PART II

Mercuric acetate oxidation of acetyl betulinic acid

Introduction

Allison and coworkers[1] carried out mercuric acetate oxidation of acetyl betulic acid 1 and obtained a γ-lactone, m.p. 315-17°, $(\alpha)_D$ + 60°, to which they assigned structure 2, but did not assign the stereochemistry of the isopropenyl substituent at C-19. Our chief objectives in investigating the lactone 2 has been to gain insight (i) into its chemistry and stereochemical behaviour and (ii) the stereochemical disposition of the isopropenyl group at C-19.

Section A

Towards this end, we prepared the lactone 2, by mercuric acetate oxidation of acetyl betulic acid 1 in chloroform and acetic acid solution, essentially following the method of Allison et al.[1] The lactone 2, m.p. 302°, $(\alpha)_D$ + 58°, showed I.R. peaks (Fig. 9) at 1780 (γ-lactone), 1730 and 1245 (acetate) and 1640 and 890 cm^{-1} (vinylidene group). Its NMR spectrum (Fig. 10), besides peaks for five methyl groups at saturated carbons at δ 0.96, δ 0.89 and δ 0.81, exhibited peaks at δ 1.98 (3H, -O.CO.CH_3), δ 1.65 (3H, C=C(CH$_3$)), δ 4.95, δ 5.35 (2H, vinylidene = CH_2) and at δ 4.4 (1H, H-C-O.CO.CH_3).

To provide reasonable evidences regarding the sterochemical assignment of the isopropenyl substituent at C-19, we thought it desirable to examine the Dreiding model of the lactone and found that if the C-18H were α-oriented the lactone could be constructed

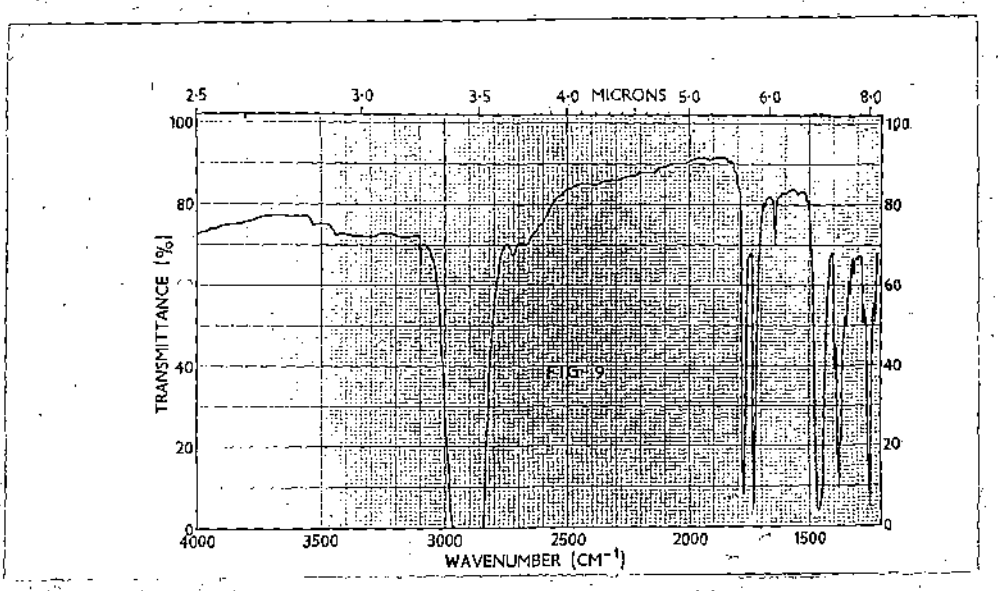


FIG-9

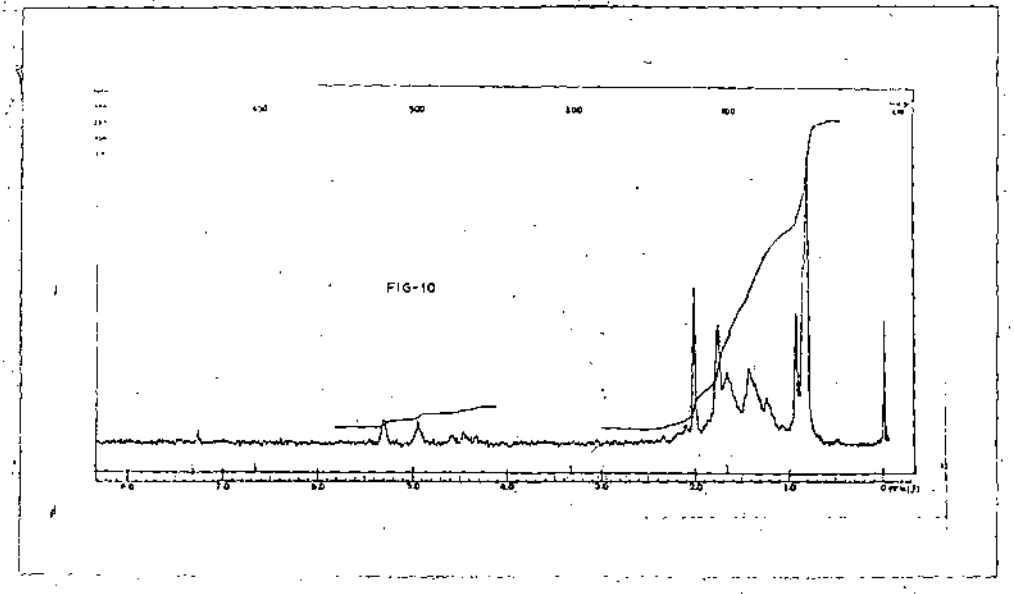


FIG-10

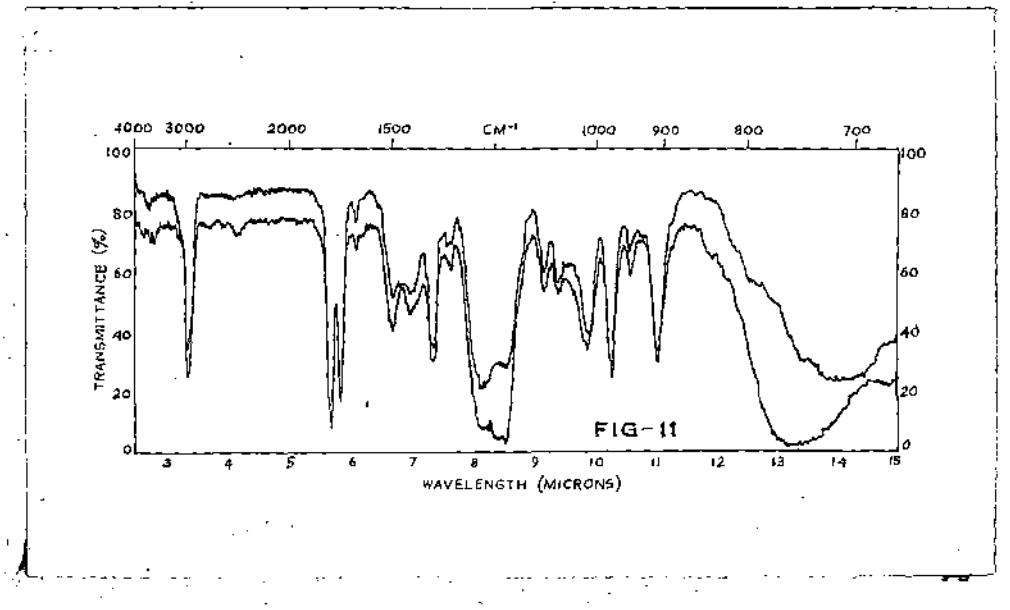
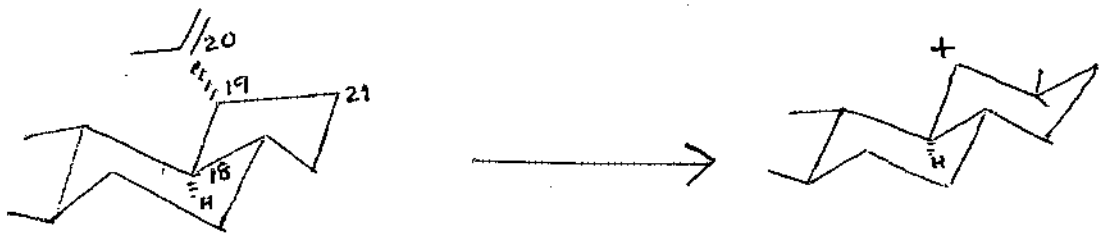
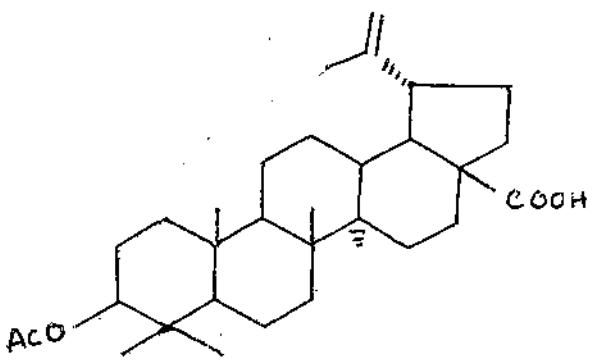


FIG-11

easily. And if the C-18H retained its original ∝-orientation and the C-19 isopropenyl group were <u>trans</u> (∝-oriented) to the C-17 group, as in lupeol - betulinic acid series, it should be amenable to acid induced isomerisation[2] as observed in the lupeol series, with expansion to six-membered ring E with chair conformation as shown below. The expansion results from the co-planarity of the



carbon atoms at C-19, C-20 and C-21. But if the orientation were <u>cis</u> no skeletal rearrangement would occur as the carbon atoms at C-19, C-20 and C-21 would no longer be co-planar. Accordingly, the lactone <u>2</u> was exposed to the action of (i) HCl-$CHCl_3$[3,4] (ii) 98% formic acid[5,6] (iii) HCl-acetic acid and in each case the starting material was recovered in good yield (mixed m.p. and I.R. comparison) (Figs. 11 and 12) and no isomerisation product could be detected. These experiments suggested that the skeleton of the lactone and the stereochemistry of the substituents in the compound is preserved unchanged.



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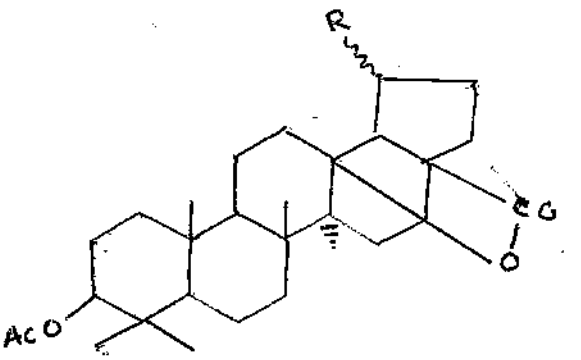


2 , R = isopropenyl
3 , R = $-CO.CH_3$
4 , R = isopropyl

The lactone 2 was next ozonised at 0°C in chloroform solution and the ozonide on decomposition under neutral conditions furnished the nor-ketolactone 3, m.p. 301-3°, ~~m.p. 301-3°~~, $(\alpha)_D$ - 9° (lit.[1] m.p. 317°, $(\alpha)_D$ - 2°). Elemental analysis and molecular weight determination by mass spectrometry established its molecular formula as $C_{31}H_{46}O_5$. The mass spectra (Fig. 13) of the keto-lactone 3, in addition to the molecular ion peak at 498, showed peaks at m/e 438 (M^+ - ACOH) and m/e 395 (M^+ - CH_3COOH - $COCH_3$), and a peak at m/e 249 resulting from the C-ring cleavage as observed in other saturated 3-acetoxy triterpenes[7]. It showed an U.V. absorption at 275 mμ (log ε 1.4) attributable to the carbonyl group. I.R. spectra (FIG.14) of the compound showed peaks at 1721 (composite for acetate and carbonyl), 1788 (γ-lactone) and 1255 cm^{-1} (acetate). The O.R.D. curve (Fig. 15) of the compound showed a negative Cotton effect with

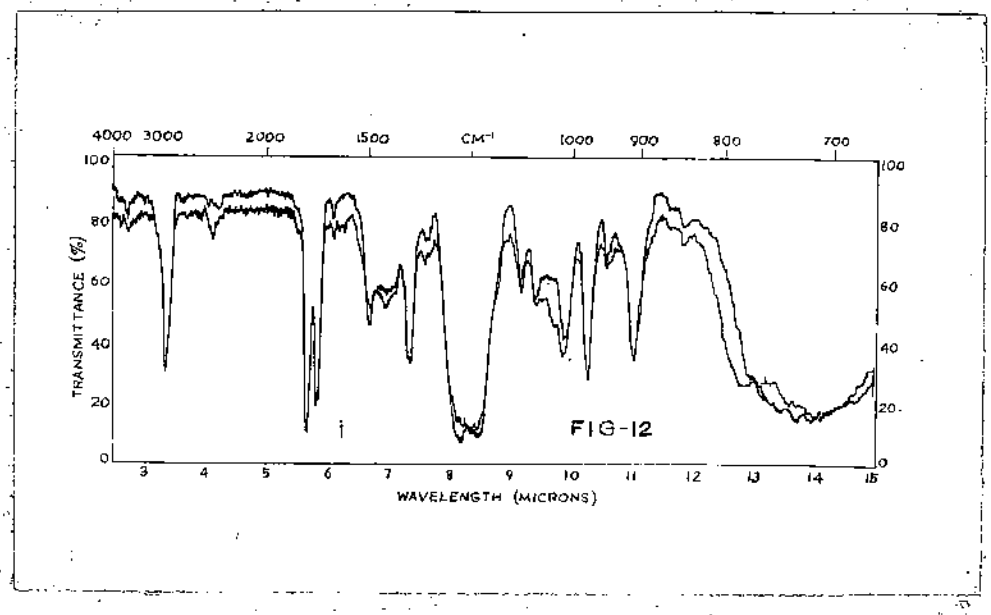


FIG-12

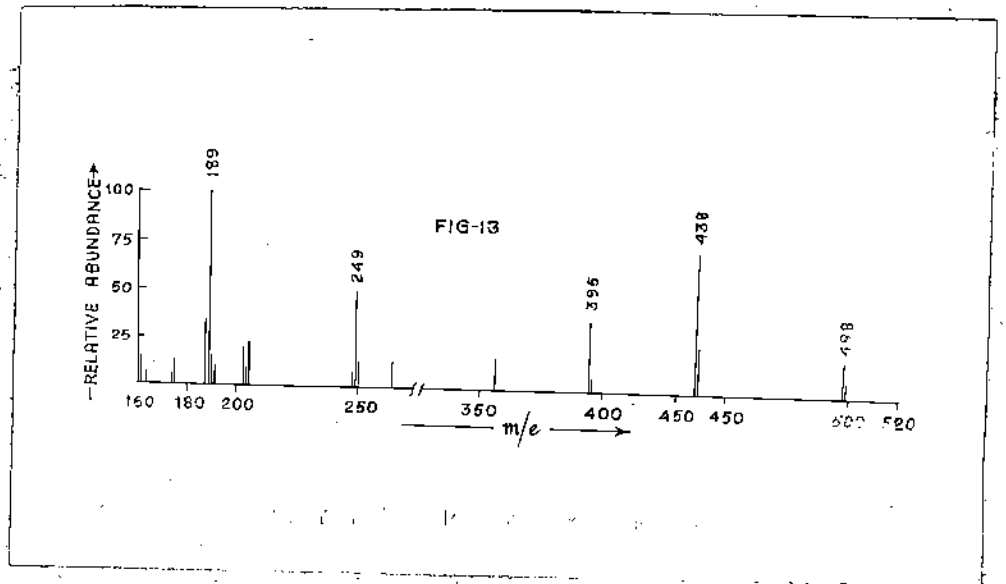


FIG-13

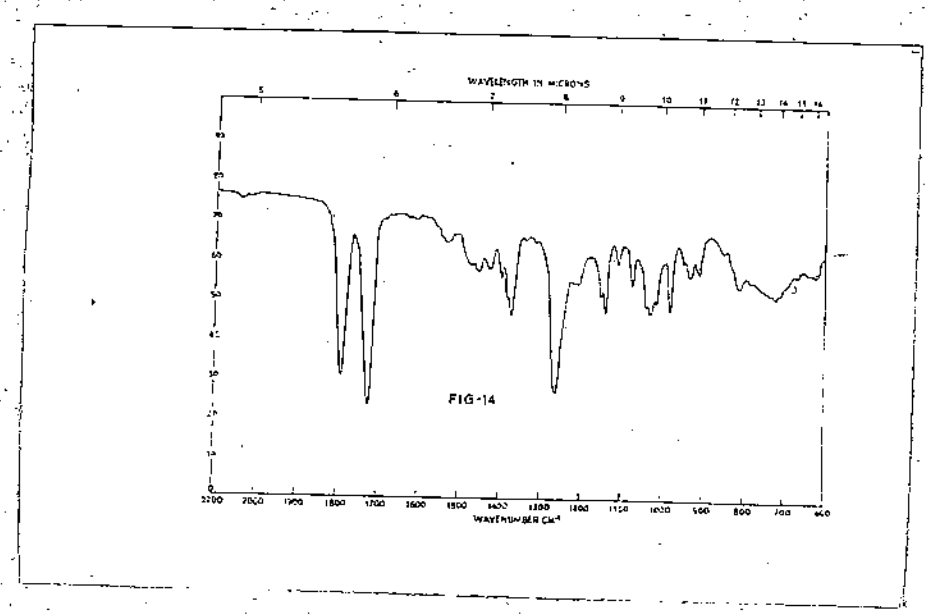


FIG-14

the following characteristics $(\phi)_{306}$ - 381° (trough), $(\phi)_{273}$ + 114° (peak) and $(\phi)_{234}$ - 1765° (trough). In NMR spectrum (Fig. 16) it exhibited peaks at δ 2.35 (3H, -CO.C<u>H</u>$_3$), δ 2.04 (3H, -O.CO.C<u>H</u>$_3$), δ 4.4 (1H, <u>H</u>-C-O-COCH$_3$) and peaks at δ 0.95, δ 0.90 and δ 0.85 attributed to five methyl groups situated at saturated carbon atoms.

Baeyer-Villiger oxidation of nor-ketone <u>3</u>

With a view to converting the acetyl side chain in <u>3</u> to the corresponding acetate (-O.CO.CH$_3$) Baeyer-Villiger oxidation was attempted. Both perbenzoic acid and trifluoro peracetic acid has been applied but no oxidation of the acetyl (COCH$_3$) side chain could be effected, indicating thereby a strong steric hindrance about the acetyl side chain.

At this stage we thought it appropriate to ask what these chemical and physical evidences mean. The facts that lactone <u>2</u> could not be isomerised under strong acid conditions and that Baeyer-Billiger oxidation was not successful on <u>3</u>, seemed most significant. Consideration of the above results led us to think that most probably the isopropenyl side chain in <u>2</u> was probably β-oriented and hence the -CO.CH$_3$ group in the nor-ketone <u>3</u> was also in the unstable β-orientation. Since equilibration by base and acid[8] is an expected process for a conformationally unstable ketone, we thought that if the side-chain at C-19 (CO.CH$_3$) in <u>3</u> were β-oriented it would undergo epimersation to the stereochemically more stable α-isomer as is observed in the case of steroids and

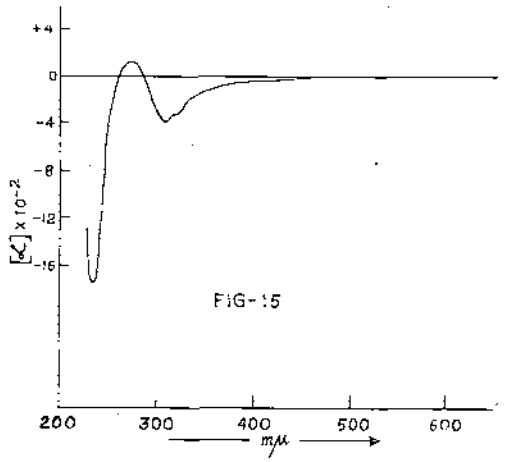


FIG-15

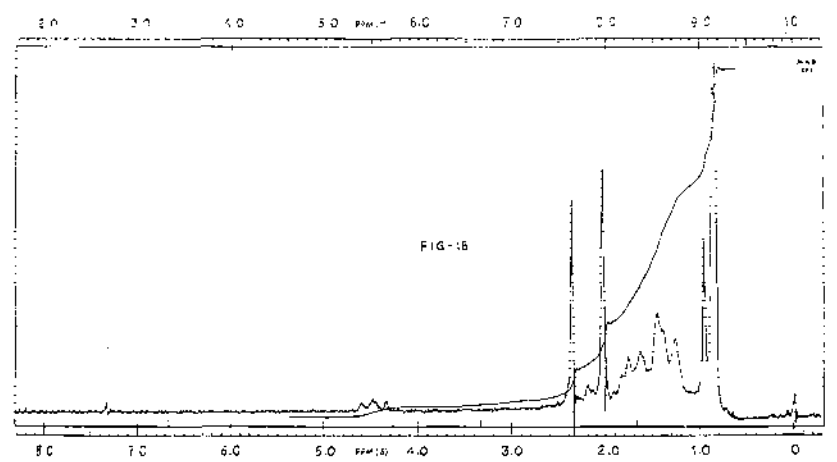


FIG-16

triterpenoids[8]. This is because, examination of Dreiding model shows that ∝-equatorial orientation of the $CO-CH_3$ group is more stable than the β-(axial) orientation, since in the former case it would experience less steric interaction with C-17 β-axial substituent.

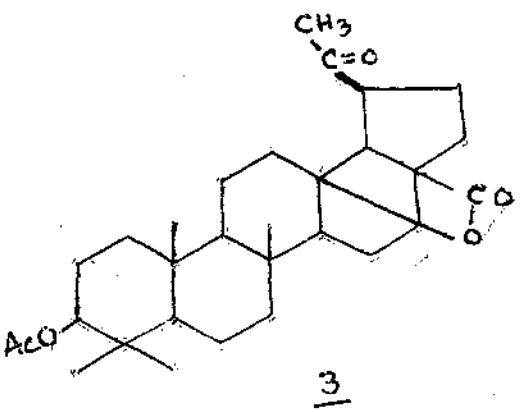
Action of 5% methanolic potassium hydroxide and 2N H_2SO_4 on the nor-keto lactone 3

The nor-keto-lactone 3 was accordingly treated with 5% methanolic potassium hydroxide solution by refluxing the reaction mixture for three hours. The crude product of the reaction on acetylation and chromatography gave back the starting material 3 in good yield (m.m.p. and I.R.). It was also treated with 2N H_2SO_4 in ethanol solution by refluxing the solution for four hours. On working up the reaction mixture, the starting material was recovered unchanged (m.m.p. and I.R. comparison).

Action of potassium tertiary butoxide - in t-butanol on 3

A more drastic basic condition was then applied. The benzene solution of the compound was added to potassium tertiary butoxide in tertiary butanol and the reaction mixture was refluxed for four hours. The crude product isolated was acetylated which on chromatography on alumina furnished a new compound m.p. 300-2°, $(\alpha)_D$ -24°. The mixture melting point of this compound with the original keto lactone showed considerable depression. TLC analysis of the two

compounds also showed that they were different, the compound 3 had R_f = 0.55 and the new compound had R_f = 0.63 in the solvent system benzene:chloroform (1:1). Both elemental analysis and mass spectroscopic molecular weight determination established its molecular formula as $C_{31}H_{46}O_5$ (M^+ 498). Its I.R. spectrum (Fig. 17A)showed absorption bands at 1765 (γ-lactone), 1725 (acetate and ketone, composite) and 1240 cm^{-1} (acetate). It also showed U.V. absorption at 290 mμ (ε 90) indicating the presence of a saturated carbonyl group. Since equilibration by base to the stereochemically more stable isomer is an expected process, the product obtained by K-tertbutoxide treatment on 3 was believed to be the epimer of 3 with acetyl side chain at C-19 α-oriented and was assigned[9] structure 4.



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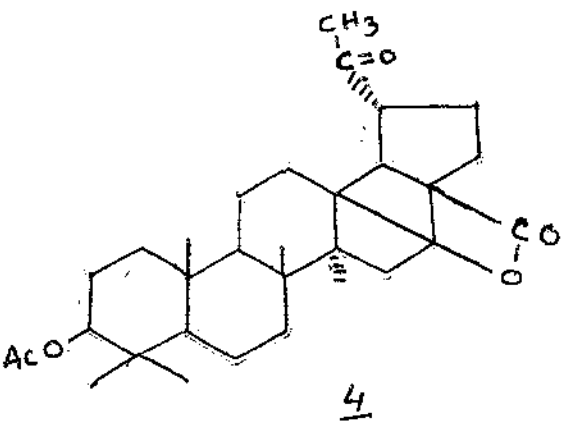

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Subsequently, the O.R.D. of the compound was measured and the O:R.D. curve (Fig. 18) revealed a strong negative Cotton effect, much more stronger than the original compound 3, and had the following

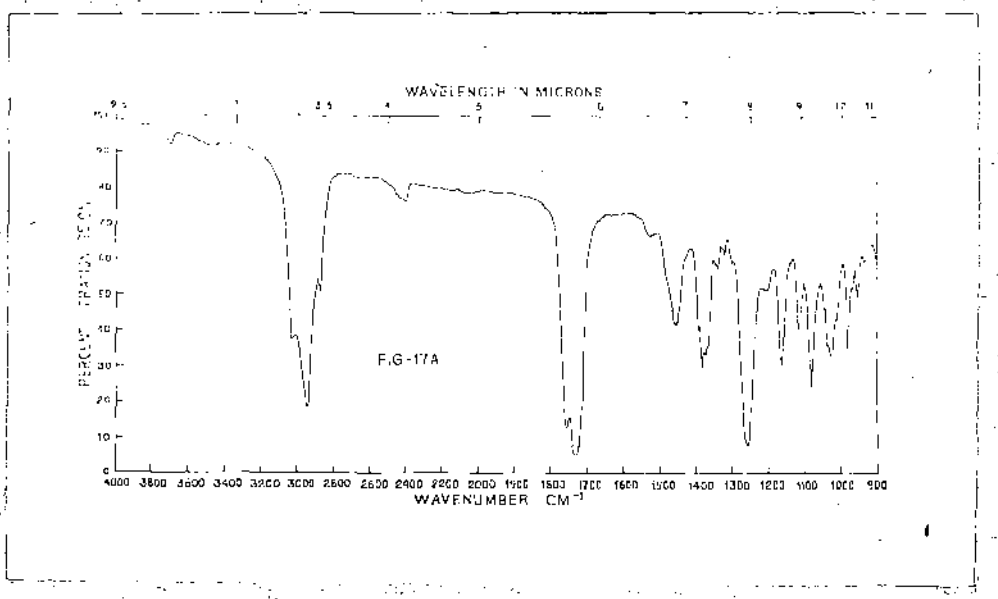


FIG-17A

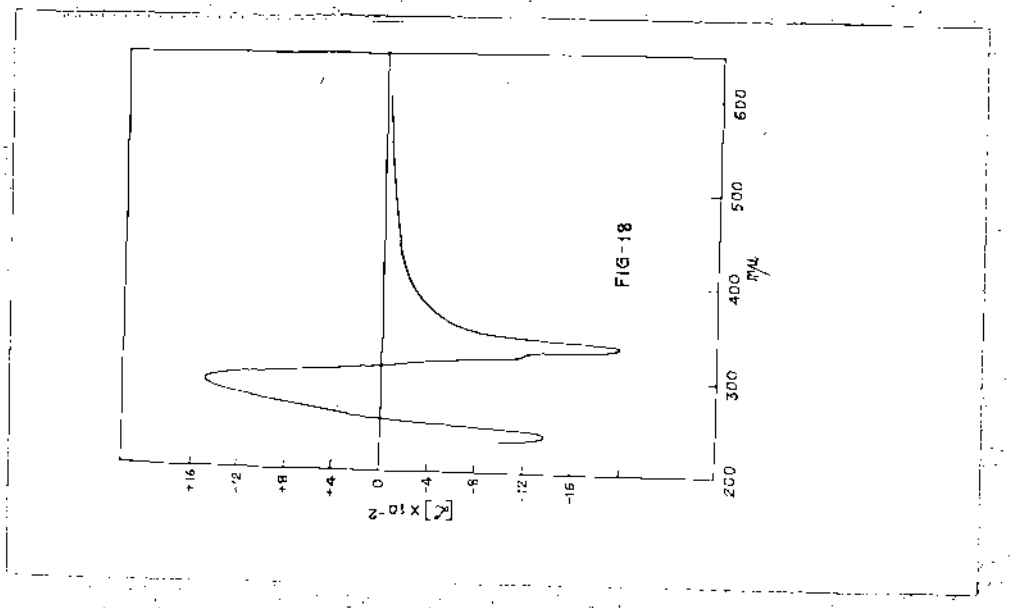
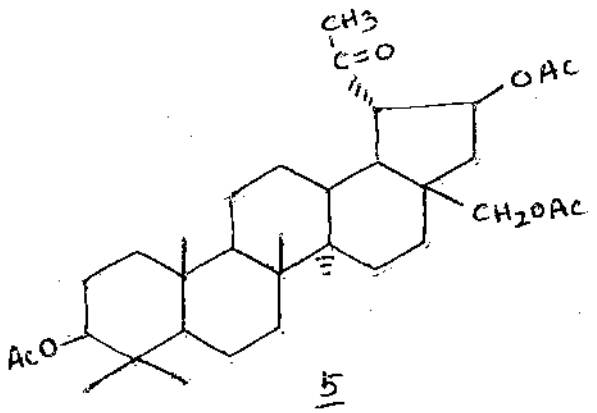


FIG-18

characteristics: $(\phi)_{335}$ - 1794° (trough), $(\phi)_{295}$ +1480° (peak) and $(\phi)_{240}$ - 1380° (trough). This result was unexpected for a structure depicted in 4. Survey of literature showed that compounds 5[10] and 6[11] obtained from thurberogenin and methyl 3β-acetoxy betulinate where the $-CO.CH_3$ group is α-oriented have been reported to exhibit positive Cotton effect. ORD curve of compound 5 showed the following characteristics[10]: $(\phi)_{589}$: 0° $(\phi)_{305}$ + 1848° (peak), $(\phi)_{305}$ + 1848° (peak), $(\phi)_{257}$ - 2184° (trough) and O.R.D. curve of 6 showed the following characteristics[10,11]: $(\phi)_{305}$ + 1368° (peak), $(\phi)_{260}$ - 1938° (trough).



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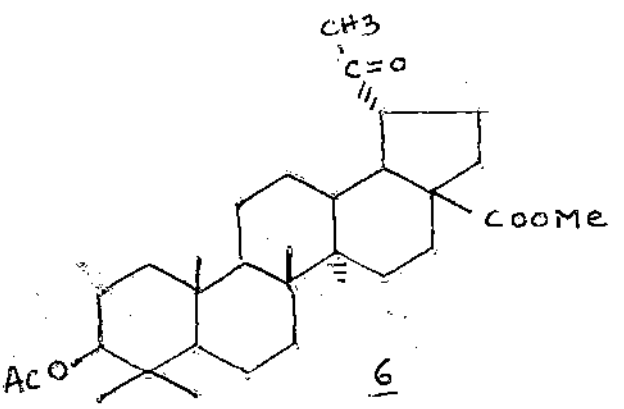

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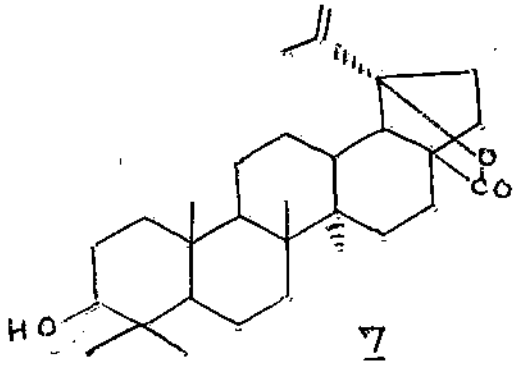
At this point we thought that there was no reason why our compound should exhibit such a strong negative cotton effect if it really represented structure 4. This consideration led us to suspect the validity of the structure 2 of the lactone put forward by Allison and coworkers[1] and raised the question that the lactone termination might not be at C-13.

Allison and coworkers[1] assigned the structure of the lactone as 2 from the following considerations. In the formation of the γ-lactone from acetyl betulic acid by mercuric acetate oxidation the lactone termination may be at C-13, C-19, C-21 or C-15. Since the triol obtained by LAH reduction gave a diacetate and not a triacetate it was concluded that the third alcoholic group was tertiary in nature and the lactone termination at C-21 or C-15 was ruled out. This was further confirmed by dehydration experiment on the above diacetate with $POCl_3$-pyridine. Thus the lactone termination could either be at C-13 or C-19. Allison et al.[1] discarded the C-17-19 lactone structure on the ground that the lactone was not identical with thurberogenin, which was previously assigned structure 7 by Djerassi and coworkers[11,12]. But recently, the structure of thurberogenin was revised[10] and was assigned the 17-21 lactone structure 8. Therefore, in the light of the recent revised



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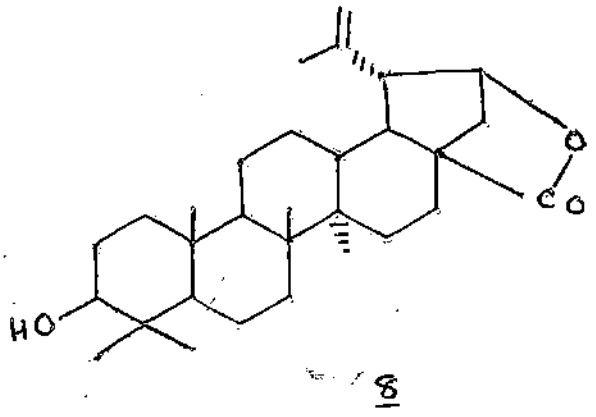
Thurberogenin (old structure)



8

(Revised structure)

structure of thurberogenin as well as our observation regarding the unexpected negative O.R.D. data strengthened our belief that the structure of the lactone in mercuric acetate oxidation of acetyl-betulic acid needs revision.

Application of circular dichroism studies

Recently a great deal of work has been done on the application[13] of circular dichroism measurements in structural elucidation and stereochemical aspects of unknown natural products, specially in the steroid and terpene series. We thought that circular dichroism measurements on the lactone might offer valuable clues not only to the structure of the lactone but also the stereochemical environment of the compound. Klyne and coworkers[14-16] have extensively applied this physical tool for the determination of nature and structure of lactones derived from various steroid and terpene derivatives. A lactone sector rule has been devised which enable the Cotton effect of many lactones to be predicted from consideration of assymetric surroundings of the chromophore. The sector rule affords a satisfactory explanation of the empirical data for the great majority of the compounds studied.

In the triterpene field Klyne et al.[16] studied the circular dichroism curves of various lactones of the β-amyrin series of both 18α and 18β stereochemistry. Chart A shows the results obtained by them for some of the lactones studied.

consideration also predicted positive Cotton effects for both the compounds. If the lactone termination were at C-13, in our compound, as depicted in 2, then by analogy with oleanane series a positive C.D. curve would be expected. But on the contrary a negative lactone Cotton effect was actually observed, for the nor-keto lactone 3 (Fig. 19A) with a strong negative maximum at 218 mμ (Δε,-7.02) and a negative Cotton effect at 290 mμ (Δε,-0.9) for the ketone carbonyl chromophore. Since the ketonic carbonyl chromophore might have some effect on the C.D. of lactone chromophore, the lactone devoid of any other chromophore was also prepared and C.D. measured. The lactone 2, was hydrogenated in the presence of PtO_2 catalyst at room temperature to furnish the dihydrolactone 9, m.p. 296-8°, $(\alpha)_D$ + 45° (lit.[1] m.p. 299-300°, $(\alpha)_D$ + 49°), I.R. 1780 (γ-lactone), 1735 and 1240 cm^{-1} (acetate) and absence of bands for vinylidene group. The circular dichroism curve of this dihydrolactone 9, exhibited (Fig. 20) a negative lactone Cotton effect with a maximum at 216 mμ (Δε = -6.05). This observation was a compelling evidence against the 13-18 lactone structure 2, and coupled with the chemical evidences so far recorded opened up the possibility that the lactone termination could be at C-19. Klyne et al.[17], have indeed, measured the C.D. of a 28-19β lactone of lupane series and found a negative lactone cotton effect which is in accord with our observation for 3 as well as 9. On the basis of these physical evidences, it may be argued that the lactone termination should be at C-19 and the structure of the lactone should be represented by 10 (dihydro lactone by 10a) and the nor-keto lactone by 11. The positive Cotton effect (Δε, + 1.58 at

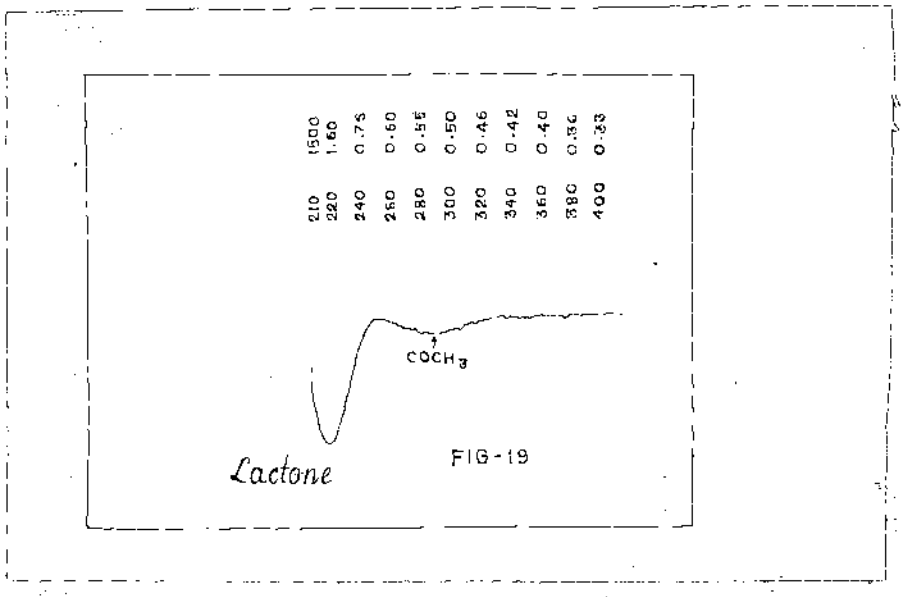


FIG-19

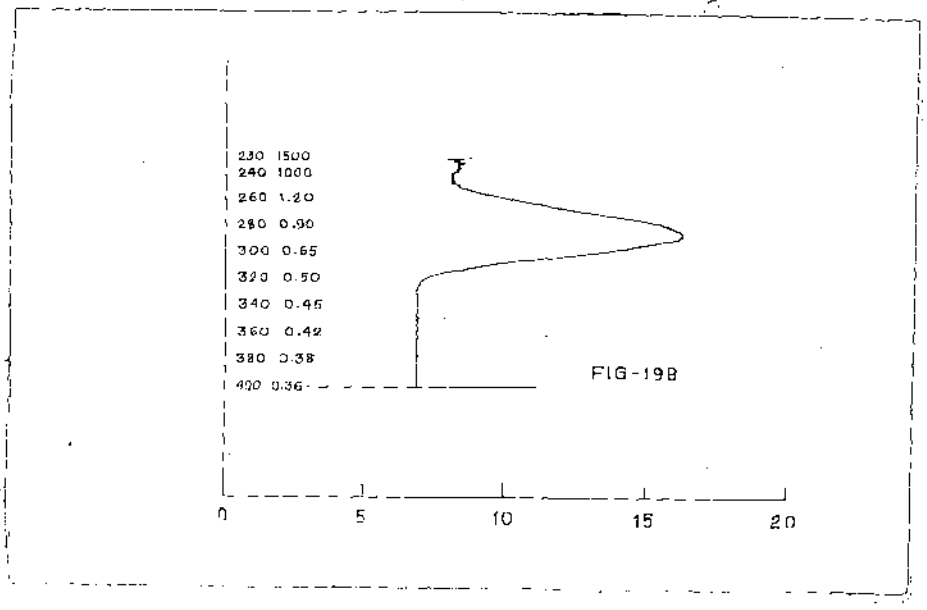
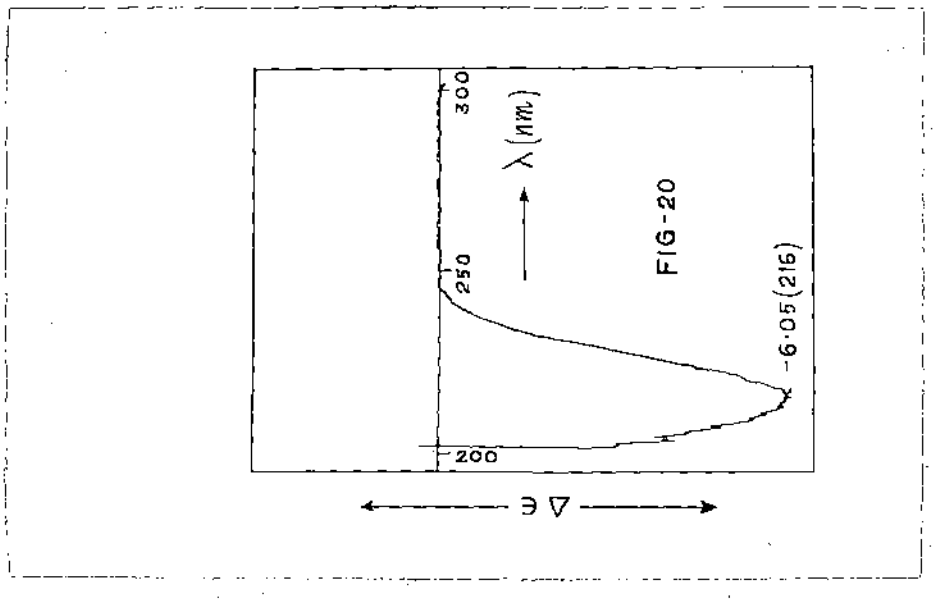
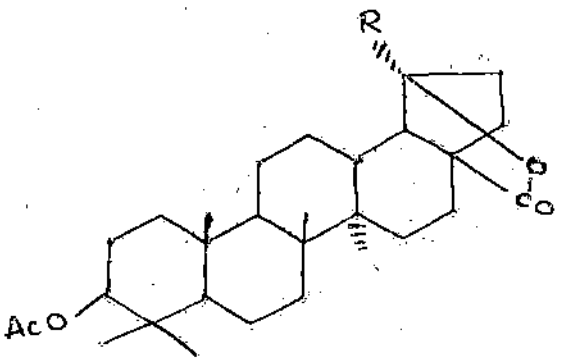


FIG-19B





10 , R = isopropenyl

11 , R = $-CO.CH_3$

10a , R = isopropyl

288 mμ, Fig. 19B) due to carbonyl group of methyl 3β-acetoxy 20-oxo-30-nor lupan-28-oate which has been recorded by us changed to a negative value in nor ketolactone 11 (Δε, -0.99 at 290 mμ; Fig. 19). This change to a negative value for the compound 11 is a familiar effect of α-substitution of methyl ketone with restricted rotation[18,19].

The resistance of the lactone 11 towards acid-catalysed isomerization can now be explained as due to considerable steric hindrance arising out of the C-17-C-19 lactone bridge. The same consideration also explains the inertness of the compound to Baeyer-Villiger oxidation even under most drastic conditions of trifluoroperacetic acid.

Section B

Structure of the product m.p. 300-2°, $(\alpha)_D$ - 24° obtained by K-tertbutoxide treatment of lactone 11

In view of the revised structure 11 of the nor-ketolactone, re-investigation of the structure of the compound m.p. 300-2°, $(\alpha)_D$- 24°, obtained by K-tertiary butoxide treatment of lactone 11, and reacetylation, was undertaken. NMR spectrum of the compound was taken and revealed a new picture with very interesting features. The spectra (Fig. 21) showed the absence of the peak at δ 2.35 due to $-CO-CH_3$ group present in the original compound 11. It also exhibited peaks at δ 2.04 ($-O.CO.C\underline{H}_3$, 3H), δ 4.4 ($\underline{H}$-C-O.CO.CH_3) and tall peaks δ 0.95 and δ 1.0. attributable to five methyl groups on saturated carbons. In addition to these, an additional peak appeared at δ 1.40 (singlet) which integrated for three protons. This observation, proved that it was not at all a simple case of epimerisation, but a skeletal rearrangement had taken place during the reaction. The molecular weight determination by mass spectra and elemental analysis showed that it had the same molecular formula $C_{31}H_{46}O_5$ as the original compound proving thereby that no elimination in the molecule had taken place. The new peak at δ 1.4 was assigned to a methyl group on a carbon bound by a oxygen bridge, in this case, probably by a lactonic oxygen bridge, which contributes to deshielding[20] and the consequent shift of the new methyl peak.

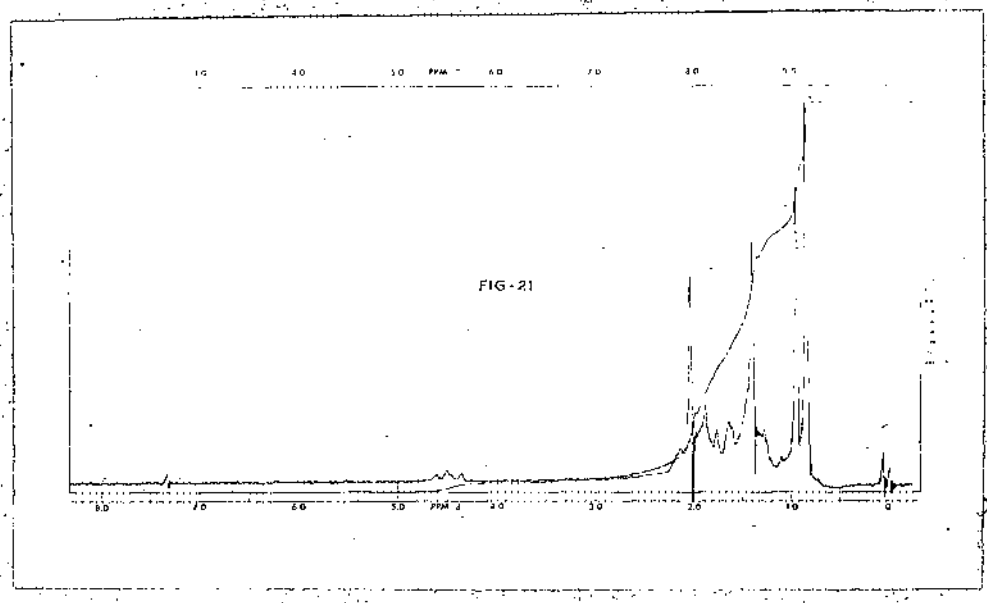


FIG-21

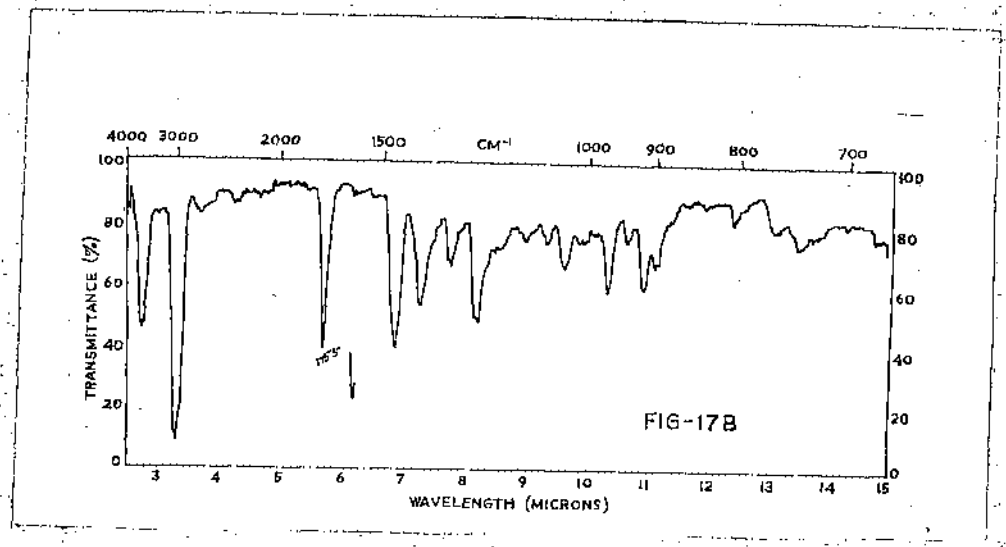
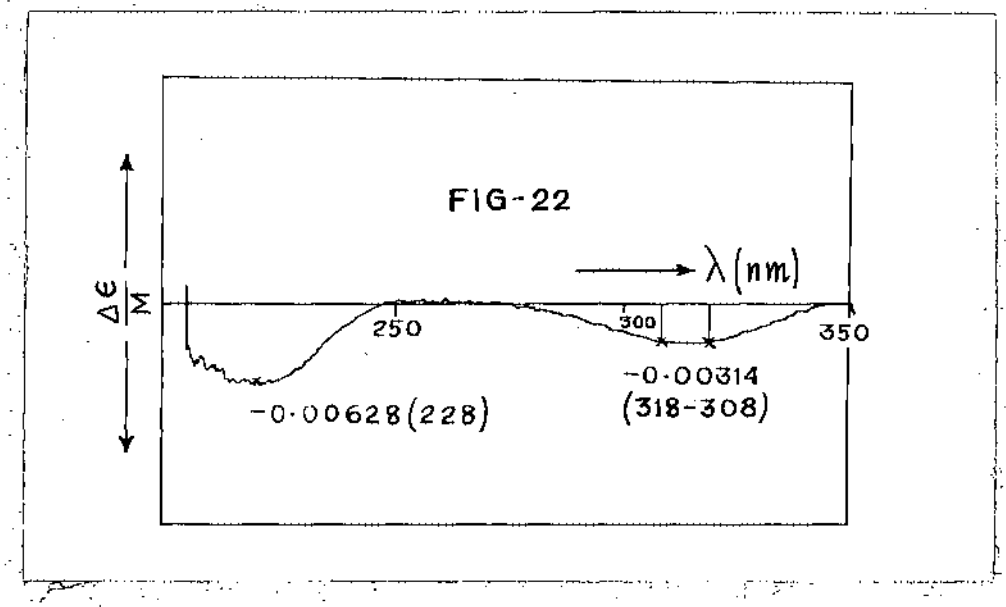
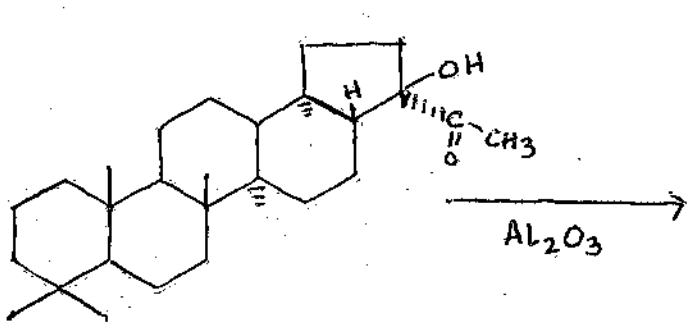


FIG-17B



It is interesting to observe that the structure 11, represented for the nor-keto-lactone, possesses all the structural requirements for ring enlargement to an E-homo derivative under basic conditions. The basic condition employed in the present case, K-tert-butoxide-tert-butyl alcohol, is sufficient to break the lactone ring and then undergo E-homo rearrangement as have been observed in the case of 17-hydroxy-20-keto steroids[21] and E-homo rearrangement[22] in 21-hydroxy ~~iso~~adiantone 12 which in contact with basic alumina furnished 13. The stereochemical requirements of this rearrangement have been considered in detail by Turner[23]. For this type of base-catalysed reaction the suggestion was made that removal of a proton from the hydroxyl group and conference of a full negative charge on oxygen will, as a consequence of an electrostatic repulsion, lead to a particular orientation (s-trans[24] i.e. trans rotational isomer about a single bond) of the carbonyl dipole in 14 and 15. Rearrangement in this case will then proceed from the 17α-hydroxy in 14 to the 17β-OH configuration in 16 and 17β- OH in 15 to 17 α-OH D-homoderivative 17 would be anticipated. This prediction was found to agree with the experimental results.



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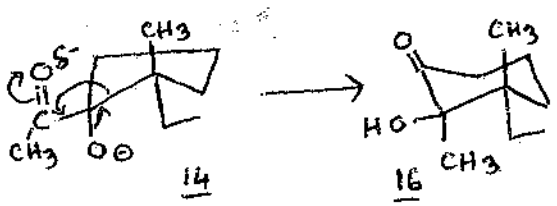

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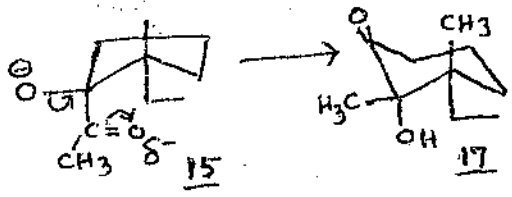
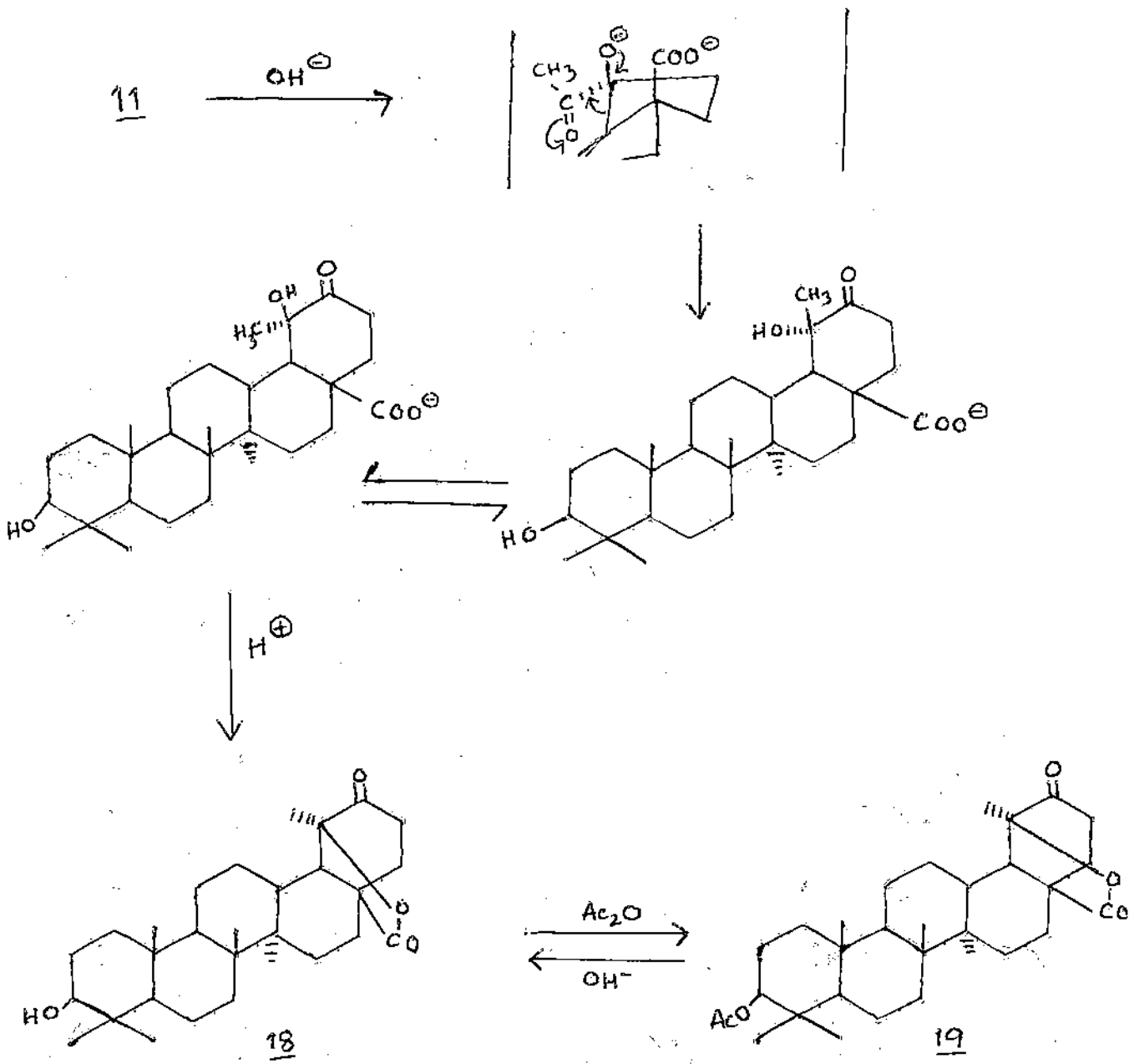


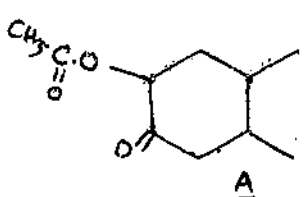
15 17

On the basis of the above argument the base catalysed ring expansion of the nor-keto lactone 11, should be represented by 18, which would be formed according to the mechanism shown below in chart B.

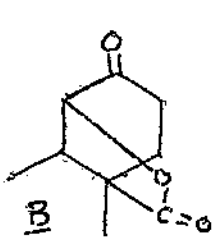
Chart B



If the above mechanism, demanding stereo specificity of the reaction, predicted by Turner[23] is applicable here, then the resulting hydroxy group at C-19 would be equatorial in the chair E-ring conformation. But as the product in the reaction obtained after acidification, was a γ-lactone (I.R. ν max 1755 cm^{-1}) (vide supra), the mechanism of lactonisation requires to be discussed. There are four possibilities: (i) The lactonisation is possible during acetylation, when the C-19 carbonium ion may be generated leading to lactone 18, (ii) during working up of the reaction mixture which involved acidification with mineral acid (see experimental) the carbonium ion at C-19 may be formed, (iii) the intermediate 19α-ketol which is first formed may undergo equilibriation to a mixture of 19α and 19β ketols under the strong basic conditions (K-t butoxide) utilised, and then undergo lactonisation to give 18. This is consistent with the observations[25] in the case of D-homo rearrangements in the steroid series where an equilibrium mixture of 17α and 17β ketols were obtained in several cases under strong alkaline conditions, (iv) ring E-may assume boat conformation which probably facilitate lactonisation between C-19 OH and C-17 COOH groups. In order to understand the lactonisation process the intermediate product 18 was isolated prior to acetylation. It had m.p. 258-60°, molecular formula $C_{30}H_{44}O_4$ and showed U.V. absorption at 280 mμ (ε84). IR spectrum of the compound (Fig. 17B) also showed a broad peak at 1755 cm^{-1} attributable to the composite contribution of a γ-lactone and a six membered ring ketone situated at the α-position to an ester group (in this case γ-lactone). Such shifts



A



B

in the I.R. absorption of ketone adjacent to ester group (acetoxy) of structural type A have been recorded in the literature[26]. Since the compound 18 has got the structural type B having the lactone termination α to the keto group of a six membered ring ketone (analogous to A structurally), the absorption at 1755 cm^{-1} is explicable for the keto-group as well. The same hydroxy lactone 18 was also isolated by mild alkaline hydrolysis of the corresponding acetate. This clearly demonstrates that lactonisation did not take place during acetylation and hence it may be concluded that lactonisation probably took place during acidification of the reaction product with mineral acid.

The structure of the rearranged 3β-acetoxy-lactone as shown in 19, from mechanistic grounds can well explain all the spectroscopic observations. The peak at δ 1.40 in the NMR spectrum (Fig.21) can be explained for the CH_3- group in the system $CH_3-\overset{1}{C}-O-C-$ in structure 19. The U.V. absorption at 290 (ϵ 95) and I.R. peak at 1755 cm^{-1} are also in accord with the above structure. Circular dichroism measurements also strongly support the structure assigned to the rearranged lactone 19. C.D. curve showed two maxima (Fig. 22), The negative lactone effect at 220 mμ ($\Delta\epsilon$ -3.12) has the sign and order of magnitude expected for a 28-19β lactone of 18α-oleanone system. The second maxima due to the ketone Cotton effect at 318-308 mμ ($\Delta\epsilon$, -1.56), however, cannot be correlated with the stereochemistry of the molecule because the octant rule is not applicable to ketones with an oxygen function (lactone) on the α-

carbon atom (P.M. Scopes, Westfield College, London, Private communication). Thus the spectroscopic evidences coupled with the mechanistic considerations of E-homo rearrangement lead to the tentative structure 18 for the rearrangement product. It also becomes clear now that this homo-rearrangement is only explicable in terms of the lactone termination at C-19 in the original nor-keto-lactone 11 and provides additional support to the structure 10 for the γ-lactone obtained by Hg(ic) acetate oxidation of adetyl betulic acid. The failure of the E-homo rearrangement with 5% methanolic potassium hydroxide may be attributed to the inability of the mild conditions used, to effect cleavage of the γ-lactone.

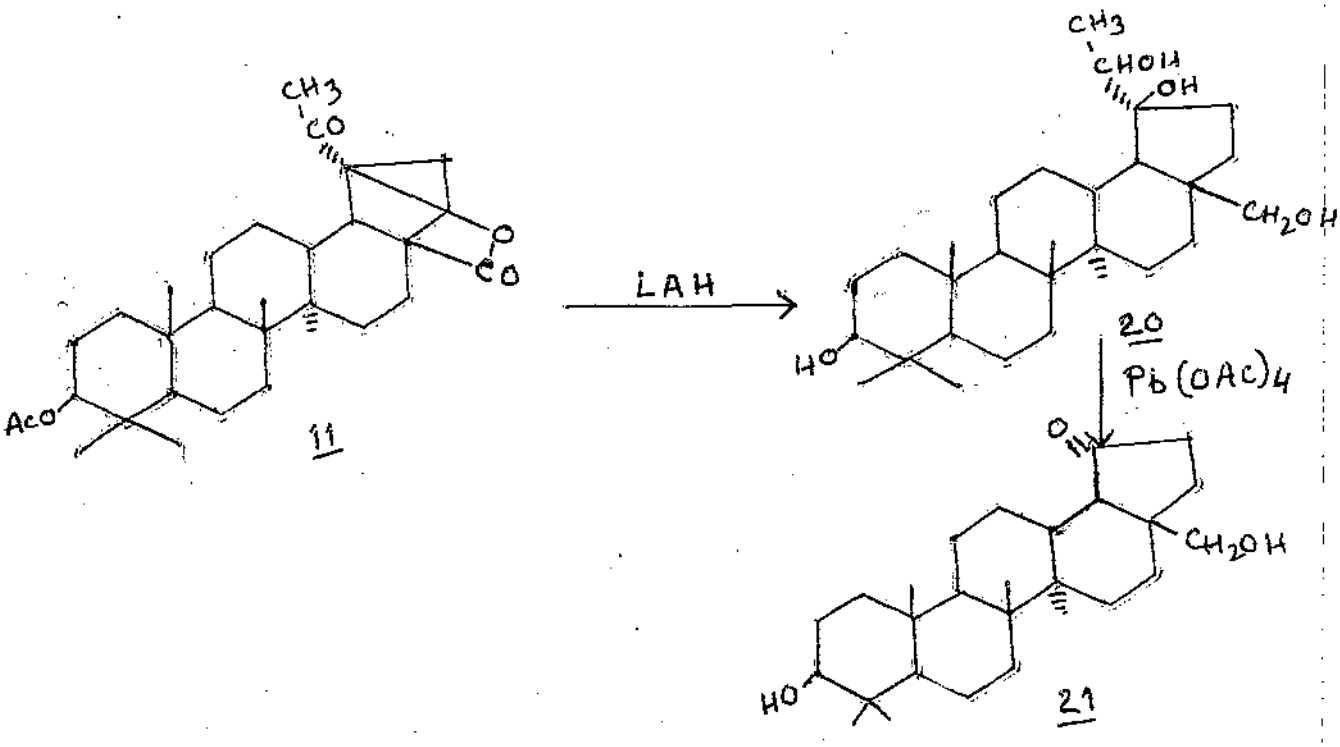
Section C

Chemical evidences for structure 10 assigned to the lactone obtained by Hg (ic) acetate oxidation of acetyl betulic acid

Additional evidence regarding the lactone termination at C-19 for the Hg(ic) acetate oxidation product of acetyl betulic acid, has been obtained from the chemical degradation studies described below.

The nor-keto-lactone 11 was reduced by lithium aluminium hydride to furnish a tetra-ol 20, $C_{29}H_{50}O_4$, m.p. 281-3°, $(\alpha)_D$ + 20°, I.R. ν max 3420 (OH), the absorption in the carbonyl region being totally absent, no U.V. absorption in the region 220-300 mμ. The tetraol was treated with sodium periodate in methanol solution at room temperature, but only the starting material could be recovered in good yield. Treatment of 20 with Pb (iv) acetate at room

temperature in acetic acid solution also resulted in the isolation of the starting material. The tetraol was then exposed to the action of Pb (iv) acetate in glacial acetic acid at 120° for six hours. The gummy reaction product thus obtained was hydrolysed with methanolic potassium hydroxide solution and then chromatographed over alumina. Elution with benzene:chloroform (1:1) resulted in the isolation of a product m.p. 240-2°, $(\alpha)_D$ +32° which analysed for $C_{27}H_{44}O_3$. Its I.R. spectrum showed a peak in the carbonyl region at 1740 cm^{-1} attributed to a ketonic carbonyl group present in a five membered ring and a peak at 3310 cm^{-1} assigned to hydroxyl stretching. In U.V. spectrum it showed abosrption at 285 mμ (ε 75) indicative of the presence of a carbonyl group. On the basis of the above evidences the Pb (iv) oxidationproduct has been assigned structure 21.

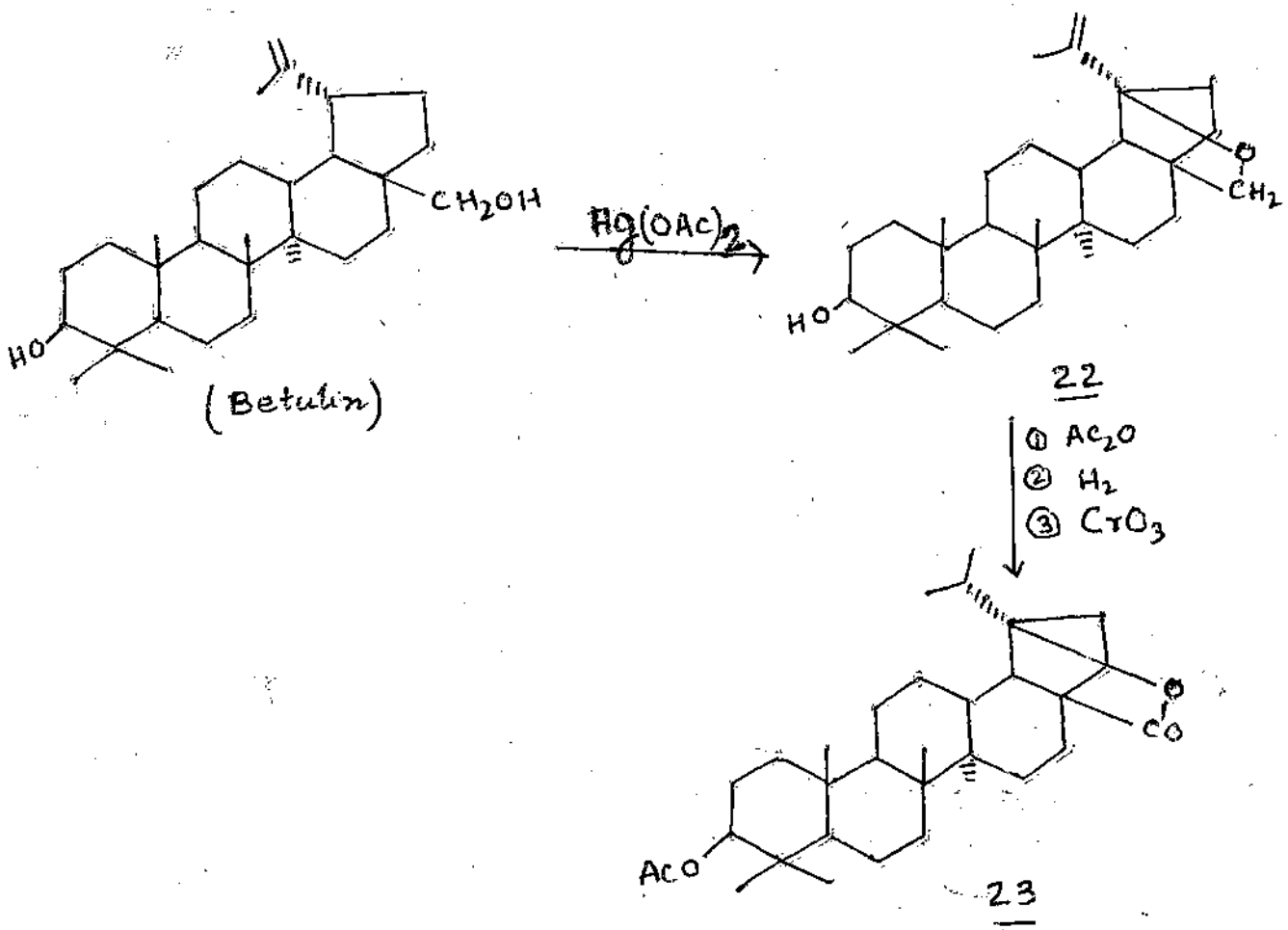


The isolation and characterisation of the tris-nor-ketone 21 establishes definitely the presence of a 1,2-glycol system in the compound. Hence the nor-keto-lactone and the parent lactone must be represented as shown in 11 and 10 respectively.

The failure of glycol cleavage by lead tetraacetate or sodium periodate under mild conditions (room temperature) must be ascribed to steric hindrance around C_{19}-C_{20} bond.

When we had completed these studies a communication[27] leading to the same conclusion regarding the structure of the lactone obtained by Hg(ic) acetate oxidation of acetyl betulic acid has been published. However, the Australian group[27] did not provide any physical evidence from circular dichroism measurements nor did they encounter the novel rearrangement, the base catalysed E-Homo rearrangement, discussed above.

Very recently Vystrčil[19] et al. have shown that the Hg(ic) acetate oxidation product of betulin was 22, the oxygen bridge being present between C-17-C-19. They have established their structure mainly from acid-induced E-ring expansion, which has been discussed in detail (vide supra). Since this ether on acetylation, hydrogenation, followed by CrO_3 oxidation afforded the same dihydro 3β-acetoxy lactone 23 as that obtained by Hg (ic) acetate oxidation of acetyl betulic acid followed by hydrogenation, the revised structure 22 of the ether lends further good support to our structure 10 having lactone termination at C-19.


CH2OH
HO
(Betulin)
Hg(OAc)2
CH2
HO
22
① Ac2O
② H2
③ CrO3
CO
AcO
23