PART - I

PREPARATION AND CIRCULAR DICHROISM STUDIES OF TRITERPENE LACTONES OF LUPANE SERIES

CHAPTER I

A short review on the structure of the synthetic lactone derived from Hg(II) acetate oxidation of acetyl betulinic acid and the closely related naturally occurring lactone thurberogenin

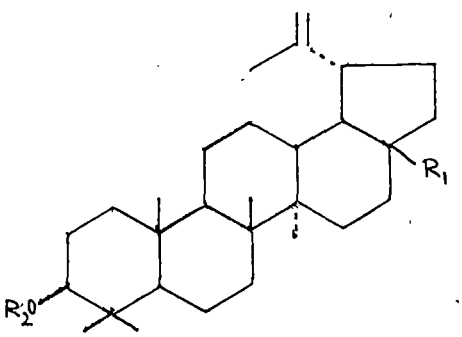
Section A. The structure of the synthetic lactone derived from mercuric acetate oxidation of acetyl betulinic acid 1b

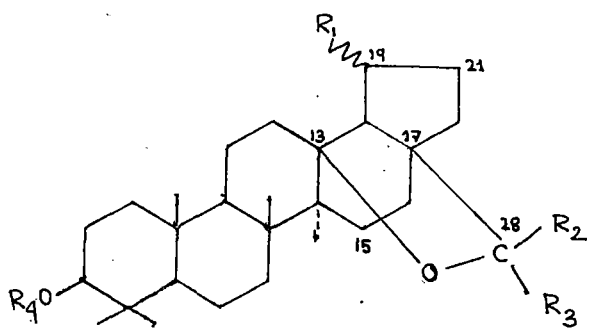
(i) The C-28, 13β-lactone structure 2a proposed initially:

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[1] first applied this reaction on α-amyrin, β-amyrin and lupene derivatives. Of those only triterpenes of the lup-20(29)-ene derivatives underwent this reaction. Betulin and lupeol gave dehydro compounds of unknown structure. Since their esters also underwent dehydrogenation, but not their dihydro derivatives, it was concluded that this dehydrogenation was associated with the presence of olefinic bond.

Allison and co-workers[2] carried out Hg(II) acetate oxidation on acetyl betulinic acid 1b and obtained a γ-lactone, assigned as 2a. IR ν_{max} 1792 cm^{-1}. This on hydrogenation gave a dihydrolactone 2b which was also obtained by Hg(II) acetate

oxidation of betulin 1c followed by hydrogenation, acetylation and oxidation. Betulin 1c afforded a cyclic ether assigned as 2c, IR ν_{max} 1630, 836 cm^{-1} (vinylidene group). NMR of the corresponding acetate 2d exhibited peaks at 63 and 89 cps ($-C-CH_2-O$). Hydrogenation of 2d followed by oxidation gave





1a R_1 = -COOH, R_2 = H

1b R_1 = -COOH, R_2 = Ac

1c R_1 = $-CH_2OH$, R_2 = H

1d R_1 = $-COOCH_3$, R_2 = Ac

1e R_1 = $-CH_2OAc$, R_2 = Ac

1f R_1 = CH_3, R_2 = Ac

2a R_1 = isopropenyl R_2, R_3 = O, R_4 = Ac

2b R_1 = isopropyl, R_2, R_3 = O, R_4 = Ac

2c R_1 = isopropenyl, R_2 = R_3 = H, R_4 = H

2d R_1 = isopropenyl, R_2 = R_3 = H, R_4 = Ac

2e R_1 = isopropenyl, R_2, R_3 = O, R_4 = H

2f R_1 = isopropyl, R_2, R_3 = O, R_4 = H

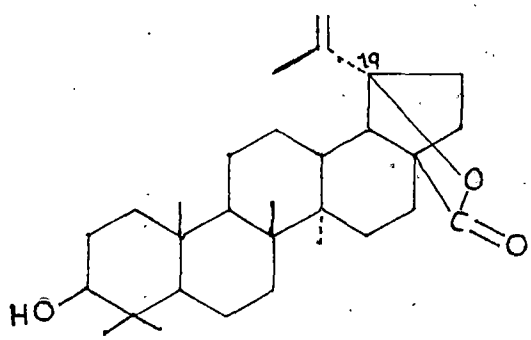
the γ-lactone 2b. The authors advanced the following arguments in favour of their structure 2b for the lactone. The termination of the lactone could be at 13, 15, 19 or 21 position. Lithium aluminium hydride reduction product of 2b on acetylation afforded a diacetate and not a triacetate, thus excluding 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 4f, λ_{max} 206 nm (ϵ = 7900), ν_{max} 1650 and 3050 cm^{-1}.

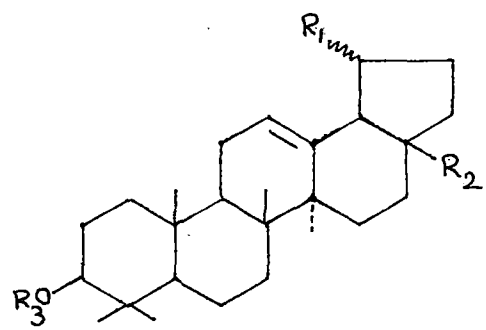
Consequently, the lactone termination in 2b and the ether linkage in 2d must be at the same point, either at C-13 or C-19. The lactone 2e, the hydroxy derivative of the lactone 2a, was found to be different from the cactus lactone thurberogenin which was previously assigned the C-28, C-19 lactone structure 3 by Djerassi et al[3,4] in 1955. Non-identity of thurberogenin with this lactone led Allison and co-workers[2] to assign structure 2a for the acetoxylactone and 2e for the corresponding hydroxy lactone in 1961.

Allison and co-workers also observed that 3β-acetoxy methyl betulinate 1d 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} (-$OCOCH_3$) and then (in 1961) proposed its structure as 4a. The corresponding hydrogenated product 4b,

λ_{max} 205 nm (ϵ = 5300), ν_{max} 876 cm^{-1}, on hydrolysis with sodium ethoxide afforded the acid 4c. The latter on treatment with hydrogen chloride in chloroform gave the lactone 2f identical with the lactone obtained from mercuric acetate reaction of 1b followed by hydrogenation and hydrolysis.

Betulin diacetate 1e on Hg(II) acetate oxidation gave a diene assigned at that time (in 1961) as structure 4d, which was also prepared from the ester 4a and the lactone 2a. Reduction of the ester 4a with LAH gave a diol which on acetylation furnished the same dienyl acetate 4d. Similar reduction of the lactone 2a followed by acetylation gave an





3. thunbergenin
old structure
advanced in 1955

4a R_1 = isopropenyl, R_2 = CO_2CH_3, R_3 = Ac

4b R_1 = isopropyl, R_2 = CO_2CH_3, R_3 = Ac

4c R_1 = isopropyl, R_2 = COOH, R_3 = H

4d R_1 = isopropenyl, R_2 = CH_2OAc, R_3 = Ac

4e R_1 = isopropenyl, R_2 = CH_3, R_3 = Ac

4f R_1 = isopropyl R_2 = CH_2OAc, R_3 = Ac

acetoxy alcohol which on $POCl_3$-pyridine dehydration furnished the dienyl acetate 4d.

(ii) Revised formulation of the lactone as C-28, 19β-lactone 5 :

The structural assignment of the lactone as C-28, C-13 lactone derived from Hg(II) acetate oxidation of acetyl betulinic acid by Allison et al[2] rested partly on its non-identity with the acetate of thurberogenin which was previously assigned C-28, 19-lactone 3. Later Djerassi et al[5] revised the structure of thurberogenin as C-28, 21-lactone 25 and this invalidated the structural assignment of the mercuric acetate oxidation product.

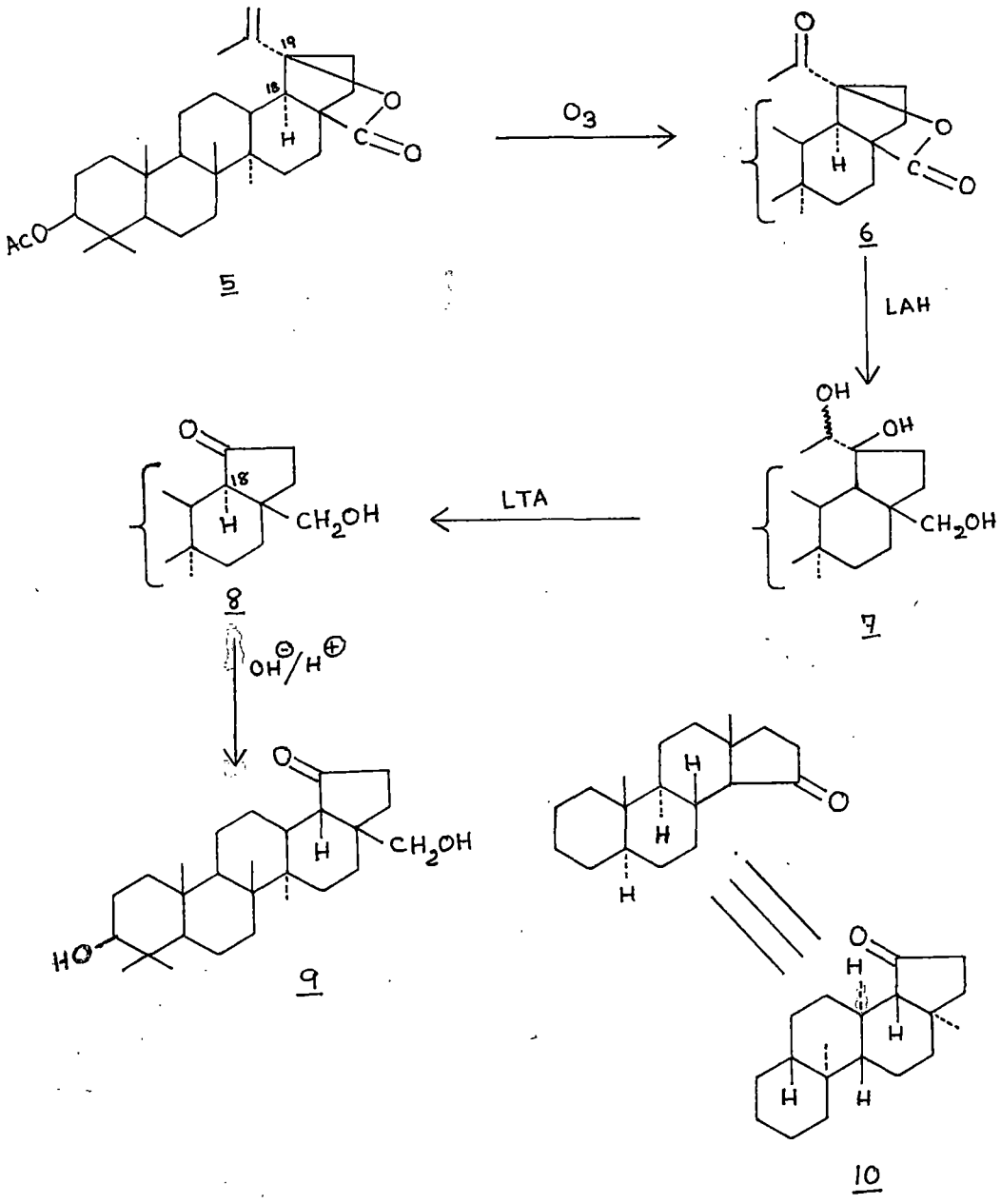
Three groups of workers almost simultaneously but independently put forwarded both spectral and chemical degradative results in support of the revised formulation of this lactone as C-28, 19-lactone.

Baddeley and co-workers[6,7] carried out ozonolysis of the lactone derived from acetyl betulinic acid 1b by Hg(II) acetate, in methanol-chloroform at -10° to give a norketone 6. The norketone 6 was reduced by LAH to the tetrol 7 which on lead tetraacetate treatment afforded the trisnorketone 8 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-18H α stereochemistry of 8 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

($[\phi]_{296}$-8220°). The 18 α(H)-trisnorketone 8 was isomerised by heating with 0.1 N methanolic KOH or in AcOH to ketone 9 epimeric at C-18. The ORD curves of the trisnorketones 8 and 9 have nearly the same amplitudes as are near reflection of, those of androstan-15-one 10 and its 14 β(H)-isomer, respectively whose ring system are essentially enantiomeric with the B/C/D/E rings in the two trisnorketones.

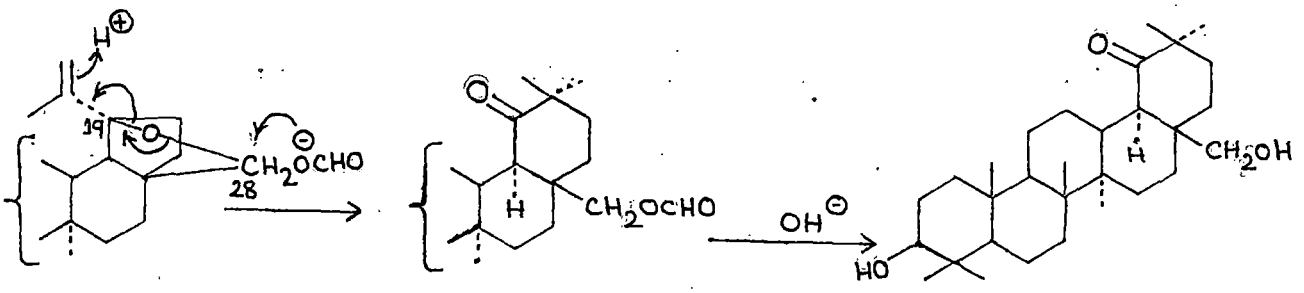
Based on this evidence they advanced the structure 5 for the lactone from acetyl betulinic acid 1b. Clearly there can be no ambiguity in the stereochemistry at C-19 in this lactone and this structure also explains the recovery of the norketone 6 unchanged after treatment with alkali (i.e. with reacetylation of the 3β-hydroxyl group where necessary) including the method of Khastgir and Bose[8]. The whole sequence may be illustrated in the scheme I.

Vystrcil and Blecha[9,10] almost simultaneously published the correct structure of mercuric acetate oxidation product of betulin 1c. They showed that the primary product of oxidation of betulin 1c by mercuric acetate had the structure 13a. They advanced the following evidence in support of their conclusion. The oxidation product 13a of betulin 1c 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[11] 11. The isomerisation


AcO
19
18
H
O
C
O
5
O_3
O
O
H
C
O
6
LAH
OH
OH
CH_2OH
7
LTA
O
18
H
CH_2OH
8
$OH^{\ominus}/H^{\oplus}$
O
H
CH_2OH
HO
9
H
H
H
O
O
H
H
H
H
10

Scheme - I

is initiated similarly to betulin[12], 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, 19-β-olide system failed to undergo isomerisation.

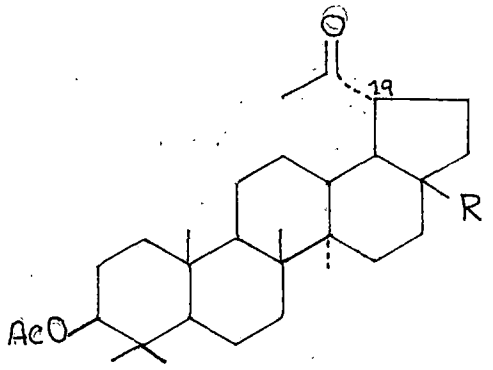


13a

11

The positive Cotton effect of 3β, 28-diacetoxy-30-norlupan-20-one 12a $[\phi]_{308}$ + 1248°, $[\phi]_{272}$ - 3925°, a = 52 (dioxane) is changed to negative value in 3β-acetoxy-19β, 28-epoxy-30 nor-lupan-20-one 13b $[\phi]_{307}$ -1883°, $[\phi]_{270}$ + 203°, a = -21 (dioxane) which is the familiar effect of α-substitution of methyl ketones with restricted rotation[13]. Whereas the ^1H NMR spectrum of the diacetyl norketone 12a contains a clear signal at 19β-H (τ 7.38, multiplet) it is completely absent in the spectrum of the acetyl epoxynorketone

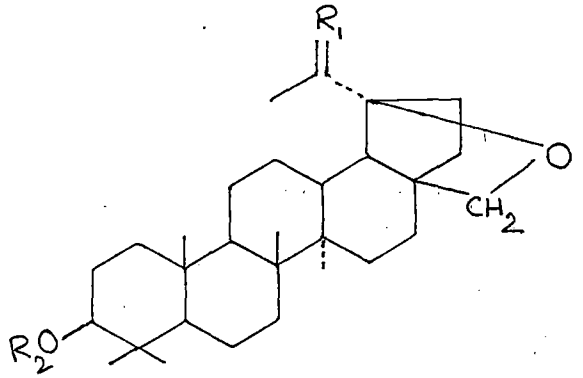
13b. Furthermore, by the condensation of 13b with benzaldehyde, the benzal derivative 14 $C_{38}H_{52}O_4$ m.p. 276-7°, $[\alpha]_D$ + 34, λ_{max} 294 nm (log ε=4.3) was obtained. This on subsequent sodium borohydride reduction affords a mixture of isomeric alcohols 15, $C_{38}H_{54}O_4$, λ_{max} 252 nm (log ε=4.3). ν_{max} 3600, 3520,



12a R = CH_2OAc

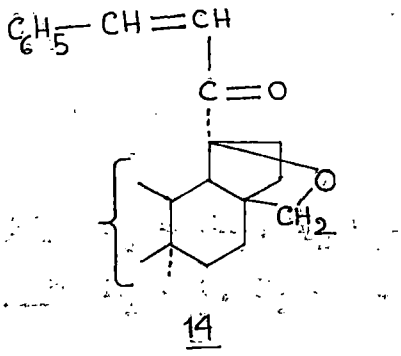
12b R = CO_2CH_3

1083 cm^{-1} (OH), which cannot be dehydrated to the phenyl butadiene system C_6H_5-CH = CH-CH = C< . Had the epoxide bridge been terminated differently from 19β, the above mentioned dehydration reaction would be expected by analogy with the literature report[14,15].



13a $R_1 = CH_2$, $R_2 = H$

13b $R_1 = O$, $R_2 = Ac$



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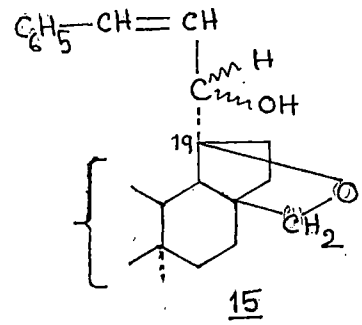
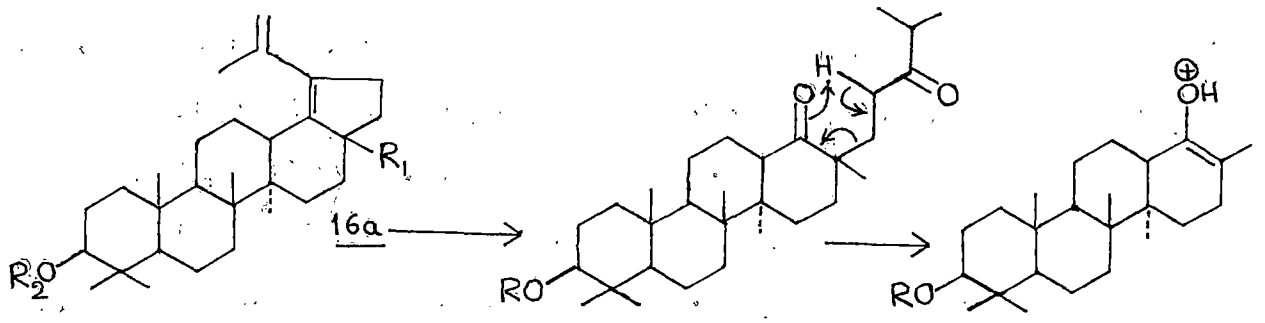


15

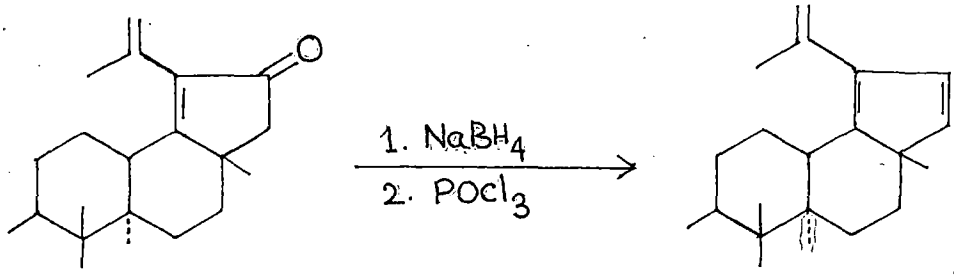
At the time when the correct structures of the lactone[6,7] and ether[9,10] were advanced, McLean and co-workers[16], the original workers, also came forward with their revised formulation on the Hg(II) acetate oxidation products of lupane series. They demonstrated that the new double bond introduced into lupeol acetate 1f, betulin diacetate 1e and methyl betulinate acetate 1d by dehydrogenation with Hg(II) acetate should be placed in conjugation with the side chain double bond and should be represented by 16a, 16b and 16c respectively. The anomalous absorption [λ_{max} 207 nm (ϵ=7000)] in the products 16a - 16c they ascribed due to the inability of the double bonds to attain coplanarity, as a result of the interaction between the C-12 hydrogen atoms and the hydrogens of the isopropenyl side chain[17]. Osmylation of dihydro dehydro lupenyl acetate 16a followed by Pb(IV) acetate cleavage afforded the dioxo acetate 17a 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 17a came from mass spectral study. 17a showed a base peak at m/z 402($C_{26}H_{42}O_3$), corresponding to the ion 18a formed as a result of a McLafferty rearrangement[18] depicted below.



16a	$R_1 = CH_3$, R_2 = Ac	17a	R = Ac	18a	R = Ac
16b	$R_1 = CH_2OAc$, R_2 = Ac	17b	R = H	18b	R = H
16c	$R_1 = COCCH_3$, R_2 = Ac				

A prominent peak at m/z 360 corresponding to the ion 18b also occurred in the mass spectrum of the corresponding dioxo-alcohol 17b.

Oxidation of 16a with chromic acid gave a mixture of products, from which the dienone acetate 19 was isolated by chromatography. Spectroscopic analysis of 19 indicated the presence of an isopropenyl group and a cyclopentenone system. ^{1}H NMR showed a sharp singlet at τ 7.75 (2H) assigned to the methylene protons adjacent to the carbonyl group and suggested that these protons were magnetically equivalent and were flanked by a quaternary carbon atom. Reduction of the dienone acetate 19 with $NaBH_4$ followed by dehydration with $POCl_3$ afforded the trienylacetate 20. Besides showing the presence of the isopropenyl group, the ^{1}H NMR spectrum of 20 exhibited a pair of doublets ($J_{AB} \simeq$ 6.0 Hz) at τ 3.73 and 3.91 indicative of a cis-disubstituted double bond in a five-membered ring and flanked



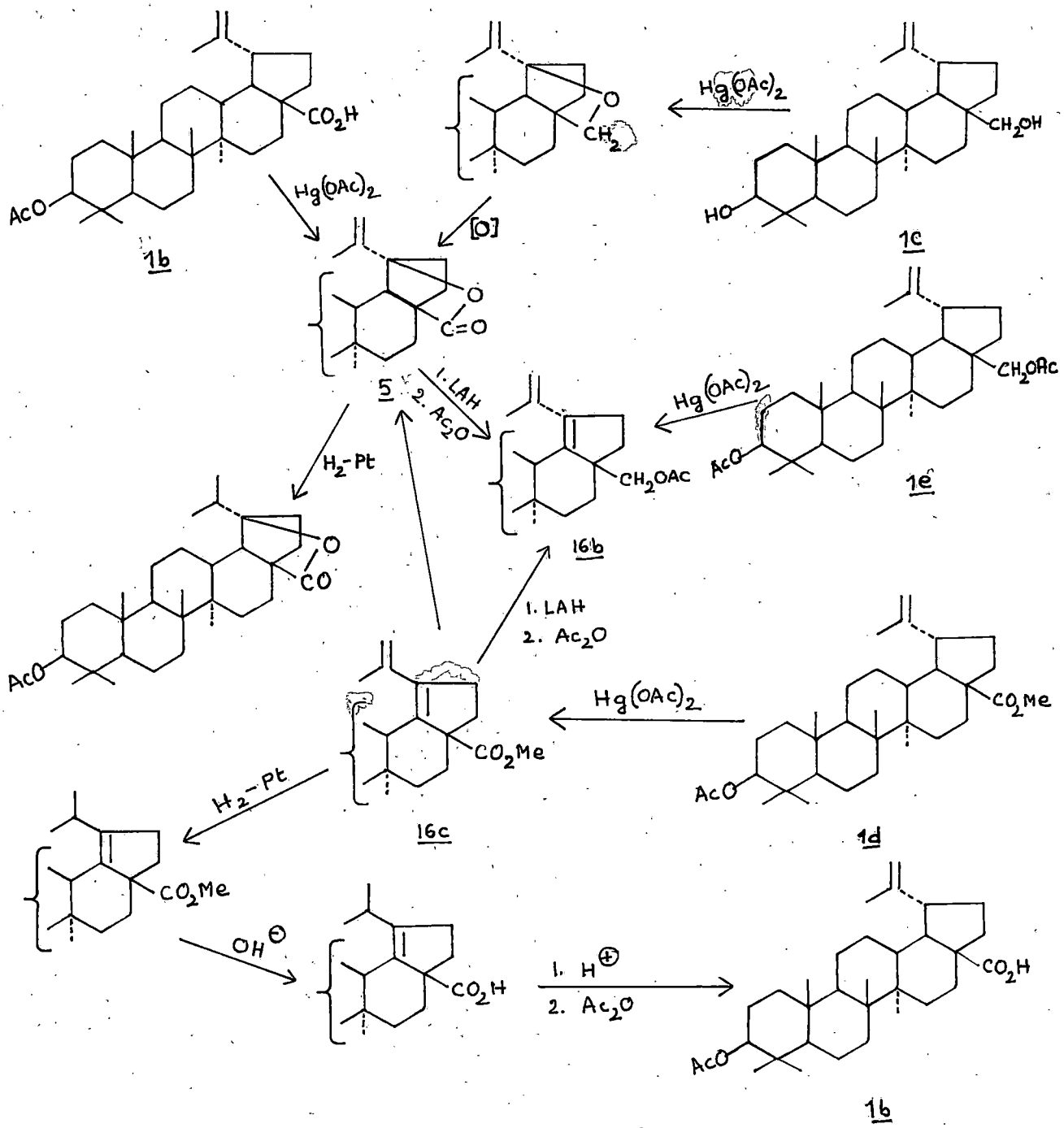
19 20

by quaternary carbon atoms. Thus, a combination of spectral and chemical evidence led them to revise their initial structures 4a, 4d, 4e and enabled them to formulate correct structures for the products of Hg(II) acetate oxidation of lupeol acetate 1f, betulin diacetate 1e and methyl acetyl betulinate 1d as 16a, 16b and 16c respectively.[9,10] Since the correct structure of ether and lactone[6,7] obtained by Hg(II) acetate oxidation of betulin and betulinic acid has already been proposed, the interconversions as shown in the scheme II, further substantiated their revised formulations.

Section B. The Structure of the Cactus Triterpene Lactone Thurberogenin

(i) The structure of thurberogenin 28, 19β-lactone 21a originally proposed:

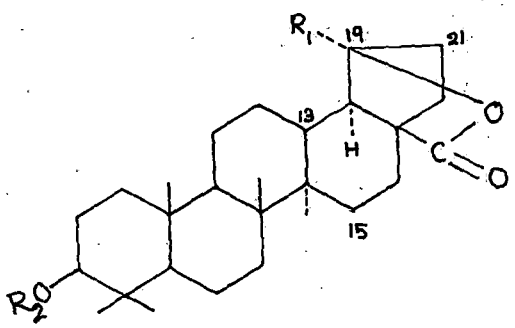
Djerassi and co-workers[19] isolated thurberogenin from the Cactus-Liemaireocerus thurberi. It possesses[3] a reduceable double bond, gave an acetate which on treatment with SeO_2 in AcOH afforded an unsaturated aldehyde (λ_{max} 222 nm, log ϵ = 4.01).

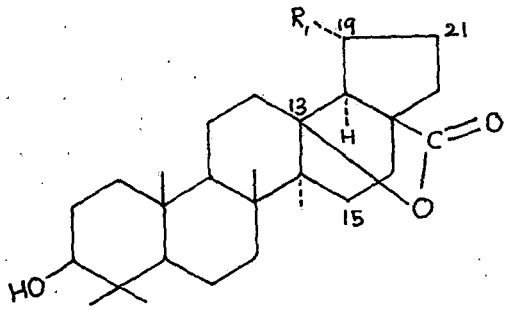

AcO
CO2H
1b
Hg(OAc)2
[O]
O
CH2
O
C=O
5
1. LAH
2. Ac2O
CH2OAc
16b
Hg(OAc)2
HO
CH2OH
1c
Hg(OAc)2
AcO
CH2OAc
1e
H2-Pt
O
CO
AcO
1. LAH
2. Ac2O
CO2Me
16c
Hg(OAc)2
AcO
CO2Me
1d
H2-Pt
CO2Me
OH⊖
CO2H
1. H⊕
2. Ac2O
AcO
CO2H
1b

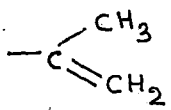
Scheme - II

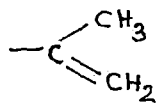
These reactions eliminate completely from consideration of an α- or β-amyrin skeleton but rather suggest that it belongs to the class of lupeol triterpenes. Thurberogenin acetate on ozonolysis produced formaldehyde and a norketone characterised as the oxime. The unsaturated aldehyde mentioned above on ozonolysis afforded a bis-nor acid characterised as the methyl ester. This behaviour was in complete analogy to betulin[20]. Oxidation of thurberogenin with CrO_3-pyridine yielded thurberogenone, which showed two carbonyl bonds, ν_{max} 5.66 and 5.90μ, the latter corresponding to a six membered (or larger) rings ketone. Thurberogenone on $NaBH_4$ treatment regenerated thurberogenin indicating the hydroxy group is most likely equatorial and hence β-oriented. Dihydro thurberogenin 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 triterpene alcohols.

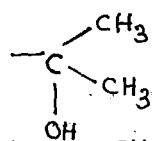
Based on biogenetic grounds they also proposed that the carbonyl group of the lactone ring of thurberogenin and also stellatogenin, another cactus lactone obtained from Lumaireocerus stellatus[21], originates at C-17. Some support for this assumption came from co-occurrence of betulinic acid and oleanolic acid with these lactons. Stellatogenin[21] 21c is a dihydroxy lactone and was correlated with thurberogenin by dehydrative elimination of the side-chain tertiary hydroxyl

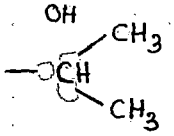


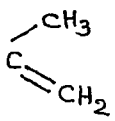


21a $R_1 =$  $R_2 = H$

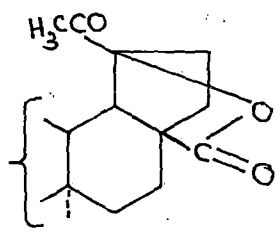
21b $R_1 =$  $R_2 = Ac$

21c $R_1 =$  $R_2 = H$

21d $R_1 =$  $R_2 = Ac$

24a $R_1 =$ 

24b $R_1 = -COCH_3$

21b $\xrightarrow{O_3}$ 

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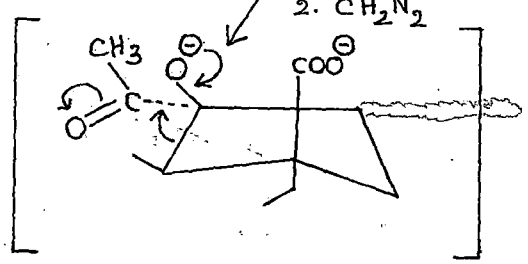
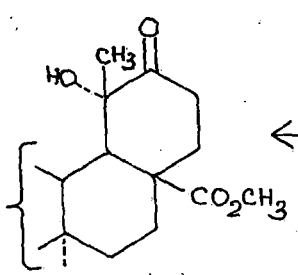


1. $OH^{\ominus}$
2. CH_2N_2



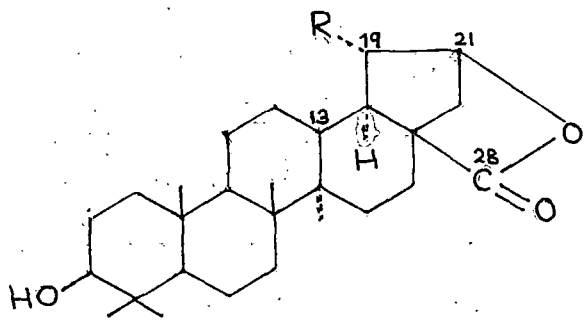
23

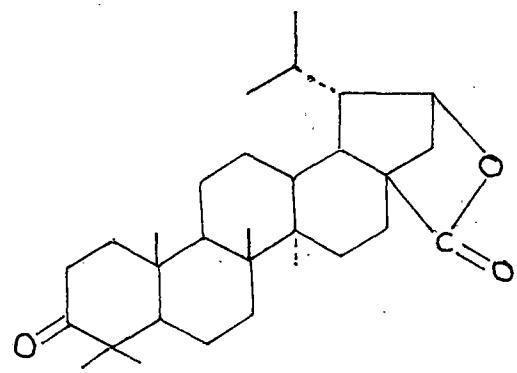
group. Four alternatives (position 13, 15, 19, 21) were considered as termination points of the five membered lactone ring. With LAH treatment, thurberogenin yielded a triol which gave a diacetate, which was resistant to CrO_3 oxidation; and this led them to propose at that time the tertiary hydroxyl rather than secondary hydroxyl function (position 15 or 21), which they found to be incorrect later[5]. Of the two tertiary positions of lactone terminations 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 22 obtained by ozonolysis of thurberogenin acetate 21b. They then formulated 23 as the structure of the rearranged product and the structure of thurberogenin as 21a. Based on a detailed spectral analysis they in a later communication[5] revised the formulation of this rearranged product as well as the structure of thurberogenin. The alternative structure 24a for thurberogenin was discarded by them as the derived norketolactone 24b would be incapable of this rearrangement. In order to establish the genetic relationship of thurberogenin with lupane triterpenes, Djerassi and Hodges[4] treated the 30-nor-20-ketone 22 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[22] 12b. Identity with an authentic specimen was established by mixture melting point determination, IR comparison and close similarity of the ORD curves. Later they explained the

formation of 23 based on the revised formulation of thurberogenin[5].

(ii) Revised structure of thurberogenin as C-28,21 lactone 25a :

After more than a decade of the original proposition of the structure of thurberogenin by Djerassi et al[3,4], the same group[5] published in 1967 a communication concerning the revised structure of thurberogenin 25a. They intensively applied ^{1}H NMR and mass spectrometry which were not available at the time when they proposed[3,4] their initial structure 21a for thurberogenin. ^{1}H NMR spectrum of dihydro thurberogenone[5] (now known to have structure 26) obtained by hydrogenation of the side chain and





25a R = $-C(CH_3)=CH_2$

25b R = $-CH(CH_3)(CH_3)$

25c R = $-C(CH_3)(CH_3)(OH)$

25d R = $-COCH_3$

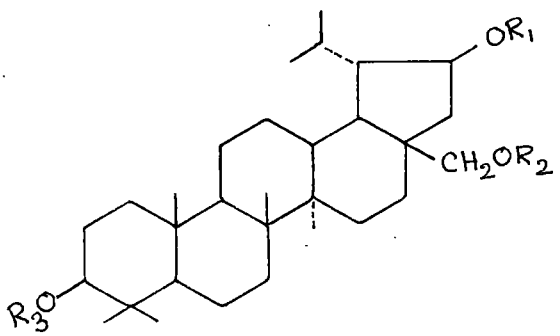
26

(revised structure of thurberogenin 25a and stellatogenin 25c proposed in 1967)

oxidation of the C-3 hydroxyl group, showed a complex resonance at δ 4.6 (integrating for one proton) indicative of the grouping H-COR. Since the other two oxygens in 26 are carbonyls, this function must involve the lactonic hydroxyl group and the hydroxy group involved in lactone formation must therefore be secondary.

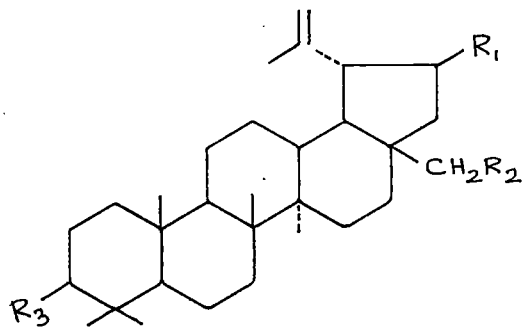
Further evidence for this conclusion came from mass and ^{1}H NMR analysis of the triacetate 27b obtained by LAH reduction and subsequent acetylation of dihydro thurberogenin 25b. The acetylation product 27b originally thought to be a di-acetate, demonstrated a molecular ion peak in the mass spectrum 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 were confirmed by ^{1}H NMR analysis, which showed signals at δ 1.96 (singlet, 3H) and 2.04 (singlet, 6H). 100 MHz spectrum resolved the complex absorption pattern in δ 4.2-5.2 region integrating for four protons to an AB quartet (2H, -C<u>H</u>$_2$OAc) and two one proton triplets (2H, -<u>H</u>COAc). The presence of a primary alcohol (from reduction of lactonic carboxyl), a secondary alcohol (ring A) and another secondary alcohol (lactonic hydroxyl) in triol 27a 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 28a under carefully controlled conditions produced a monoacetate 28b, which on Jones



27a $R_1 = R_2 = R_3 = H$

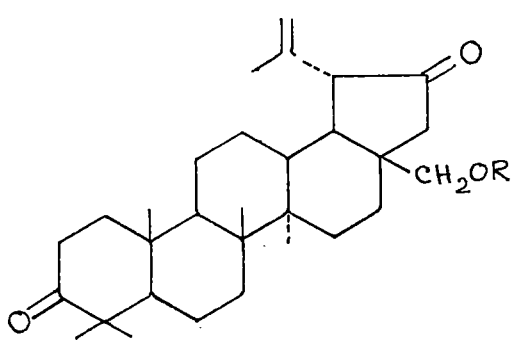
27b $R_1 = R_2 = R_3 = Ac$



28a $R_1 = R_2 = R_3 = OH$

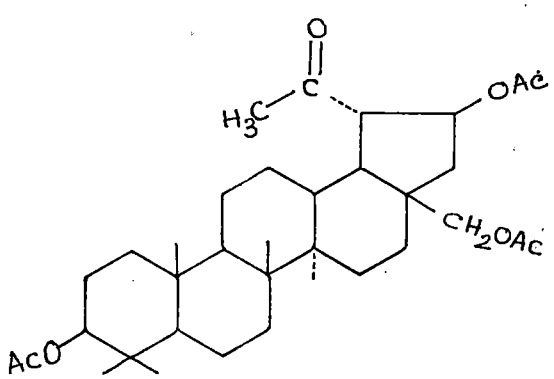
28b $R_1 = R_3 = OH$ $R_2 = OAc$

28c $R_1 = R_3 = H$ $R_2 = OH$



29a $R = H$

29b $R = Ac$



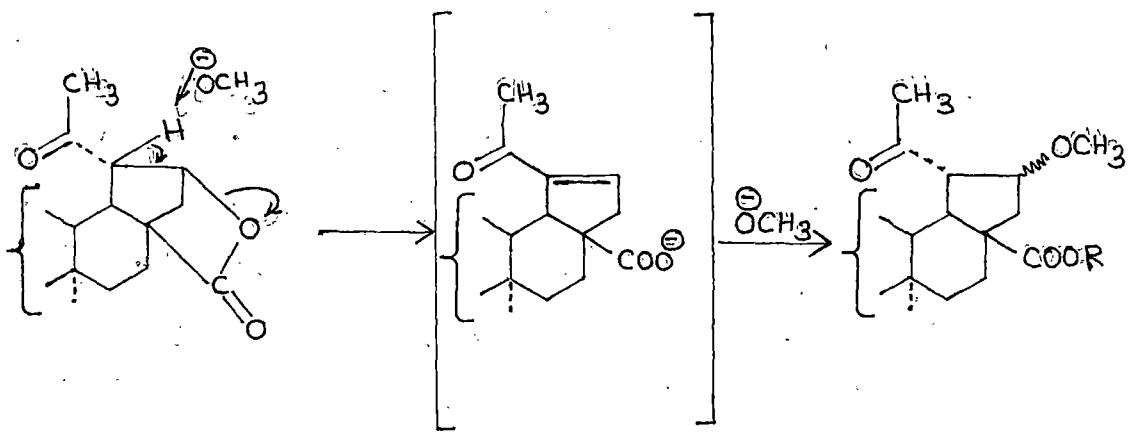
30

oxidation and mild hydrolysis afforded the diketo alcohol 29a, $C_{30}H_{46}O_3$ (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 wave length due to cyclopentanone. This led to the structure of the diketo alcohol as 29a, the triol 28a and its progenitor, thurberogenin 25a. A correlation with a known compound of lupene series was also achieved by them. The acetate 29b from the diketo alcohol 29a on Wolf-Kishner reduction afforded the known compound 3-deoxybetulin 28c.

They compared the ORD spectra of the known 30-nor keto ester 12b with that of 30-nor ketone 30 derived from the oxidation of the triacetate of thurberogenin triol 28a. The ORD spectra of 12b and 30 are very similar, both exhibiting modarately strong positive CE. This demonstrates that the isopropenyl group at C-19 in thurberogenin 25a possesses α-stereochemistry.

They made an indepth study of the base catalysed rearranged product which was formulated previously as 23. The mass spectrum showed molecular ion peak M^+ 502 m/z ($C_{31}H_{50}O_5$) 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 31 differed from that of previous one 23 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 31. The presence of an ethereal $-OCH_3$ group was confirmed by a peak at δ 3.21 (3H, singlet), besides showing signals at δ3.70 (3H, singlet

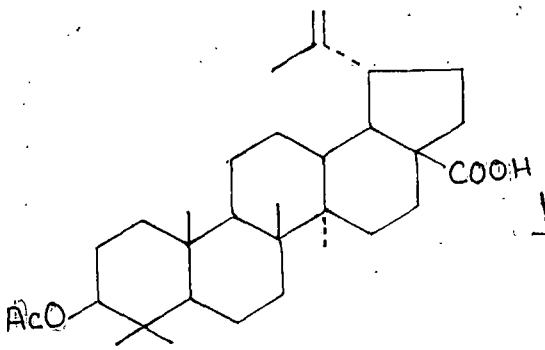
$-COOCH_3$) and δ 2.17 (3H, singlet, $-COCH_3$). Since with their revised formulation of thurberogenin 25a the base-catalysed E-homo rearrangement of the derived nor-ketolactone 25d cannot occur, they proposed[5] the following mechanism for the formation of 31.



25d

31

Interestingly enough, the nor-ketolactone 6 derived from ozonolysis of mercuric acetate oxidation product 5 (revised structure) of acetyl betulinic acid 1b, possesses the necessary structural requirements for E-homorearrangement and should undergo this ring expansion reaction under appropriate reaction condition. Indeed, Vystrcil and Blecha[23] and Khastgir et al[24], independently advanced the structure of the E-homorearranged product 32 based on a combination of physical and chemical studies.



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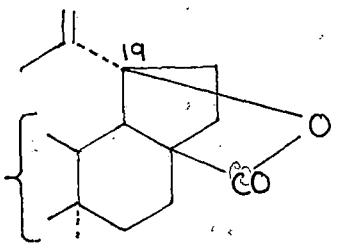
$Hg(OAc)_2$



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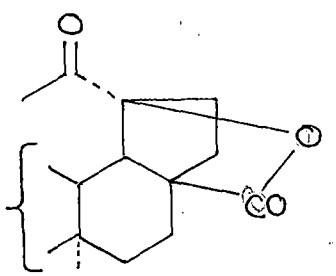
O_3



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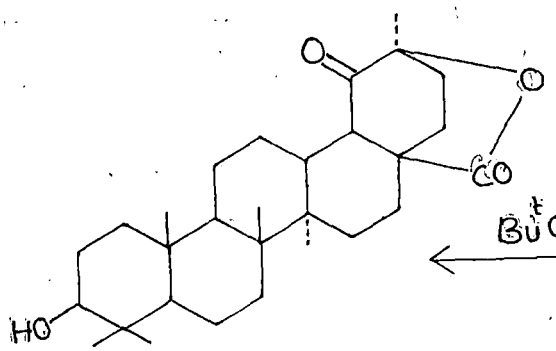
Bu^tOK



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