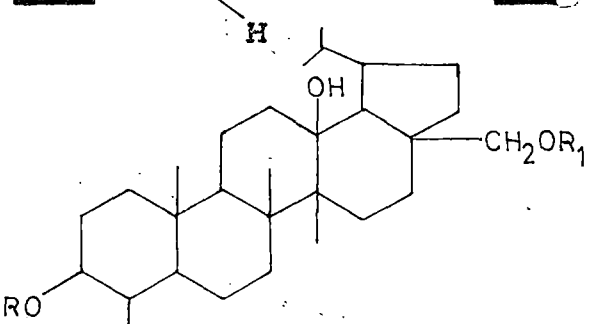


103a R = 

103b R = 

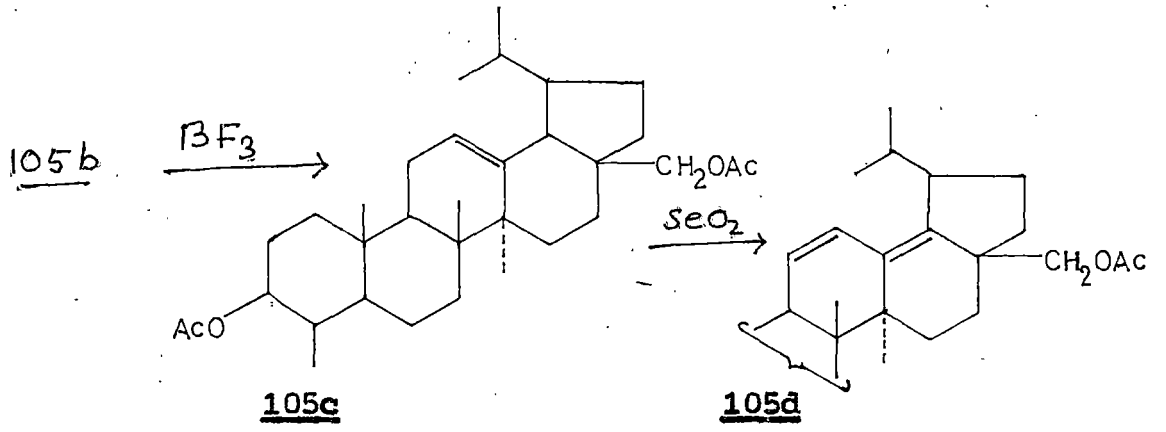
103c R = 

104 R = O



105a R = R_1 = H

105b R = R_1 = Ac

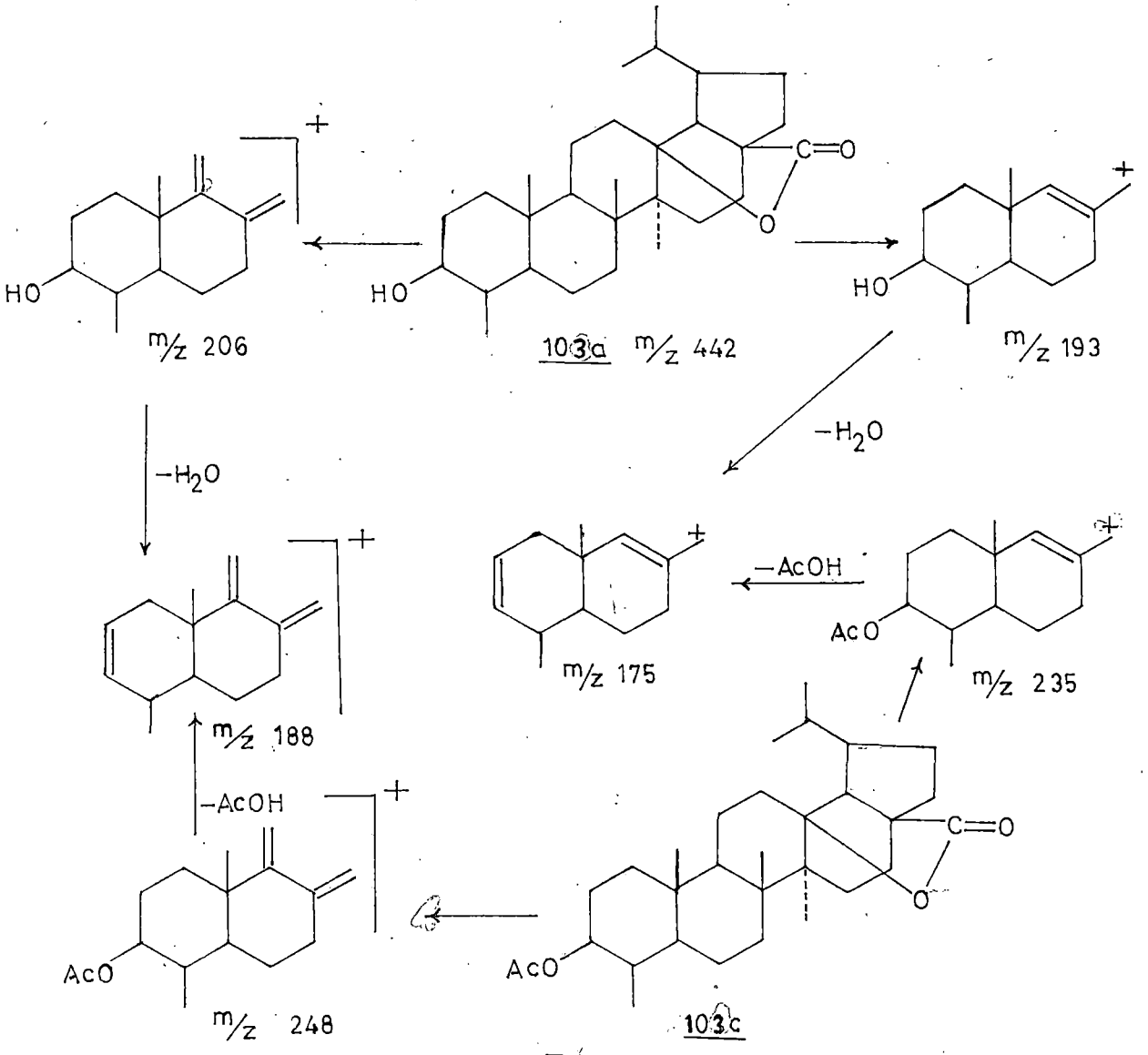


105c 105d

The high field ^1H NMR (400 MHz) spectrum of caltholide acetate 103c showed the most downfield signal in the methyl region at δ 1.16 due to the C-26 methyl group which was ascribed to the diamagnetic anisotropic effect of the lactone ring while C-27 methyl group appeared at 1.13. The two methyls of isopropyl group resonated as a singlet at 0.87. The appearance of a doublet (J = 7 Hz) at 0.85 due to a secondary methyl confirmed C-24 as the nor-position thus excluding the possibility of nor-methyl at the C-8 and C-10 positions. The C-25 methyl group appeared at δ 0.82. The carbinolic proton geminal to the acetoxy group appeared as a doublet of triplets at 4.79 due to coupling with methine at C-4 and methylene protons at the C-2 position which was in agreement with its α (axial) disposition. The multiplicity ($J_{3ax,4ax}$ = 12 Hz) also showed the equitorial methyl group at the C-4 position.

The fragmentation pattern[76] (Scheme VII) in the mass spectra of caltholide - 103a and its acetate derivative 103c

was indicative of lupane series. The fragment ions at m/z 206.1686 ($C_{14}H_{22}O$) and 193.1512 ($C_{13}H_{21}O$) from 103a and corresponding ions at m/z 248 and 235 from 103c and the common ions 188.1537 ($C_{14}H_{20}$) and 175.1504 ($C_{13}H_{19}$) from both 103a and 103c comprise of ring A/B and hence the presence of the lactone group in C/D rings was confirmed. The location of the hydroxyl group at C-3 and the nor-position in rings A/B were suggested from the base peak at m/z 206.



Scheme VII

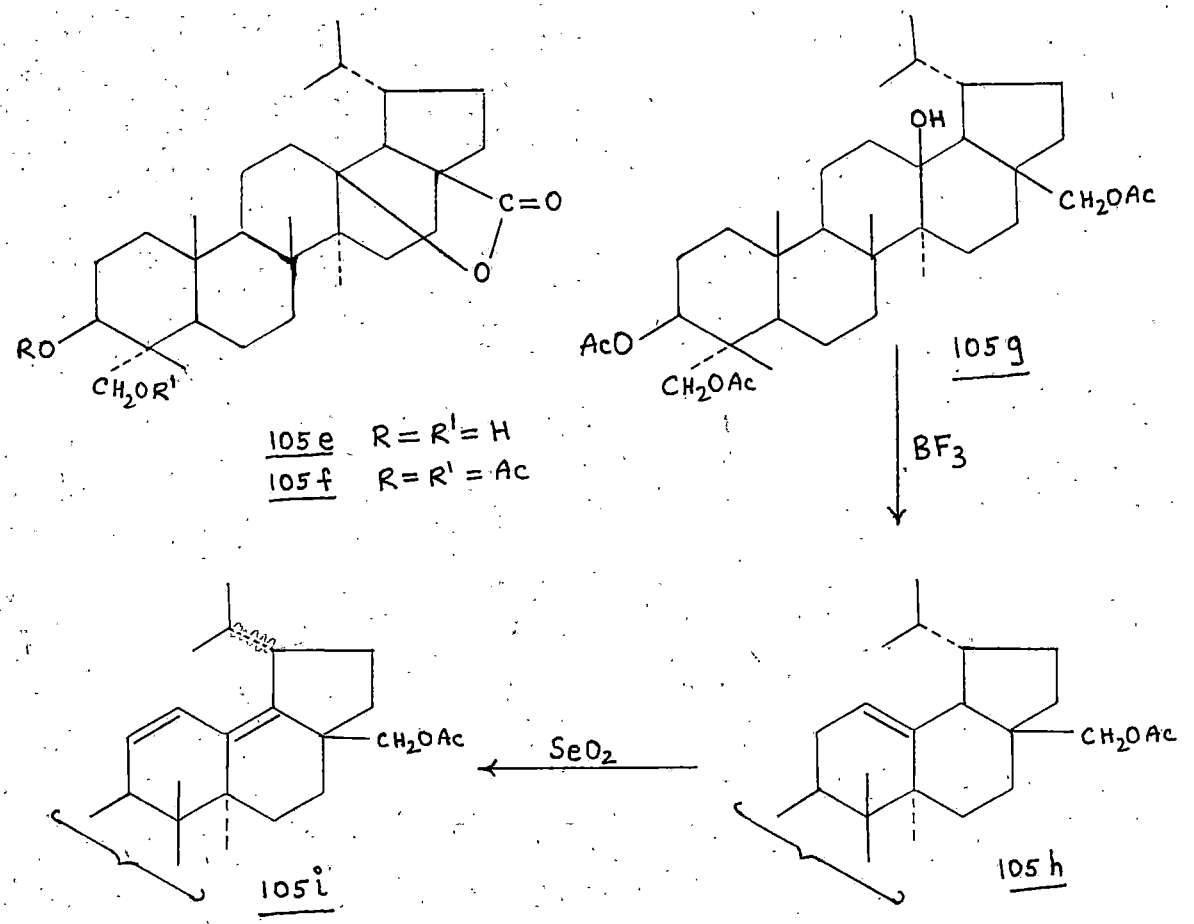
On oxidation caltholide 103a furnished a ketone 104 ν_{max} 1700 cm^{-1} (six membered ring ketone) and on LAH reduction afforded a triol 105a whose IR spectrum was devoid of carbonyl absorption. The triol 105a yielded a diacetate 105b, ν_{max} 3480 cm^{-1} indicated the presence of a tertiary hydroxyl function. ^1H NMR showed a singlet for two acetoxy methyls at δ1.96 and AB quartet at 4.08 and 4.65 (each d, J = 12 Hz, $-CH_2OAc$) and at 4.72 (1H, -CHOAc). The diacetate 105b was dehydrated with BF_3 - etherate to afford 105c which gave a yellow colour with TNM and showed IR absorption for trisubstituted double bond. ^1H NMR exhibited a signal for an olefinic proton at δ 5.1. It exhibited molecular ion peak at m/z 512 and the base peak at 203 in the mass spectrum was explained from retro Diels-Alder fragmentation. 105c on SeO_2 oxidation gave a heteroannular diene 105d, λ_{max} at 243, 252 and 262 nm. Thus, the position of double bond at Δ^{12} in 105c was established and also the presence of a tertiary hydroxyl group at C-13 in 105b. These results confirmed the structure of caltholide 103a as 24-nor-3β-hydroxy lupan-13β, 28-lactone.

Epicaltholide 103b showed m.p. 242-46°, M^+ (442 m/z, $C_{29}H_{46}O_3$), IR ν_{max} 3250 (OH) and 1742 cm^{-1} (γ-lactone). The ^1H NMR spectrum of 103b showed a carbinolic proton at δ3.72 and its acetate at 4.72 with $W_{1/2}$ = 7 Hz which was indicative of its equatorial configuration. The hydroxy group, therefore,

must be α (axial). The mass spectra of epicaltholide 103b and its acetate were found to be identical with those of caltholide 103a and its acetate. 103b on pyridinium chlorochromate oxidation afforded a ketone 104 (M^+ 440 m/z) which was found to be identical with the ketone obtained from 103a by similar oxidation. Thus, epicaltholide was assigned structure 103b, 3α hydroxy epimer of Caltholide 103a.

The structure of a new triterpene lactone, Palustrolide 105e obtained from Calthapalustris (Ranunculaceae).

The structure of a new triterpene lactone, palustrolide 105e obtained from Caltha palustris (Ranunculaceae) $C_{30}H_{48}O_4$, ν_{max} 3300, 1760 cm^{-1} (a hydroxy-γ-lactone), was deduced by Bhandari and Rastogi[76a]. Corresponding diacetate 105f in ^1H NMR spectrum displayed signals for six methyls between δ 0.78-1.20, two acetoxy methyls at δ1.95 and δ1.78 and the corresponding carbinolic protons a, an ABq (J = 12 Hz) at δ3.72 for a primary acetoxy methylene group and the signal for a methine geminal to an acetoxy group appeared as a dd (J = 10, 6 Hz) at δ 4.7 confirming one primary and one secondary hydroxyl groups in 105e. Mass spectral fragmentation of 105e and 105f established that primary and secondary hydroxyl groups were located in rings A and B and also restricted the location of the lactone function to rings C, D and E in a lupane skeleton.



The stereochemistry of the hydroxyl at C-3 was deduced as β (equatorial) since the carbinolic proton appeared as a double doublet (J_{aa} = 10 Hz, J_{ae} = 6 Hz) at δ 4.7 in ^{1}H NMR of diacetate 105f. 105e gave a monoacetonide which confirmed the presence of a primary hydroxyl at the C-23 position. 105e on reduction with LAH followed by acetylation gives the triacetate 105g which showed ν_{max} 3480 cm^{-1} indicating the presence of

tertiary hydroxyl group and hence the lactone 105e is a tertiary γ-lactone. The triacetate 105g on BF_3 treatment yielded a dehydrated product 105h which in ^1H NMR showed a peak at 5.13. Coupled with mass fragmentation enabled them to place lactone ring at C-13. The hetero annular diene 105i obtained by SeO_2 on 105h established conclusively the structure of Palustrolide as 3β, 23-dihydroxy-lupan-13β → 28 lactone 105e.