CHAPTER II

Studies on autoxidation : Isomerisation in ring A of β-amyrone

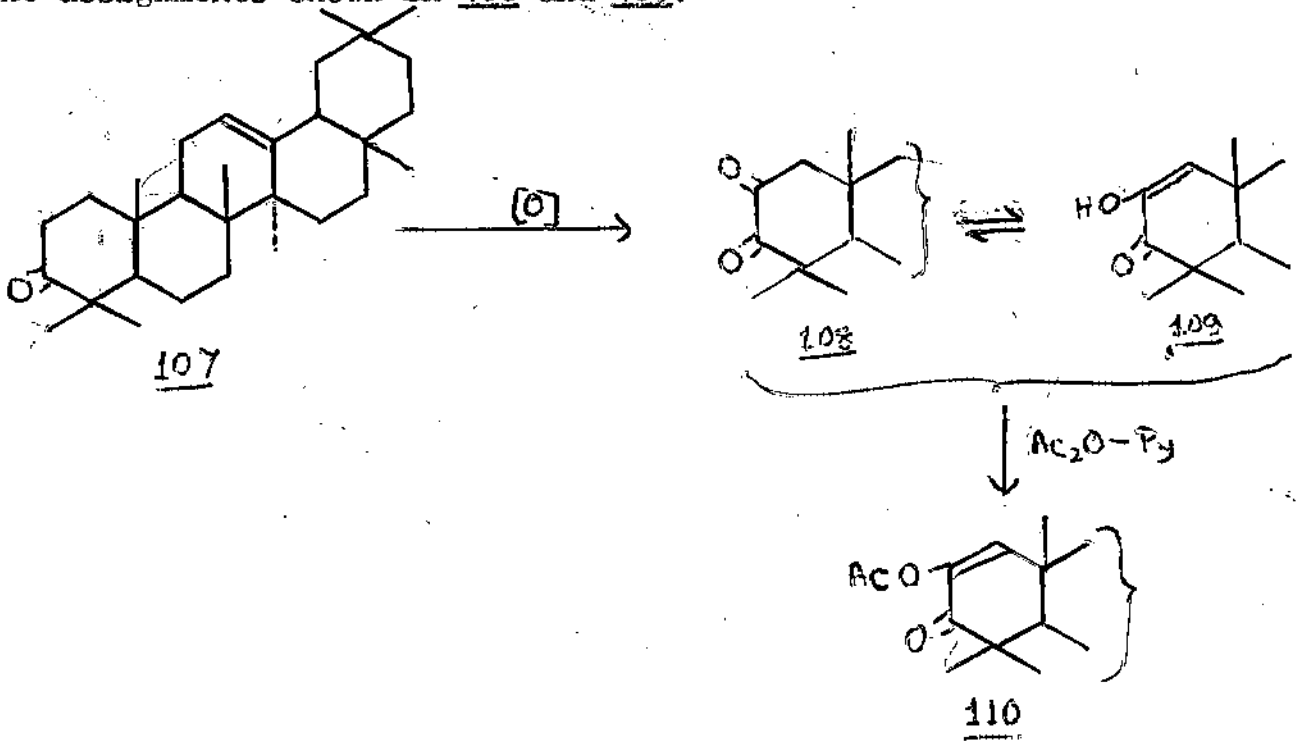
Section A : Introduction

It has been reported[23] that triterpenoids possess a selective action on neoplastic cells and display antitumor activity. Certain Euphorbia species seem to have a limited antitumor activity as indicated by tests reported in literature[20]. Certain fractions of the resins isolated from Euphorbia resinifera have been found to have certain preliminary promising results[21]. Lavie and co-workers performed several experiments with euphorbia lattices to determine their tumor promoting action, they have been compared to croton oil obtained from Croton tiglium (Euphorbiaceae) and a similar action has been discovered with certain species[22]. The occurrence of an α-hydroxy ketone or of a diosphenol grouping in ring A of certain cucurbitacins seemed to be one of the attributes for their activity which has been extensively studied by Lavie and co-workers[24,25]. These authors also studied oxidation of ring A of Euphol, the major constituent of Euphorbia resinifera[23].

In our attempt for introducing more oxygen functions in a triterpenoid molecule, we undertook the autoxidation experiment and we chose β-amyrone as the starting material. This part describes mainly the reactions encountered during the preparation of oxygenated derivatives of β-amyrone (which unlike euphol does not contain any double bond in 8,9 position).

Section B : Discussion

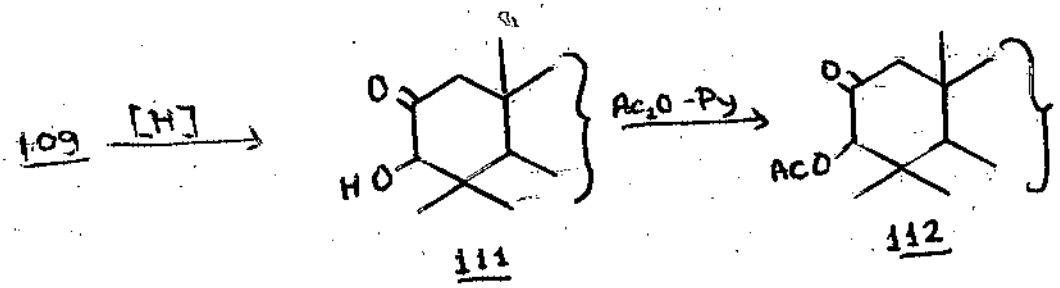
The starting material β-amyrone 107 was obtained by the acid isomerisation of Taraxerone[26] isolated from the bark of Sapium baccatum Roxb,[27] (for isolation of isomerisation see part IV of this thesis). The introduction of an oxygen function α- to the C-3 carbonyl group was carried out by stirring β-amyrone in an atmosphere of oxygen in dry tertiary butyl alcohol containing potassium tertiary butoxide. One mole of oxygen was rapidly absorbed by the compound giving a α-diketone derivative, m.p. 200-2°, $(\alpha)_D$ + 124.27°. The compound showed two spots on the chromatoplate indicating the presence of a mixture of two compounds. Investigations described below established that the above compound was a tautomeric mixture of the diketone 108 and the diosphenol 109. The compound showed positive ferric chloride coloration. Its UV spectra exhibited maxima at 270 mμ, ε= 7932, IR spectra showed peaks at 1100, 1650, 1670, 1716, 2960 and 3570 cm^{-1}. These data were in complete agreement with the assignments shown in 108 and 109.



However, acetylation of the above compound with acetic anhydride-pyridine at room temperature gave the corresponding acetate 110, m.p. 172-73°, $(\alpha)_D$ + 107.69°, which on chromatoplate showed a single round spot. The acetate showed an absorption in its UV spectrum at λ max 236 mμ, ϵ = 9915 and IR absorption at ν_{max}^{nujol} 1205, 1685, 1720, 2950 cm^{-1}. These spectral data clearly established the structure 109 and 110 for the diosphenol and the diosphenol acetate respectively.

In order to prepare the α-hydroxy ketone derivative in ring A, catalytic hydrogenation of the diosphenol 109 was investigated and several interesting results were obtained. Hydrogenation of diosphenol[109] in presence of 10% palladium-on-charcoal catalyst gave a semi-solid mass which after crystallisation from chloroform and methanol mixture gave crystals m.p. 180-82°, $(\alpha)_D$ + 101.70°. The enolic double bond in ring A was reduced during the process, yielding a hydroxy ketone derivative which was found to be homogeneous on chromatoplate. The compound showed UV absorption at λ max 270 mμ, ϵ = 43 and IR of the compound showed peaks at 1700 (carbonyl) and 3500 (hydroxyl) cm^{-1}. NMR spectra (fig. 7) of the compound showed a broad peak at 3.88 ppm accounting for one proton associated with the hydroxyl group containing no neighbouring proton (-CO-C<u>H</u>(OH)-C-), broad peak at 3.44 ppm due to proton associated with the hydroxyl group (-CH-O<u>H</u>) and two AB type doublets at 2.42 and 2.55 ppm (J_{AB} = 12 cps) accounting for two hydrogens (-CO-C<u>H</u>$_2$). The hydroxy ketone on acetylation with acetic anhydride-pyridine gave a crystalline solid m.p. 276-78°, $(\alpha)_D$ + 127.08°, UV λ_{max} 276 mμ, ϵ = 81, IR

chloroform ν_{max} 1725, 1740, 1235 cm^{-1}. It gave a positive Zimmermann test (-CO-CH_2-) and in the NMR spectra (fig. 8) it had a sharp singlet at 4.95 ppm ascribed to the C-3 proton (-C<u>H</u>-OAc) and a broad doublet at 2.49 and 2.37 ppm for two protons adjacent to a carbonyl group (-CO-C<u>H</u>$_2$-). These spectral data can be explained in terms of structure **111** for the hydroxy ketone and 112 for its acetate derivative.



111 112

The formation of 3-hydroxy-2-keto derivative 111 during the process of hydrogenation may be explained by the following way. During the process of hydrogenation a rear attack of the hydrogen from the less hindered side of the molecule may take place, resulting in the formation of the 2-hydroxyl group in β-axial orientation. It has to be borne in mind that the conformation of ring A in triterpenoids as well as 4,4-dimethyl steroids[28-30] are dependent on the 1,3-homoannular interactions of the methyl groups at C-4 and C-10. Therefore, the C-2 β-axial hydroxyl group, which may be formed by rear attack of hydrogen, would produce further 1,3-diaxial interactions resulting in a great strain in the molecule. This strain is released by ready conversion to 3-hydroxy-2-keto derivatives 111 through enolisation of the 2β-hydroxy-3-keto derivatives. It is expected

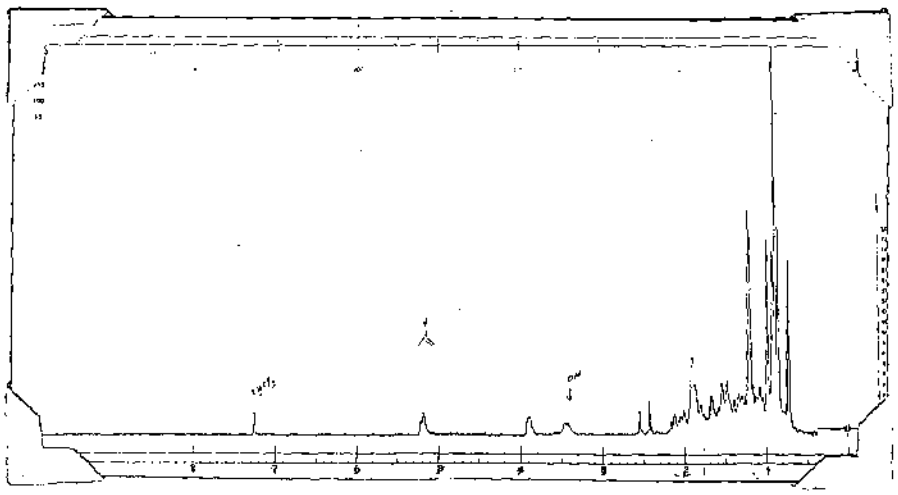


Fig. 7 : NMR spectra of 2keto - β-amyrin.

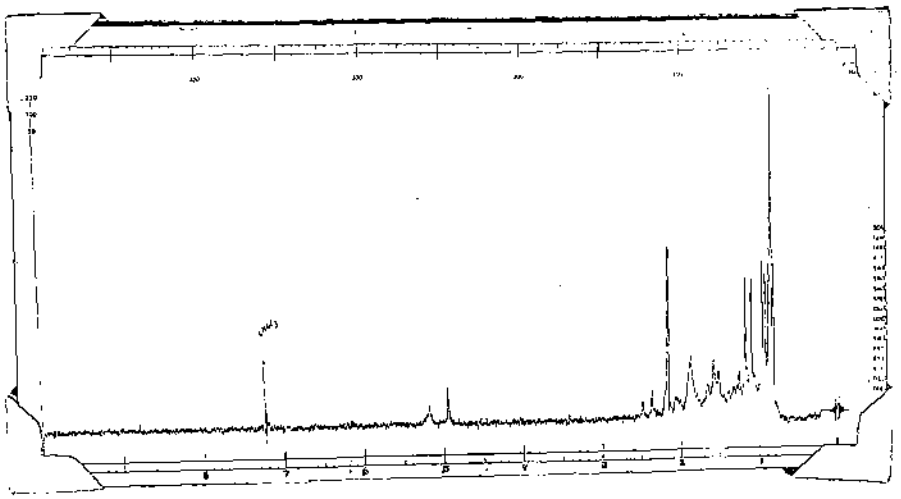


Fig. 8 : NMR spectra of 2keto - β-amyrin acetate.

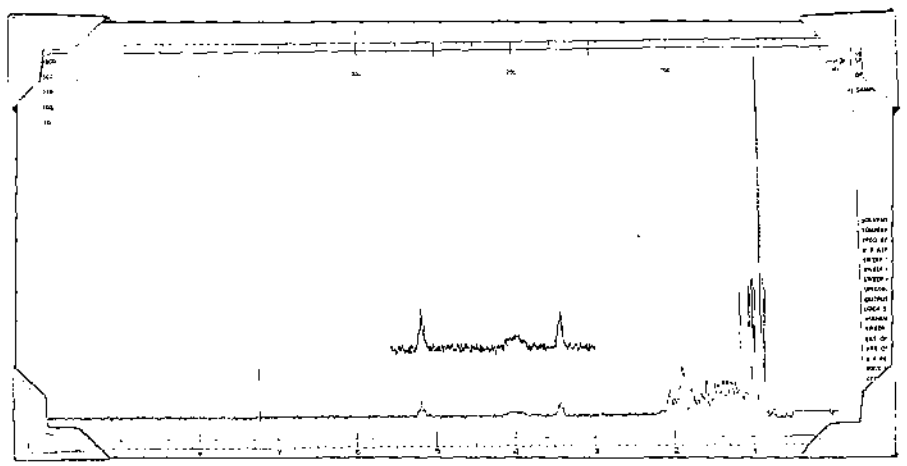
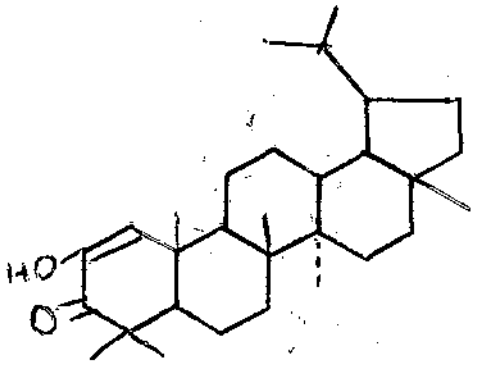


Fig. 9 : NMR spectra of 2hydroxy- β-amyrone.

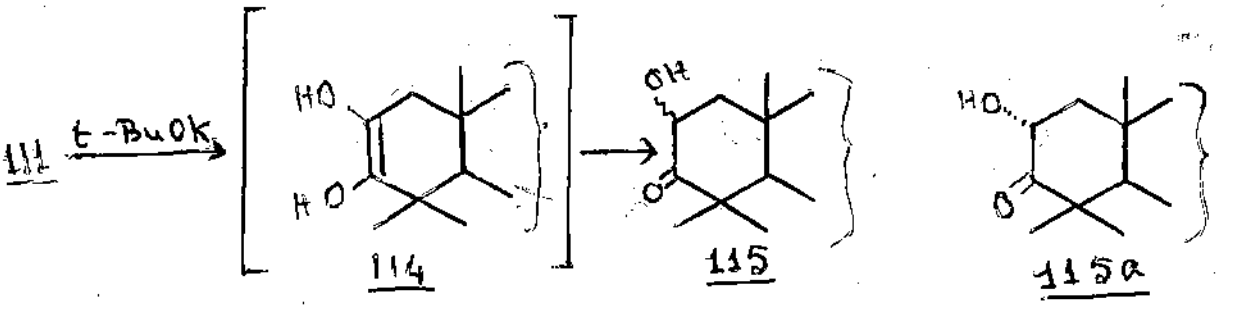
that such a mechanism would lead to the thermodynamically more stable β-equatorial configuration of the hydroxyl group at C-3. An alternative possibility for the formation of 3β-hydroxy derivative is that 1,4-addition of hydrogen takes place during the process of hydrogenation. It may be mentioned in this connection that Govindachari et al.[31] failed to isolate the hydrogenated product of diosphenol of lupanone 113 in pure crystalline form but they assumed that 1,2-addition of hydrogen took place during the process of hydrogenation which is contrary to our observation. In view of our results, a re-examination of their work seems necessary.



113

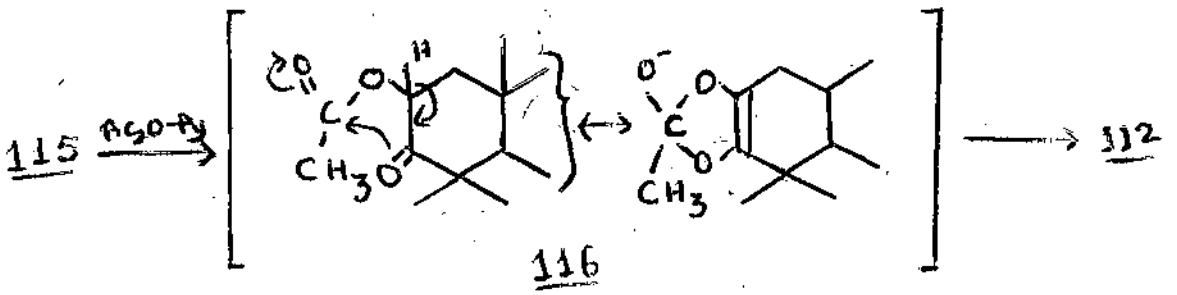
The study of the behaviour of 2-keto-3-hydroxy derivatives 111 towards potassium tertiary butoxide in nitrogen atmosphere gave interesting results. 2-keto-β-amyrin 111 on refluxing with potassium tertiary butoxide in benzene in nitrogen atmosphere gave a compound m.p. 256-8°, $(\alpha)_D$ + 76.47°, UV absorption λ max 276 mμ, ϵ = 82, IR peaks at ν max 3460 (hydroxyl), 1705 (carbonyl) 1650 cm^{-1}. NMR spectrum of the compound (fig. 9) instead of showing peaks at 3.88 and 3.89 ppm for the C-3 proton and a doublet at 2.42 and 2.55 ppm for $-CO-CH_2-$ showed a triplet of a doublet at 3.88, 3.92, 3.96, 4.00,

4.04 and 4.08 ppm (J_{ae} = 8 cps; J_{aa} = 13 cps). The spectral data specially the NMR peaks and the coupling constants indicate that the 2-keto-β-amyrin has undergone isomerisation to a 3-keto-2-hydroxy derivative 115. The nature of the peaks and the coupling constants suggest that an axial proton at C-2 couples with the protons (Ha and He) at C-1 and forms an ABX system[31-34,35]. On the basis of these lines of reasoning the structure 115 has been assigned to the isomerised product. The J values indicate that the 2-hydroxyl group is most probably in the more stable equatorial conformation 115a. The mechanism of the formation of the product 115 from 2-keto-β-amyrin 111 may be explained if it is assumed that the reaction proceed via the enediol 114[36]. Acetylation of the compound 115 with acetic



anhydride and pyridine at room temperature yielded a crystalline solid m.p. 276-78°, which was found to be identical with 2-keto-β-amyrin acetate 112 in all respects. The formation of 2-keto-β-amyrin acetate 112 after acetylation of the 2-hydroxy-β-amyrone 115 can be assumed to have proceeded by the migration of the acetoxy group, the acyl group migrating via the cyclic intermediate 116. This type of acyl migration[23,37] has been reported in literature both in steroid

and triterpenoid field. 2-acetoxy-3-keto compounds are thermodynamically less stable than the 3β-acetoxy-2-keto compounds. 2-acetoxy-

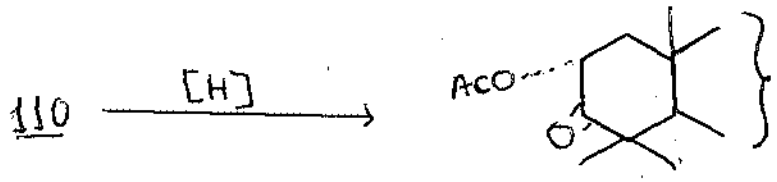


116

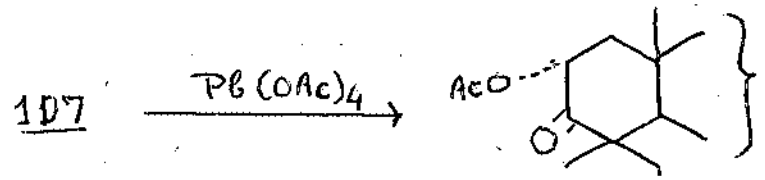
3-keto compound encounters considerable 1,3-interactions with C-10 and C-4 methyl groups. In order to release the strain due to these homoannular and heteroannular 1,3-interactions it readily isomerises to 3β-acetoxy-2 keto compound 112. However, on refluxing the compound 111 with potassium tertiary butoxide in the absence of nitrogen gave a compound which gave positive ferric chloride coloration and was found to be identical in all respects with the diosphenol[37] 109 (m.m.p., UV and superimposable IR).

Diosphenol acetate 110 on hydrogenation in the presence of 10% palladium-on-charcoal catalyst gave a product of m.p. 158-60°, $(\alpha)_D$ +108.57°, IR, ν max 1225, 1238, 1730 and 1750 cm^{-1}. NMR spectrum (fig. 10) of the compound was consistent with the structure 117a, the acetoxy group being at C-2 and its configuration as α. The proton at C-2 displayed a quartet of lines centered at 5.6 ppm, no signals were detected in the region 4.95 ppm characteristics for protons α- to a keto group (-CO-$\underline{CH}_2$-) as was observed in the case of compound 112. The low field signal, forming the X part of an ABX system, may be assigned to a methine proton α both to an acetoxy- and a carbonyl

group. The coupling constants (J_{ae} = 8 cps and J_{aa} = 12 cps) suggests an axial configuration for this hydrogen[38,39]. NMR data are thus in accord with formulation of this product as the 2α-acetoxy-3-ketone 117a. To correlate the 2α-acetoxy-3 keto compound 117a with an authentic specimen, acetoxylation of ε-amyrone using lead tetraacetate-BF_3 etherate was carried out. Acetoxylation at the α-position to the 3-keto group in ring A of triterpenoids with lead tetra-acetate leading to 2α-acetoxy-3-keto compound is well documented[40-42]. This reaction, thought to proceed via the enol[12] is expected to yield a product resulting by attack from the less hin-



117a



117b

dered α-side. In the event, under the mild conditions in which boron trifluoride catalyst was used, an acetoxy ketone 117b m.p. 158-60° was obtained. The mixed melting point of the latter was not depressed when mixed with the acetoxy ketone 117a m.p. 158-60° prepared

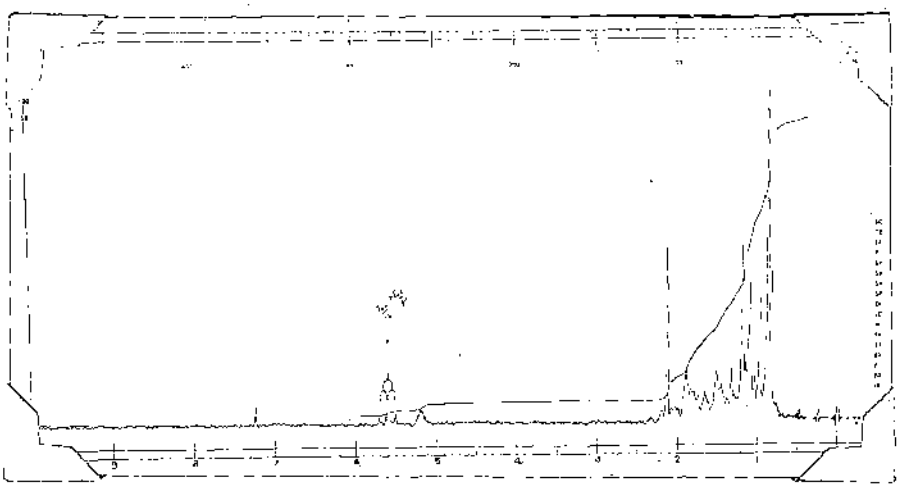


Fig.10 : NMR spectra of 2α-acetoxy - β-amyrone.

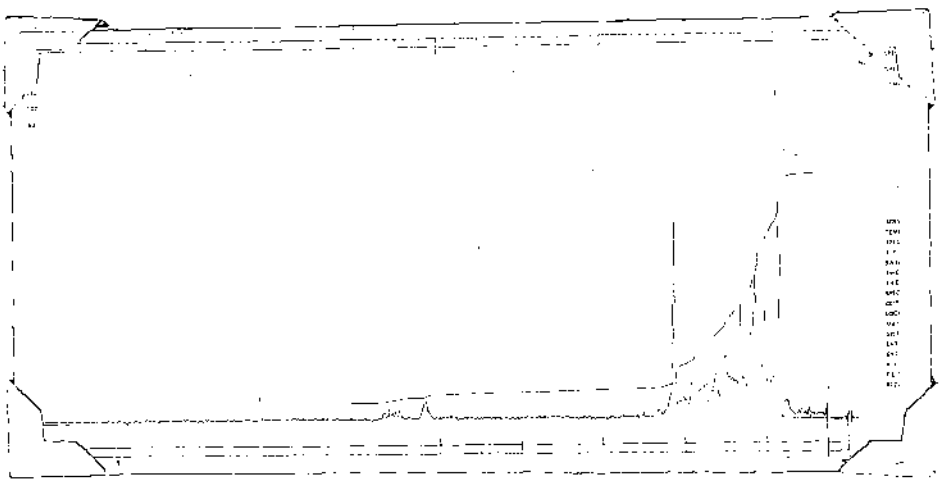


Fig. 11 : NMR spectra of 2α-acetoxy -β-amyrone.

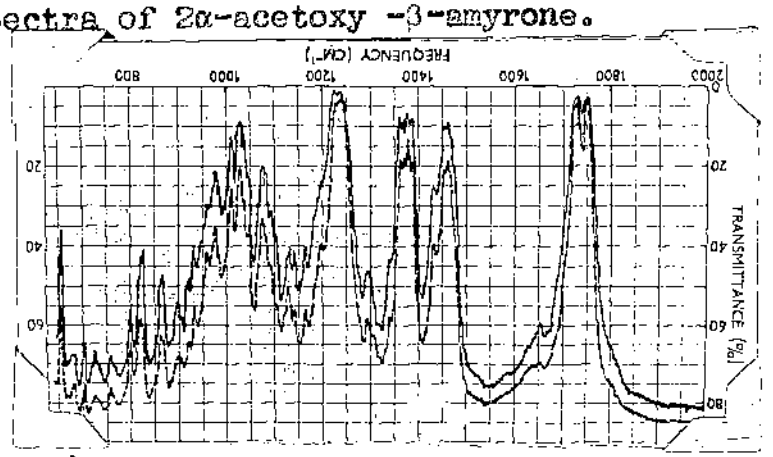
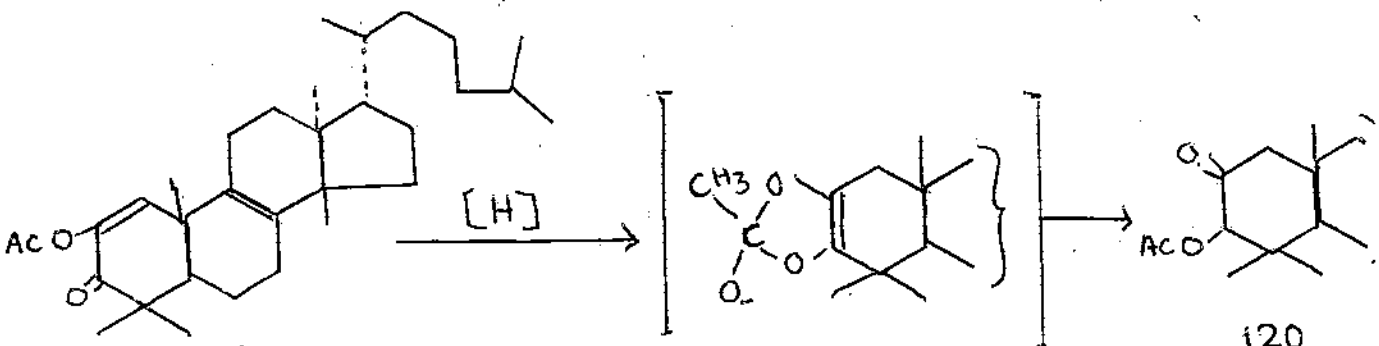


Fig. 12 : IR comparison
Solid line - 2α- acetoxy-β-amyrone obtained by hydrogenation of diosphenol acetate.
Dotted line - 2α- acetoxy -β-amyrone obtained by $(Pb(OAc)_4$ treatment of β-amyrone.

by hydrogenation of diosphenol acetate 110. The IR spectra of both the compound 117a and 117b were run on the same paper for comparison purposes. A close observation of the spectras (fig. 12) revealed slight deviations in the regions 1225-1245 cm^{-1} and 1070-1095 cm^{-1}. This significant observation lead us to believe that most probably the lead tetra-acetate acetoxylation product could be a mixture of conformational isomers. Relevent to this observation is the paper of Jones et al.[43] in which they stated that acetoxylation of 3-keto-triterpenoids can cause conformational changes in ring A. NMR spectra of the compound (fig. 11) was taken. Careful analysis of the NMR peaks of 117b showed that the peaks associated with the C-2 axial proton were slightly different from the spectra of 117a (fig. 10). In the spectra of 117a the resonance of peaks of the axial proton on C-2 appears as a clear quartet due to X proton of the ABX system[38,39]. The coupling constants of 117a (J_{aa} = 12 cps, J_{ae} = 8 cps) indicate that it is predominantly in a single conformation probably chair conformation. But the region of peaks associated with C-2 proton in 117b was complex and showed more paaks. The coupling constants showed two sets of values (a) J_{aa} = 11.5 cps, J_{ae} = 8 cps and (b) J_{ae} = 6 cps, J_{aa} = 13 cps; the values in (a) being like those observed for 117a. The coupling constant values J_{aa} = 13 cps and J_{ae} = 6 cps probably arise due to a isomer having deformed chair conformation in ring A[44]. Those observations seemed to indicate that 117b was a mixture of conformational isomers.

In this connection it is necessary to mention that Lavie and coworkers during the studies on oxidation in euphol series claimed

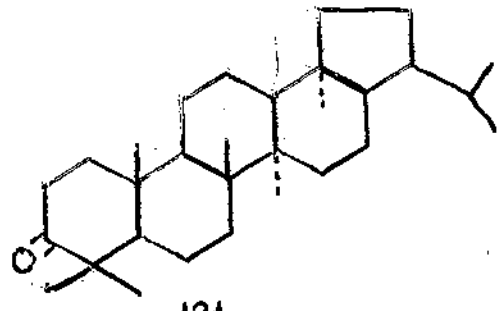
that hydrogenation of diosphenol acetate of dihydro Euphone 118 gave a product which was identical with the C-3 acetate 120, i.e. a 2-keto-3-acetoxy derivative. Migration of the acetoxy group from the C-2 to C-3 position was proposed through the cyclic intermediate 119[45]. Their results are contrary to our observations on the β-amyrone series where we obtained a 2α-acetoxy-3-keto compound 117a on hydrogenation of diosphenol acetate 110. In our case 1,2-addion of hydrogen takes place leading to a stable 2α-acetoxy-3-keto



118 119 120

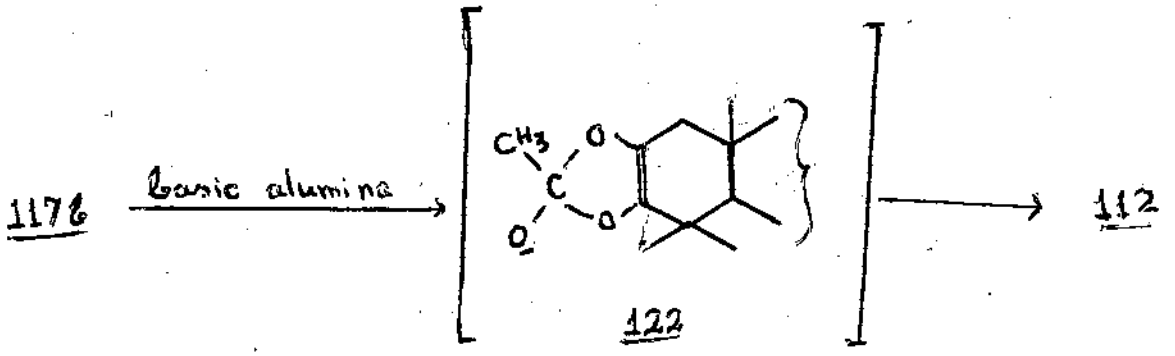
compound. In order to provide a reasonable explanation for the different behaviour shown by the two types of compound (Euphol and Oleanene) we have examined the Drieding models of both euphol and β-amyrin derivatives. In the euphol series the presence of a double bond in 8,9 position causes deformation in ring B[46] and has a modified chair conformation which confers additional strain in the molecule, whereas in the β-amyrin series (having A/B chair-chair conformation) this strain is not present. Most probably the 2α-acetoxy-3-keto compound which presumably is formed at first on hydrogenation of 118 isomerises to 119 to release the additional strain in the

molecule. In order to confirm our observation that 2-acetoxy-3-keto derivative is formed in the hydrogenation of diosphenol acetate 110 (having A/B chair-chair- conformation) we extended our studies with the diosphenol of moretanone, 121 where the A/B ring has the same chair-chair conformation. The results obtained in this series were similar to those described earlier in the β-amyrone series. The details of the work in the moretanone series is described in section C.



121

The isomerisation of the 2α-acetoxy group from 2α-equatorial acetoxy ketone to 3-acetoxy-2-ketone was also observed. The compound 117a was adsorbed on a column of basic alumina and kept overnight (18 hours). After elution with benzene the crystalline solid isolated had m.p. 276-80 and was found to be identical with 2-keto-β-amyrin acetate 112. This can be explained by the isomerisation and migration of the acetoxy group via the cyclic intermediate 122. Such isomerisation of 2α-equatorial acetoxy ketone is believed to give the thermodynamically most stable product, 3β-acetoxy-2-keto compound 112. On the basis of the above fact, the acetoxy group in 112 was assigned 3β-configuration. Furthermore, since the same 3-acetoxy-2-ketone

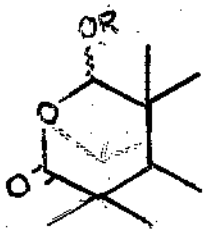


compound 112 was obtained from the 2-keto-3-hydroxy compound III which was obtained by hydrogenation of diosphenol 109, the hydroxyl group in 111 may also be assigned β-configuration.

In order to obtain the 2-hydroxy-3-keto and 2-keto-3-hydroxy compounds from their corresponding acetoxy ketones, hydrolysis was attempted, but the results were not satisfactory. Alkaline hydrolysis of both the compounds 112 and 117a gave in each case an intractable gum which could not be crystallised. However, the acid hydrolysis of the compounds 112 and 117a in nitrogen atmosphere gave a solid compound which on chromatoplate showed two spots, suggesting that it was a mixture of two compounds. From the R_f values it appeared that it was probably a mixture of keto-alcohol 111 and 115. This fact again suggests that one form can easily be isomerised to th other from through the intermediate 114. However, acetylation of the above mixture gave a single compound (TLC) m.p. 275-77°, identical with the 2-keto-β-amyrin acetate 112 (m.m.p. and IR comparison).

During the process of autoxidation a second mole of oxygen was absorbed by the disophenol 109 and an another product m.p. 262-5°,

$(\alpha)_D$ + 66.66° was isolated from the reaction mixture. This has been identified as the lactol 123 (1-hydroxy-2-oxo-3-amyrone). The compound 123 did not show any UV absorption in the region 220-300 mμ and IR showed peaks at 1720 and 3350 cm^{-1}. The formation of such a lactol has already been described and was interpreted through the formation of a ring A seco-2-nor-aldehydo carboxylic acid which cyclizes upon acidification[47]. Acetylation of 123 with acetic anhydride-pyridine gave an acetate 124 m.p. 186-8°, $(\alpha)_D$ + 117.64° IR, νmax 1740, 1752, 1462, 1458, 1380, 1360 cm^{-1}. The loss of a carbon atom (C-2) during this process and the formation of the hetero cyclic six membered ring is now shown unequivocally by examining the NMR spectrum of the compound. A characteristic singlet related to the C-1 proton was found at 5.25 ppm; this peak was not sharp due to coupling with the proton of the adjacent hydroxyl group. However, in the NMR spectrum of its acetate (fig. 13), this signal was found to be shifted downfield to 6.18 ppm and appeared now as a sharp peak. From this observation it can be deduced that no protons are present adjacent to C-1 hydrogen as would be expected from a lactol derivative derived from 2,3-seco-aldehydo-acid.



123, R = H
124, R = Ac

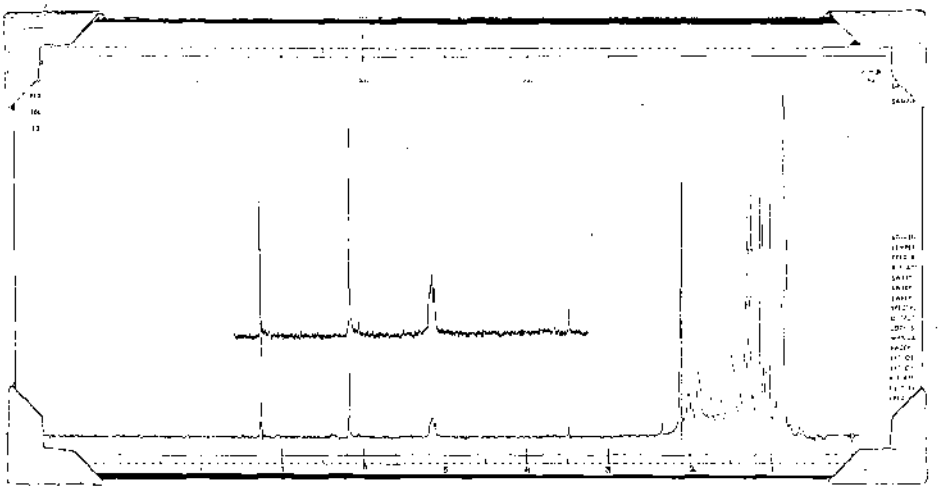
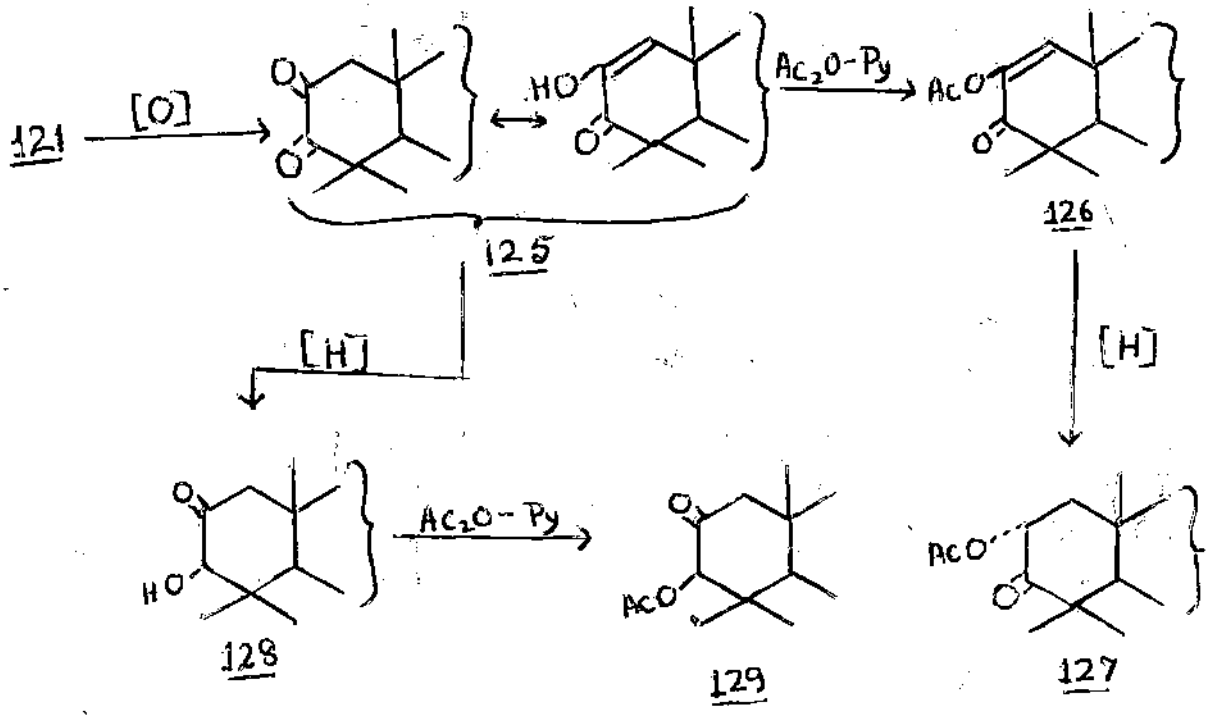


Fig. 13 : NMR spectra of lactol acetate.

During this process of autoxidation, no other positions were oxidised as it did not show the presence of any other oxygen function (elemental analysis) and the presence of the vinyl proton could be discerned in all the NMR spectras of the compounds reported herein.

Section C : Studies on autoxidation in moretanone series

Moretanone 121 obtained by hydrogenation of moretenone[48] was autoxidised by passing oxygen through a suspension of 121 in dry tertiary butanol-containing potassium tertiary butoxide. The corresponding diosphenol 125 m.p. 190-2°, UV, λmax 269, ϵ= 5104 was obtained. The solid gave positive ferric chloride coloration and showed two spots on a chromatoplate. Acetylation of the above diosphenol afforded the diosphenol acetate 126 as a viscous oil which could not be induced to crystallisation. The oil showed uv absorption at 234 mμ, ϵ = 6514 consistent with structure 126. Disophenol acetate 126 on hydrogenation in presence of palladium-on-charcoal catalyst in ethanol solution gave the reduced compound 127 m.p. 179-81°, UV, λ max 276 mμ, ϵ = 82. Diosphenol 125 on hydrogenation in presence of palladium-on-charcoal catalyst in ethanol solution afforded a solid 128, m.p. 181-3° which on acetylation afforded the corresponding acetate 129, m.p. 264-7°.



The acetate 127 was definitely different from the acetate 129 and isomerised to 129 when it was adsorbed on a column of basic alumina. This observation was similar to that observed in the auto-xidation of β-amyrone series. Therefore the isomerised product 129 above should have the stable 2-keto-moretanyl acetate structure.

The above chemical evidences establish that during the hydro-genation of diosphenol acetate a 1,2-addition of hydrogen takes place whereas in the hydrogenation of diosphenol either 1,2-addition of hydrogen followed by isomerisation to the stable 2-keto-moretanol 128 takes place or direct 1,4-addition of hydrogen takes place to give the same compound. This is consistent with our previous observations in the β-amyrone series.