CHAPTER-II

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

Section A: Introduction

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

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

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

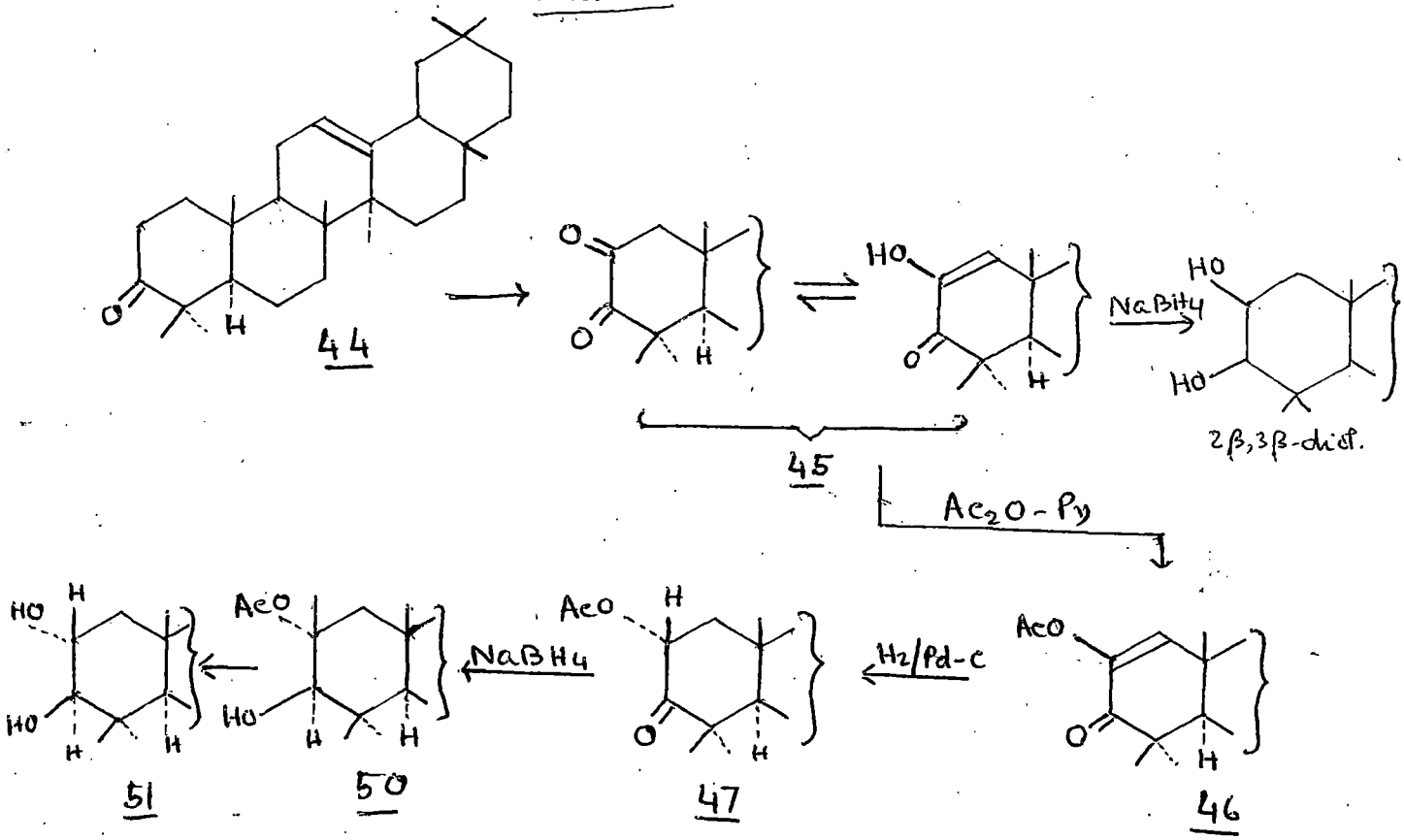
Section B: Autoxidation Studies on β-amyrone:

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

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

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

Chart-I



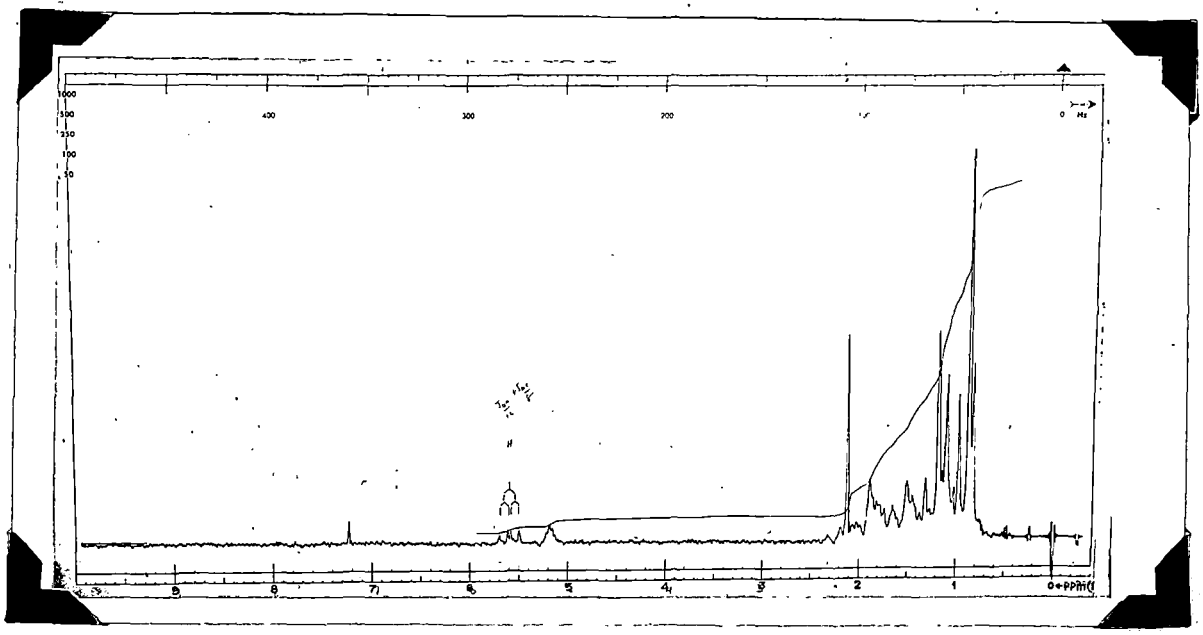
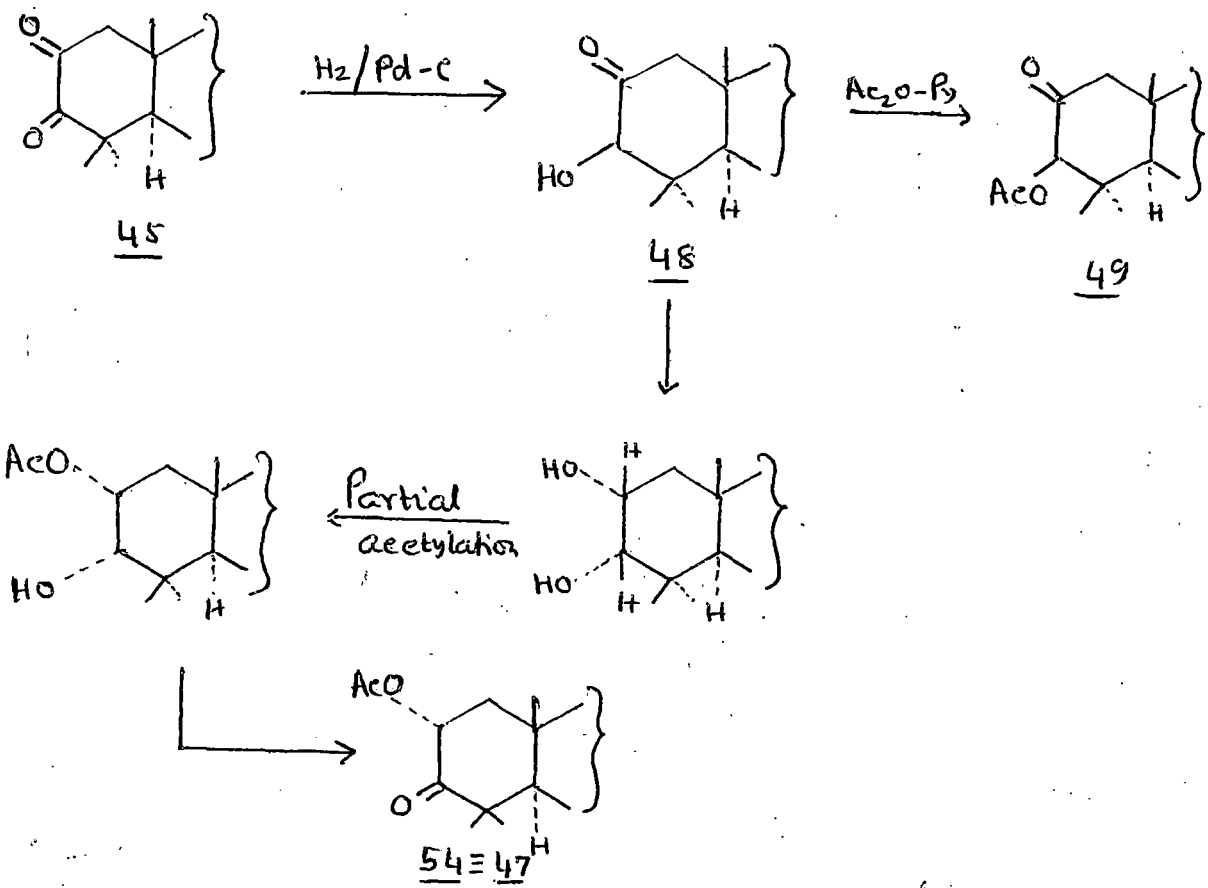


Fig. 16 NMR spectrum of 2α-acetoxy-β-amyrone 47



45 48 49

54 ≡ 47

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

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

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

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

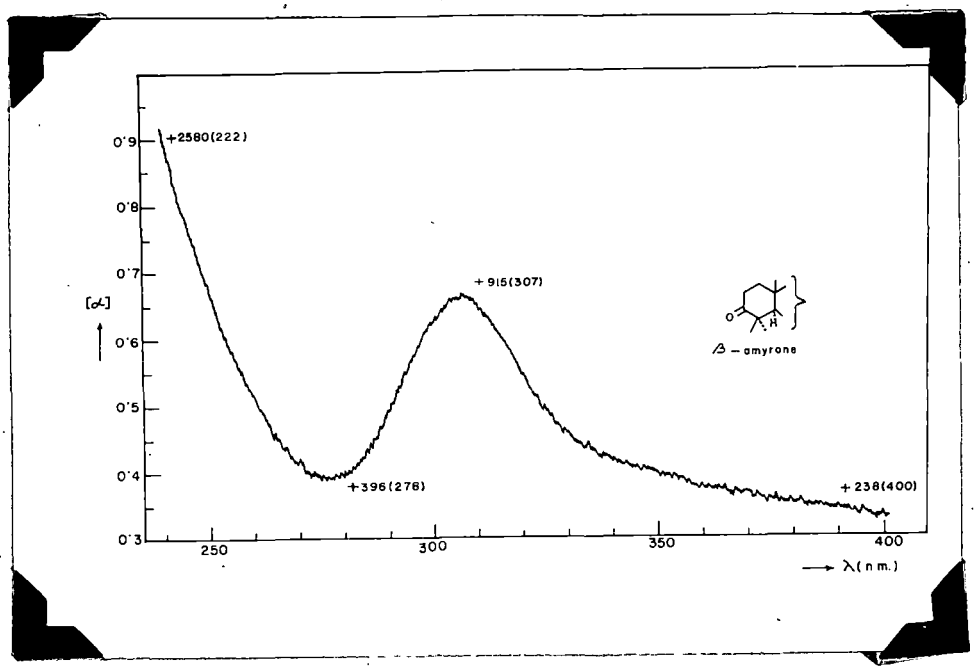


Fig. 17 ORD curve of β -amyrone 44

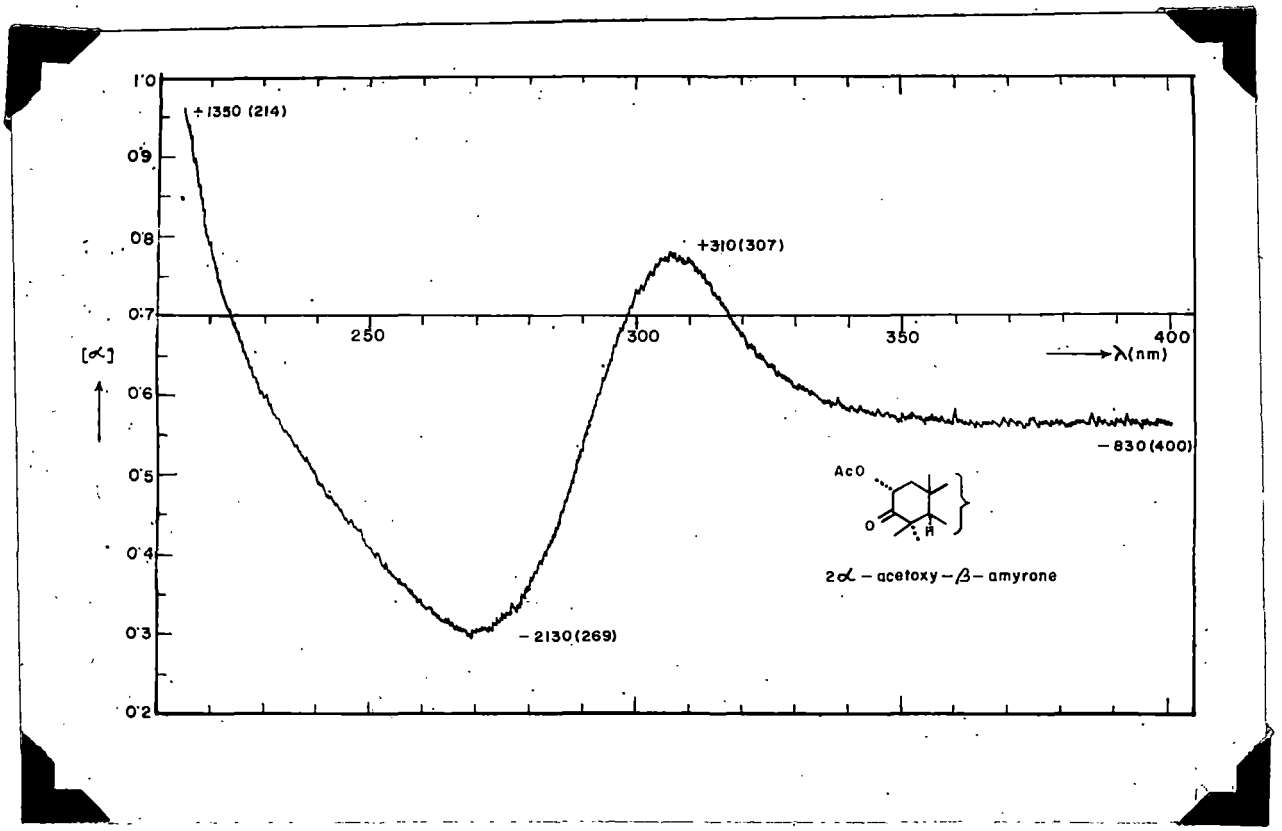


Fig. 18 ORD curve of 2α-acetoxy β -amyrone 47

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

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

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

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

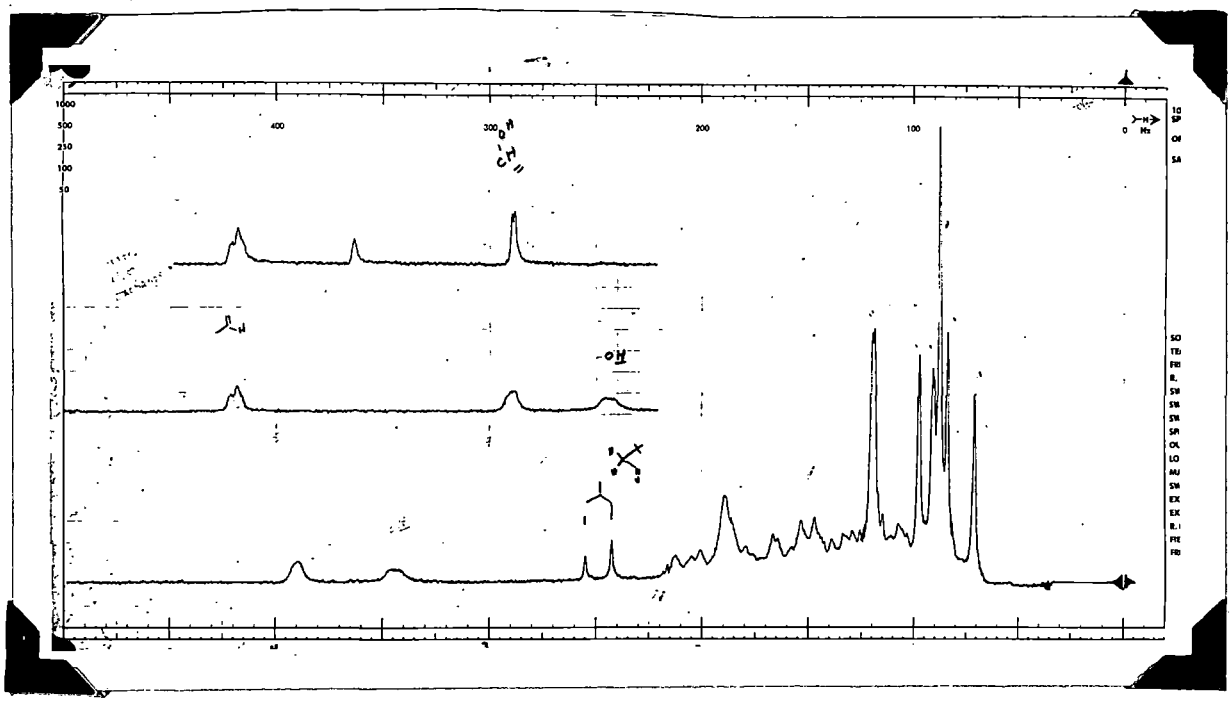


Fig. 19 NMR spectrum of 2 keto-β-amyrin 48

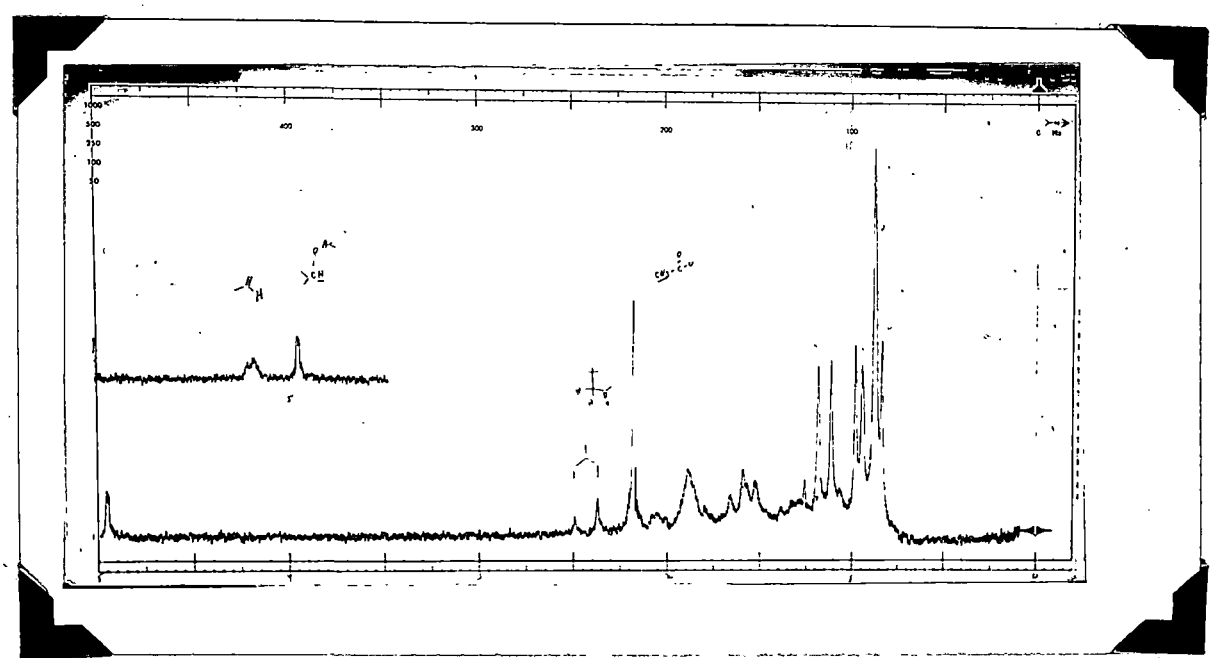


Fig. 20 NMR spectrum of 2 keto-β-amyrin acetate 49

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

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

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

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

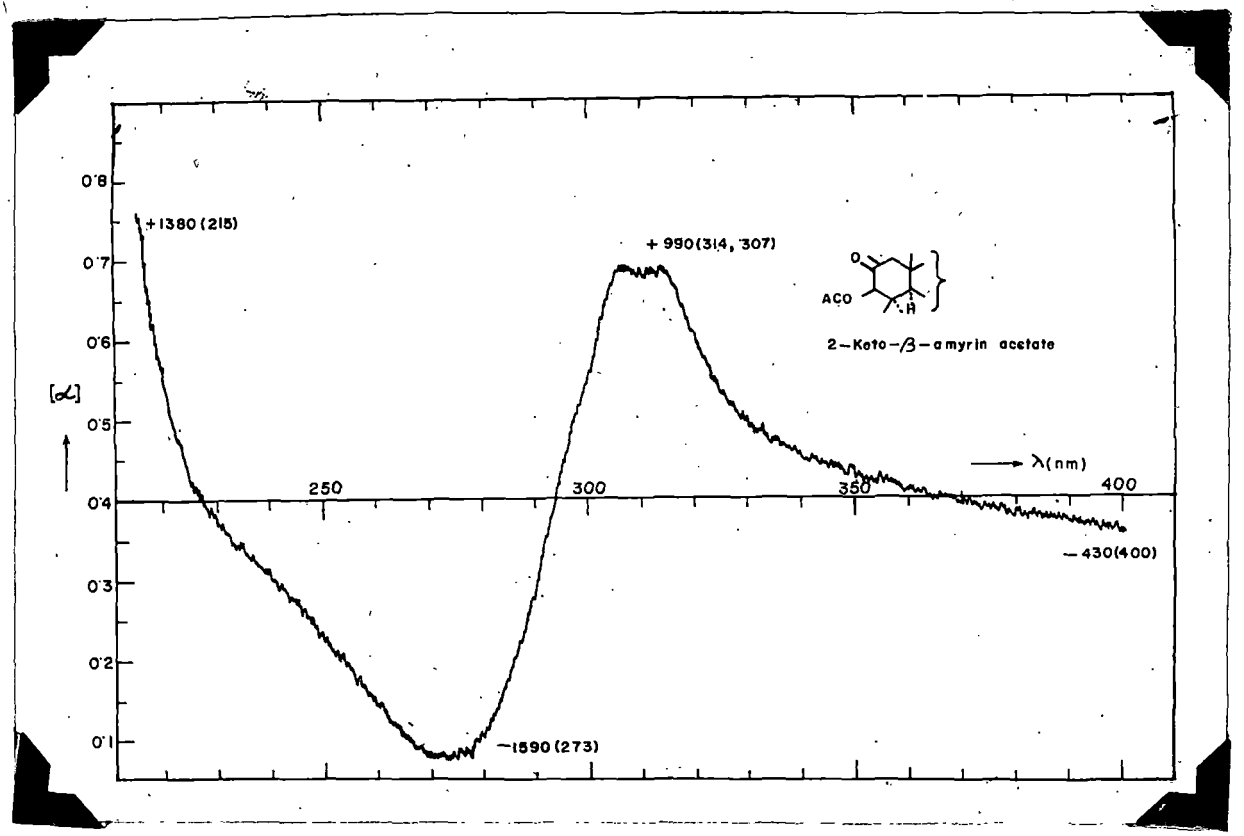


Fig. 21 ORD curve of 2 keto-β-amyrin acetate 49

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

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

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

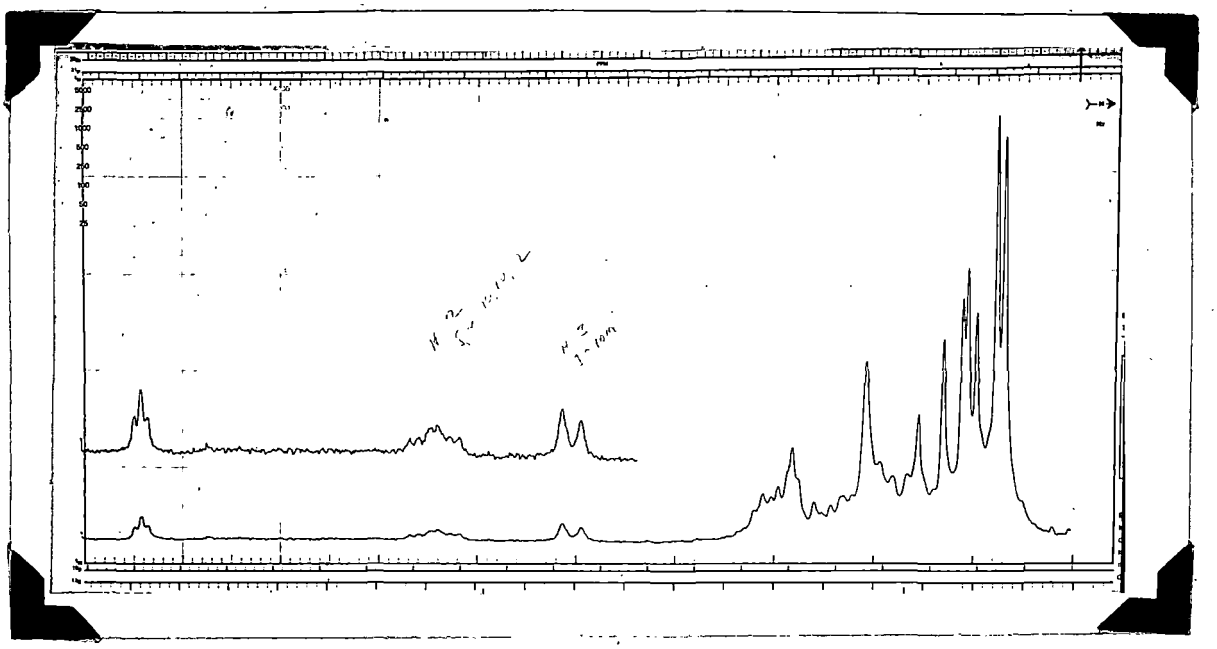


Fig. 22 NMR spectrum of 2α , 3β -diol 51

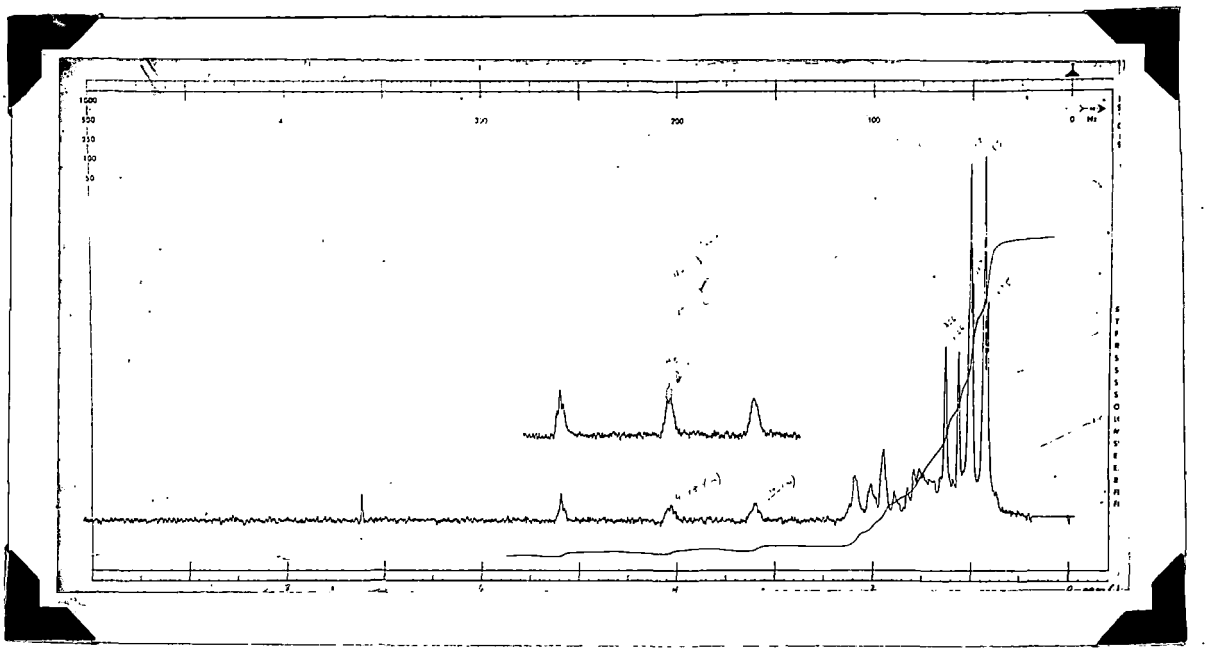


Fig. 23 NMR spectrum of 2α , 3α diol 52

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

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

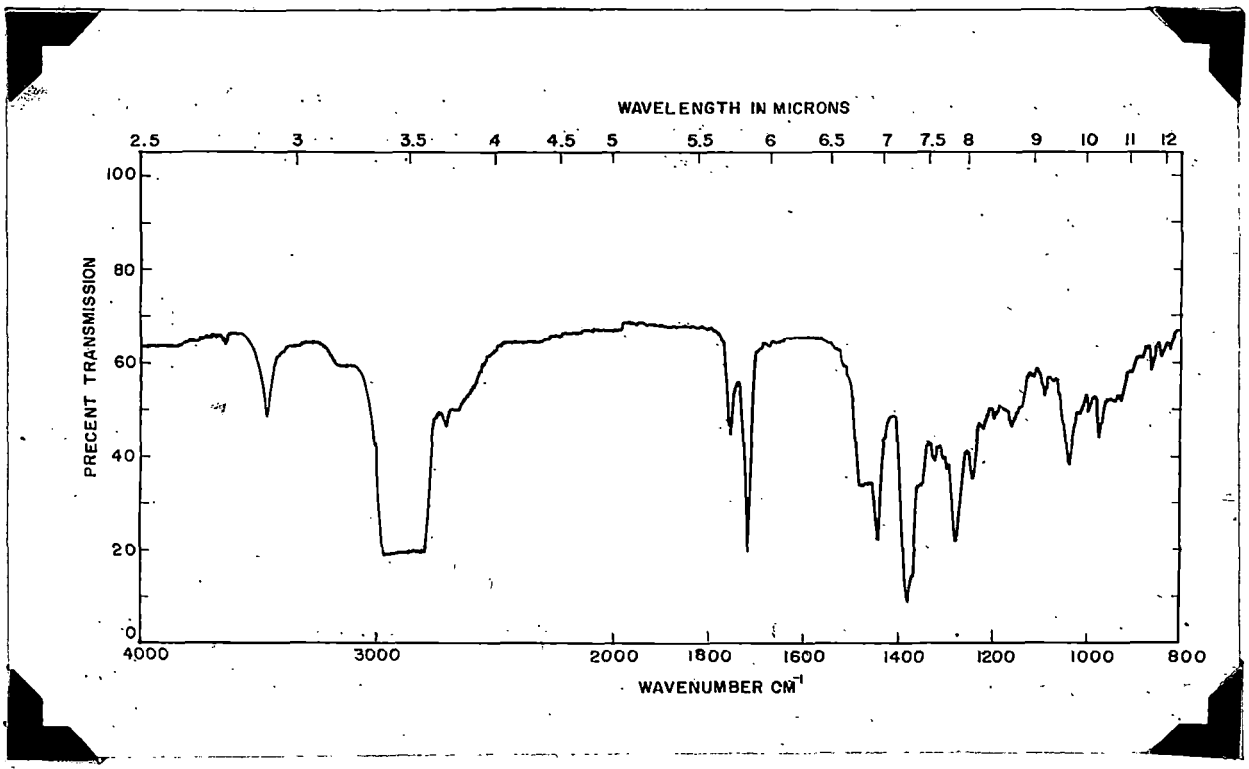


Fig. 24 IR spectrum of 2α-acetoxy-β-amyrin 53