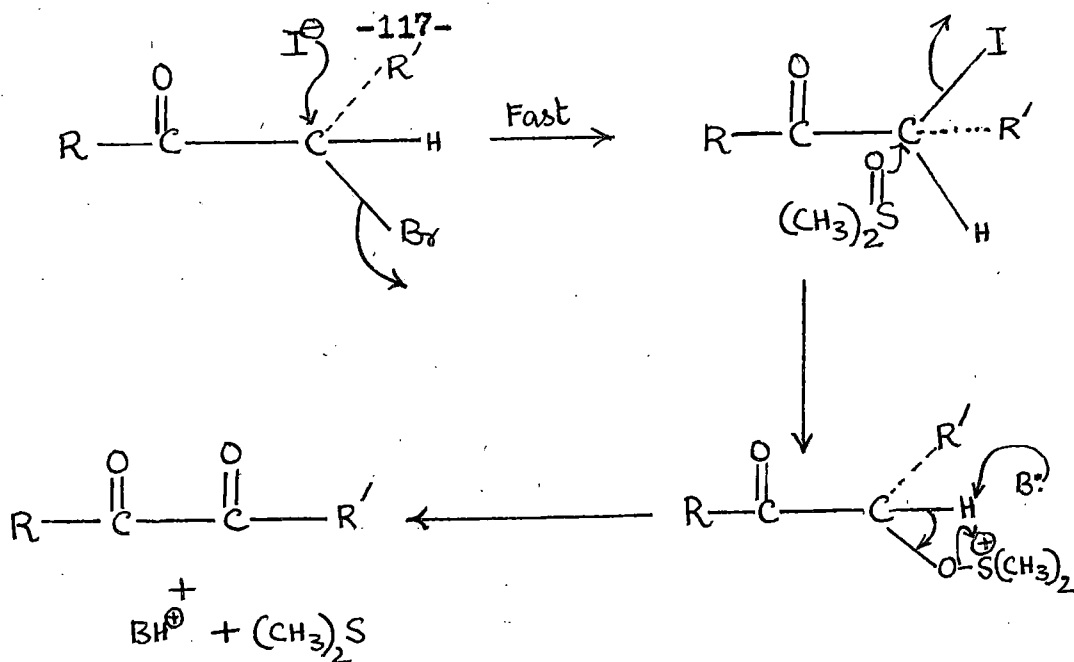


CHAPTER - II

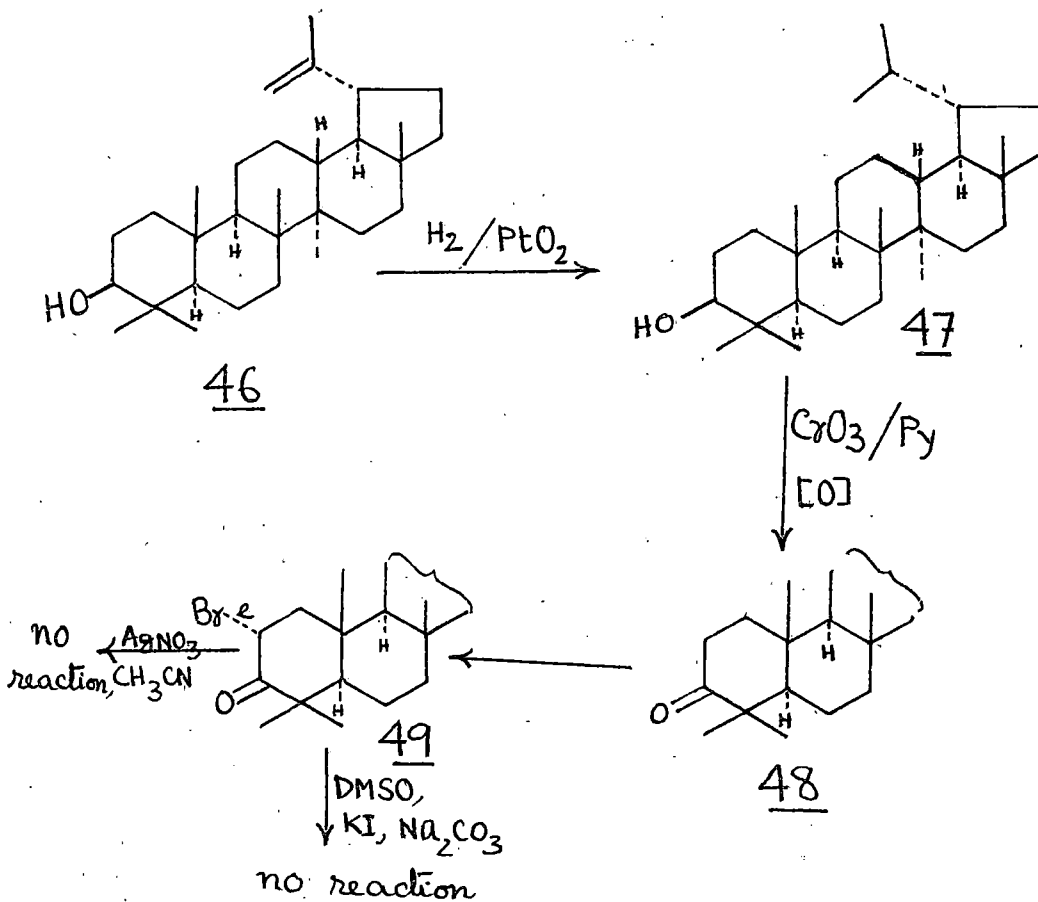
Discussion

Section A

Lupeol 46 m.p. 215-216°, $[\alpha]_D^{25}$ 33° was isolated from the neutral part of the benzene extract of the bark of Xanthoxylum budrunga^{30a,b}. It was hydrogenated in an atmosphere of hydrogen in presence of Adam's Catalyst (PtO₂) under atmospheric pressure to lupanol 47 m.p. 206°, $[\alpha]_D^{25}$ -17.8°. This on oxidation with chromium trioxide-pyridine complex⁴⁵ afforded lupanone 48 m.p. 209-210°, $[\alpha]_D^{25}$ 16.2°; tlc showed single spot on the chromatoplate. Infrared spectrum exhibited band at 1690 cm⁻¹ indicating the presence of a carbonyl group (Fig. 1). 48 on refluxing with cupric bromide in presence of chloroform and ethyl acetate mixture²³ afforded colourless crystals of 2-bromolupanone 49, m.p. 226-227°, $[\alpha]_D^{25}$ -20.6°. An attempt was made to apply the iodide catalysis oxidation of 2-bromolupanone 49 with dimethyl sulfoxide¹⁸. The reaction failed. The reason for this failure could be ascertained as DMSO oxidation required SN₂ attack by the sulfoxide oxygen at brominated carbon, it is sensitive to the steric environment of that center. The mechanism runs as follows:



Thus reaction could be feasible if the halogen atom (Br) assumed the axial conformation with respect to the carbonyl group in case of 2-bromo lupanone 49.

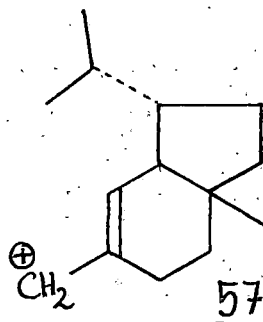


It is known that the special spatial relationship of the carbonyl function in cyclohexanone to the equatorial and axial groups on the adjacent (α) carbon gives rise certain peculiarities in the infrared spectra of these compounds when the substituent is a polar group such as halogen. In the infrared spectra, the carbonyl stretching frequency is increased by the nearly parallel dipole of an equatorial α -halogen by about 20 cm^{-1} , whereas the dipole of an axial α -halogen is so oriented that it leaves the carbonyl stretching frequency nearly the same as in the unsubstituted ketone³¹.

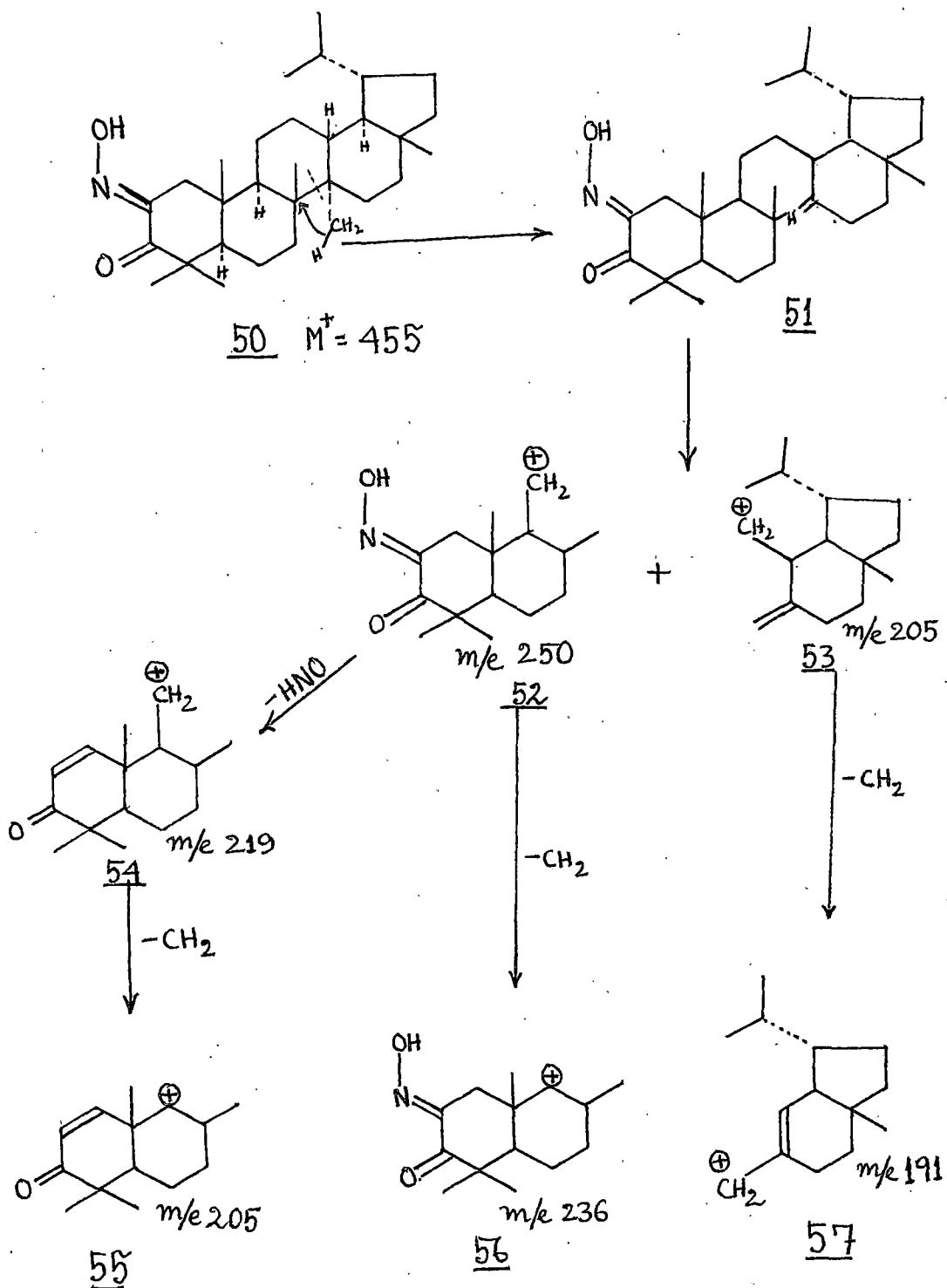
Infrared spectrum of 2-bromo lupanone 49 clearly showed an increase of carbonyl stretching frequency by about 20 cm^{-1} from that of lupanone 48 [Fig. 1] indicating thereby that the bromine atom is equatorial in respect of carbonyl group. Another attempt to prepare the nitroester of the corresponding 2-bromo-lupanone 49 by treatment with silver nitrate in presence of acetonitrile²² proved to be unsuccessful. The reason stands same as in the above case. Thus the stereochemistry of bromine atom at C-2 is equatorial in 2-bromolupanone 49. The introduction of C-2 carbonyl group in lupanone 48 was then carried out as follows:

Lupanone48 in presence of potassium tert. butoxide was stirred with freshly prepared and distilled isoamyl

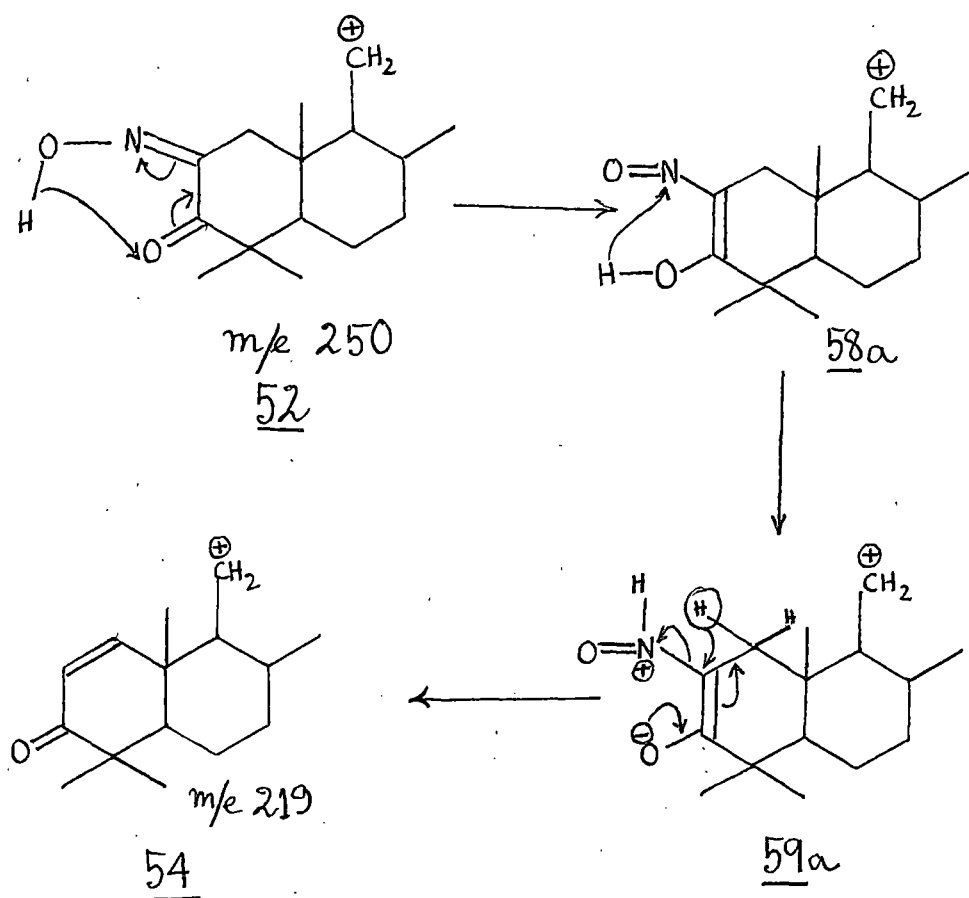
nitrite in an inert atmosphere of nitrogen³². The reaction mixture after acidification with cold dilute hydrochloric acid afforded 2-oximino lupanone 50 m.p. 260°, $[\alpha]_D^{25} 72.34$; tlc showed it to be homogeneous. Infrared spectrum showed peaks at 1700 ($\text{C}=\text{O}$), 1615 ($\text{C}=\text{N}$) and at 3240 cm^{-1} (N-OH) respectively [Fig. 2]. Its UV spectra exhibited absorption maxima at 240 nm ($\log \epsilon = 4.1$) and an alkali shift at 291 nm ($\log \epsilon = 4.3$). Further insight into the structure of 2-oximino lupanone came out from a study of the fragmentation pattern in the mass spectra (Fig. 3). The mass spectrum by chemical ionisation method showed MH^+ to be 456 i.e. the molecular ion M^+ at 455. The peaks at m/e 440 and m/e 438 were due to the fragments formed from the molecular ion by the loss of 15 and 17 mass units respectively — attributable to the loss of a methyl and hydroxyl units respectively from the molecular ion. The peaks at m/e 424 and 412 were attributed to an ion formed by the loss of (N-OH) and isopropyl units respectively from the molecular ion. The most abundant fragment at m/e 191, characteristic of lupane series^{34a-c} was due to the fragment 57



Other peaks at m/e 250, m/e 236, m/e 219 and m/e 205 could only be accounted for if the fragmentation followed the path as given below:



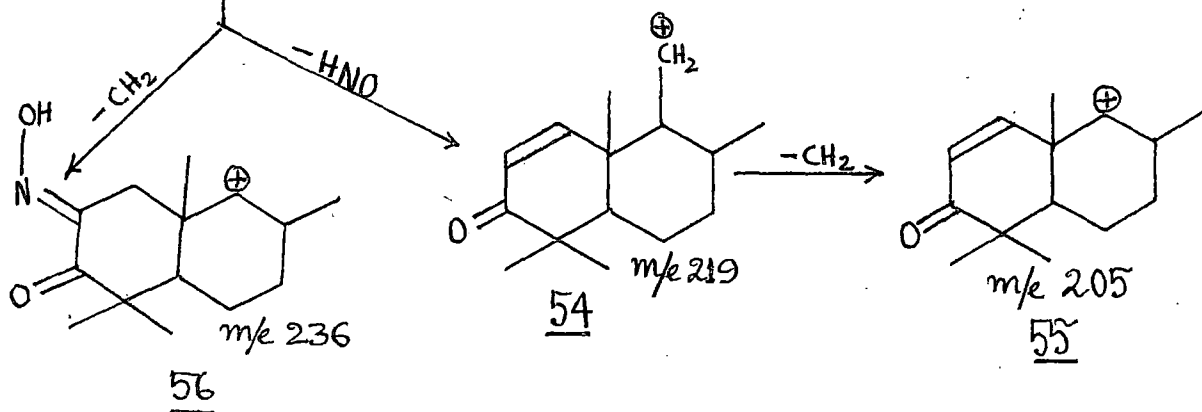
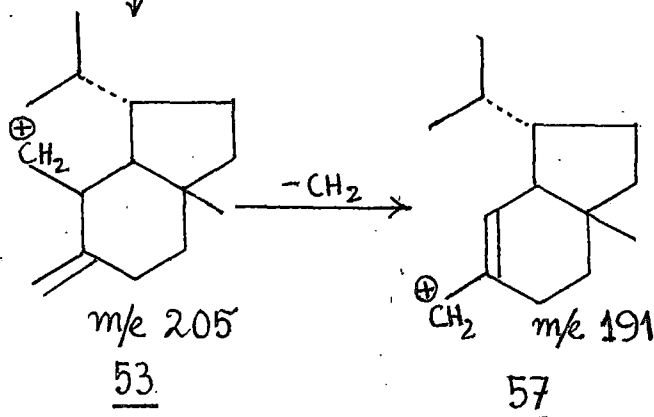
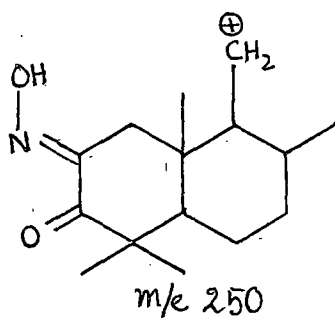
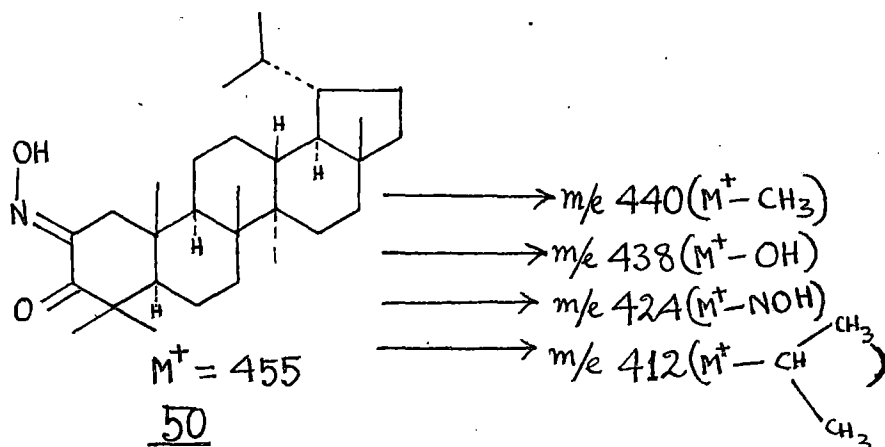
A plausible mechanism suggested for the loss of HNO from fragment 52 m/e 250 to form the fragment 54 m/e 219 has been put forward as



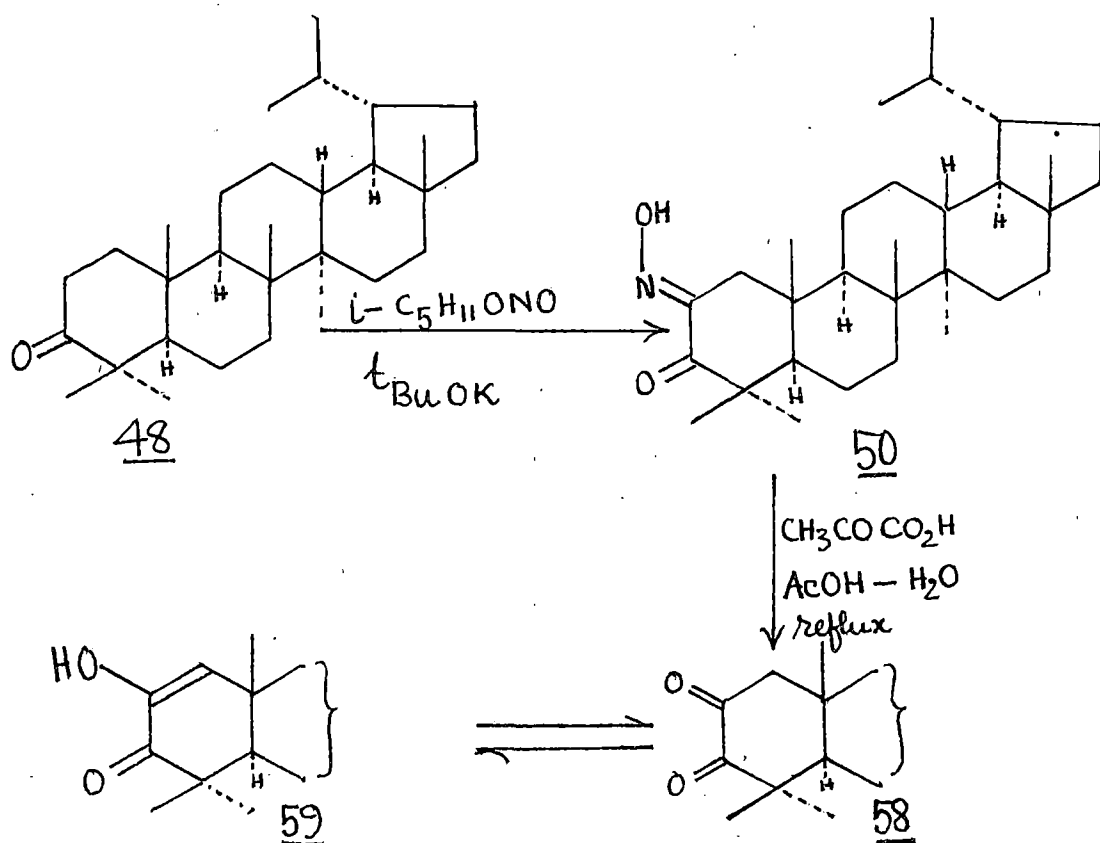
The peak at m/e 410 was probably due to the loss of $(-\text{CH}_3, -\text{NOH})$ from MH^+ (456). All the mass fragmentation of 2-oximino-lupanone 50 have been shown in Chart-I.

Chart - I

Mass fragmentation of 2-oximino lupanone 50.



The resulting 2-oximinolupanonone 50 was hydrolysed with freshly distilled pyruvic acid²⁸ in presence of dilute acetic acid to a α -diketone derivative 58 m.p. 210-213^o, $[\alpha]_D^{25}$ 24.75^o. This compound showed intense violet colour with neutral ferric chloride solution. The compound showed two spots on the chromatoplate indicating the tautomeric mixture of the diketone 58 and the diosphenol 59. Infrared spectrum showed bands at 3440, 1660 and at 1640 cm^{-1} (Fig. 4). Its UV spectra indicated absorption maxima at 272 nm ($\log \epsilon = 3.69$) and an alkali shift 324 nm ($\log \epsilon = 3.56$). These physical and chemical evidences were in complete agreement with the assignments shown in 58 and 59.



Section B

Moretenone³³ 60, m.p. 198-199°, $\left[\alpha \right]_D^{25} 50^\circ$ was isolated from the neutral part of the benzene extract of the bark of Sapium sebiferum Roxb (for isolation see experimental portion page 85 of Part I of this thesis); Infrared spectrum showed carbonyl stretching frequency at 1705 cm^{-1} and at 875 cm^{-1} indicating isopropenyl side chain ($\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{C} = \text{CH}_2 \end{array}$) $\left[\text{Fig. 5} \right]$.

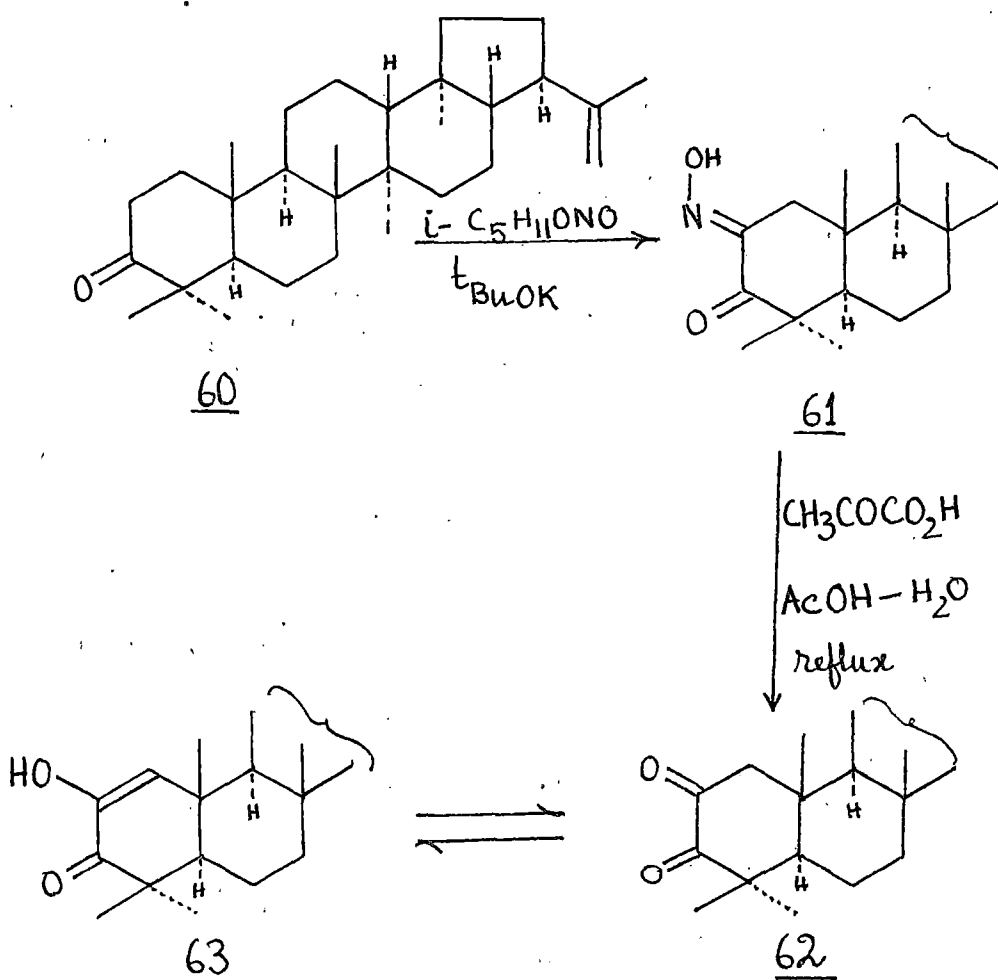
Moretenone 60 in presence of potassium tert. butoxide was stirred with freshly prepared and distilled isoamyl nitrite³² in an inert atmosphere of nitrogen for 24 hrs.

The reaction mixture after acidification with cold dilute hydrochloric acid furnished 2-oximino moretenone 61, m.p. 255-256°. TLC showed the compound to be homogeneous. Infrared spectrum showed peaks at 1700 ($\text{C} = \text{O}$), 1605 ($\text{C} = \text{N}$), 3200 ($\text{N} - \text{OH}$) and at 875 ($\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{C} = \text{CH}_2 \end{array}$) cm^{-1} respectively $\left[\text{Fig. 6} \right]$. Its UV spectra showed absorption maxima at 240 nm ($\log \epsilon = 4.06$) and an alkali shift at 290 nm ($\log \epsilon = 4.08$) respectively.

The resulting 2-oximino moretenone 61 was hydrolysed with freshly distilled pyruvic acid²⁸ in presence of dilute acetic acid to a α -diketone derivative 62, m.p. 155-158°. The compound indicated two spots on the chromatoplate indicating the tautomeric mixture of the keto 62 and diosphenol 63 forms. It developed intense violet colour

with neutral ferric chloride solution. Its UV spectra showed absorption maxima at 270 nm ($\log \epsilon = 3.52$) and an alkali shift at 327 nm ($\log \epsilon = 3.41$). Infrared spectrum exhibited peaks at 3420, 1700, 1660 cm^{-1} [Fig. 7] respectively.

These physical and chemical evidences were in complete agreement with the assignments shown as 62 and 63.



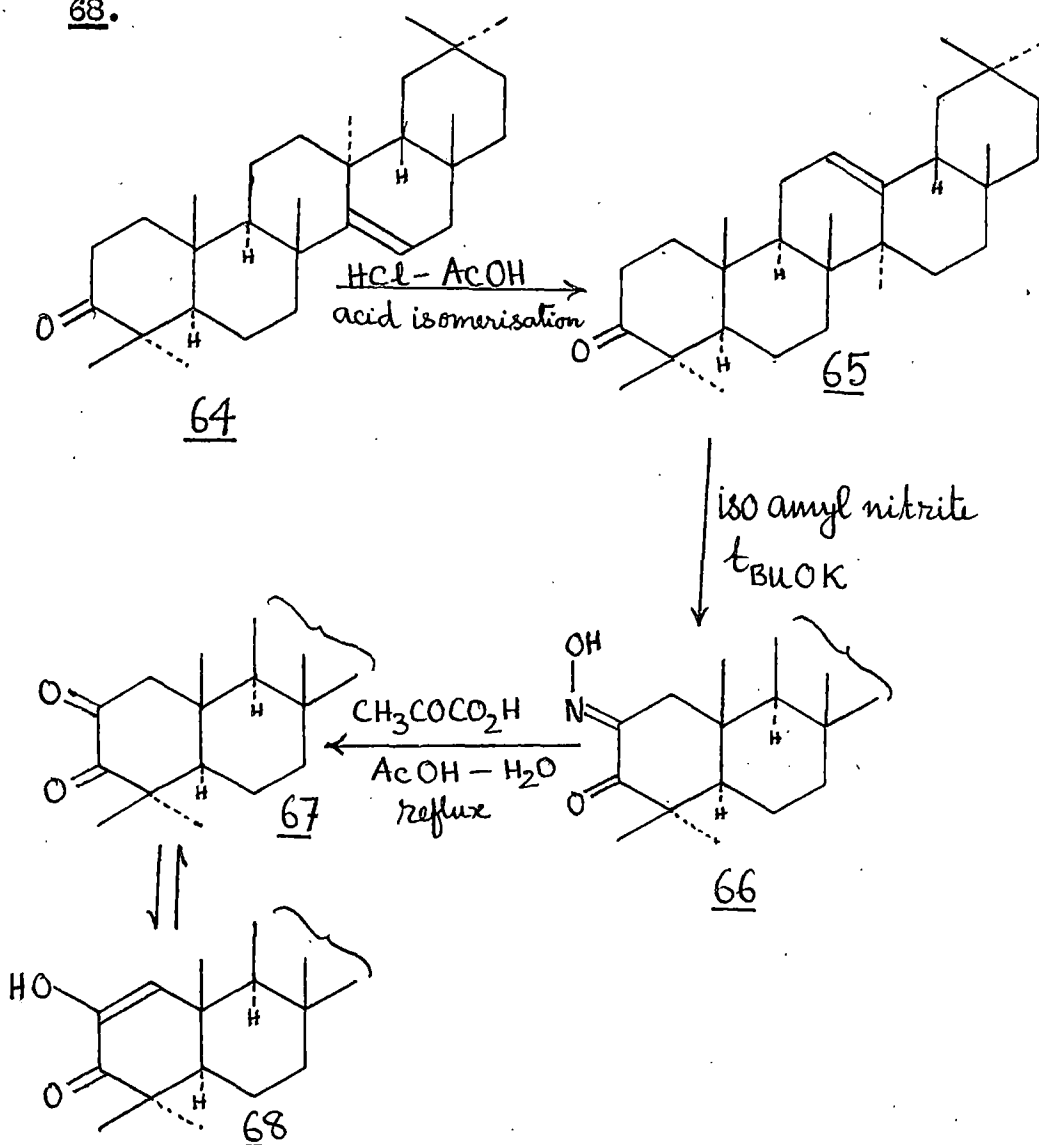
Section C

The starting material β -amyrone 65, m.p. 174-176°, $[\alpha]_D^{25}$ 105.6° was obtained by the acid isomerisation³⁵ of taraxerone³⁶ 64, m.p. 238-240°, $[\alpha]_D^{25}$ 10.8° isolated from the neutral part of the benzene extract of the bark of Sapium baccatum Roxb³⁶ (for isolation and isomerisation see experimental section of this Part II in page 57 of the thesis). Infrared spectrum of 65 showed strong carbonyl stretching frequency at 1700 and trisubstituted double bond at 815 cm^{-1} [Fig. 8]. The introduction of C-2 carbonyl group to β -amyrone 65 was carried out as follows.

β -amyrone 65 in presence of potassium tert. butoxide³² was stirred with freshly prepared isoamyl nitrite in an inert atmosphere of nitrogen for 24 hrs. The reaction mixture after acidification with cold dilute hydrochloric acid afforded 2-oximino β -amyrone 66, m.p. 200-205°, which was not crystallisable from any usual solvent. Infrared spectrum of this compound exhibited bands at 3400 (N-OH), 1700 ($\text{C}=\text{O}$), 1620 (C=N) and at 815 (trisubstituted double bond) cm^{-1} [Fig. 9]. Its UV spectra showed absorption maxima at 238 nm and an alkali shift at 239 nm respectively.

The crude 2-oximino β -amyrone 66 was hydrolysed with freshly distilled pyruvic acid²⁸ in presence of dilute acetic acid to a α -diketone derivative 67, m.p. 200-202°.

$[\alpha]_D^{25}$ 124.27°. The compound developed two spots on the chromatoplate indicating a mixture of two components for both the keto 67 and the diosphenol 68 forms. It produced intense violet colour with neutral ferric chloride solution. Infrared spectrum showed peaks at 3400, 1710, 1660 cm^{-1} [Fig. 10]. Its UV spectra exhibited absorption maxima at 270 nm ($\log \epsilon = 3.90$) and an alkali shift at 328 nm ($\log \epsilon = 3.84$) respectively. These data were in complete agreement with the assignments shown in 67 and 68.

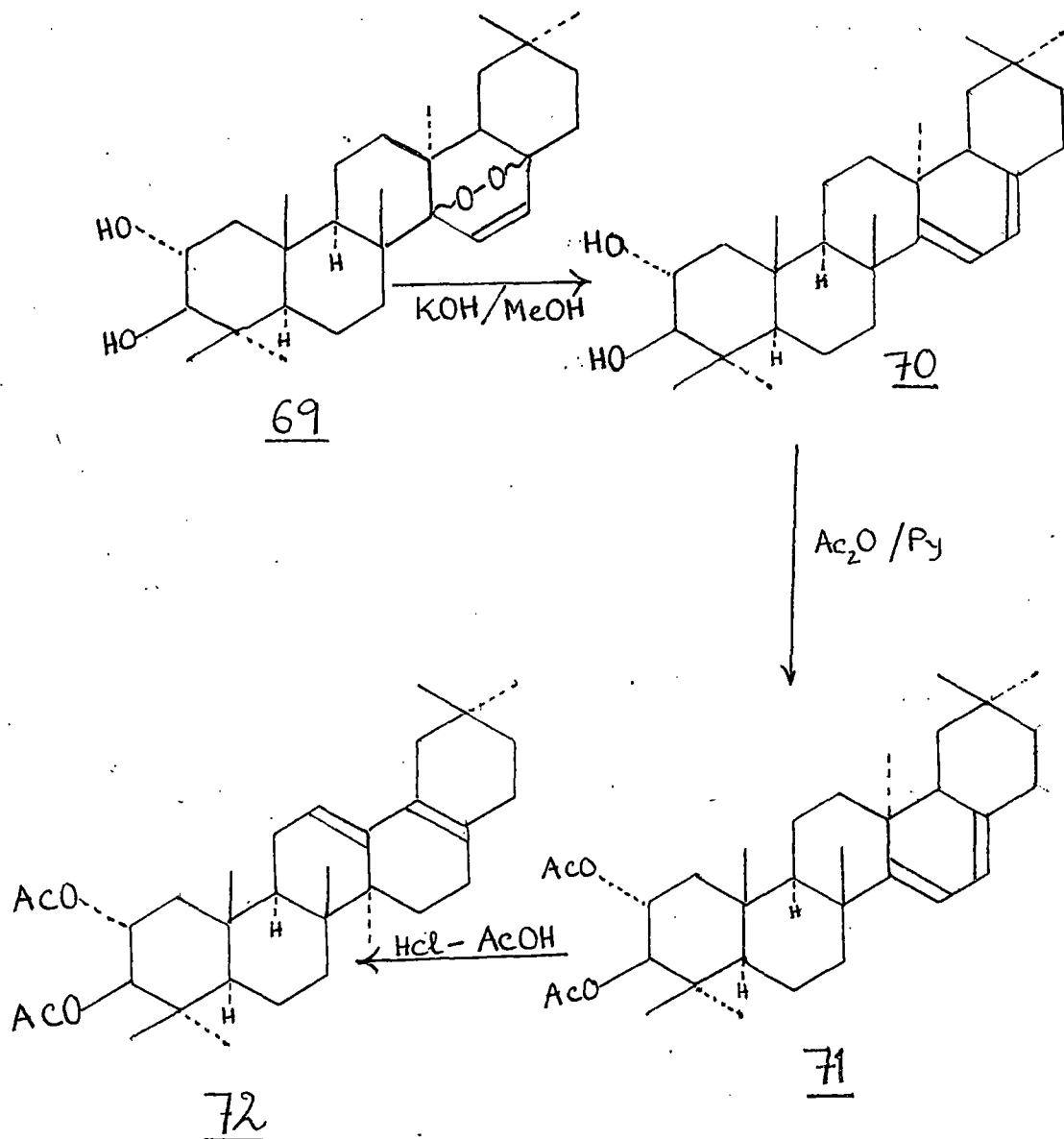


Section D

Partial synthesis of the degraded product from a nor-triterpene, baccatin, starting from oleanolic acid.

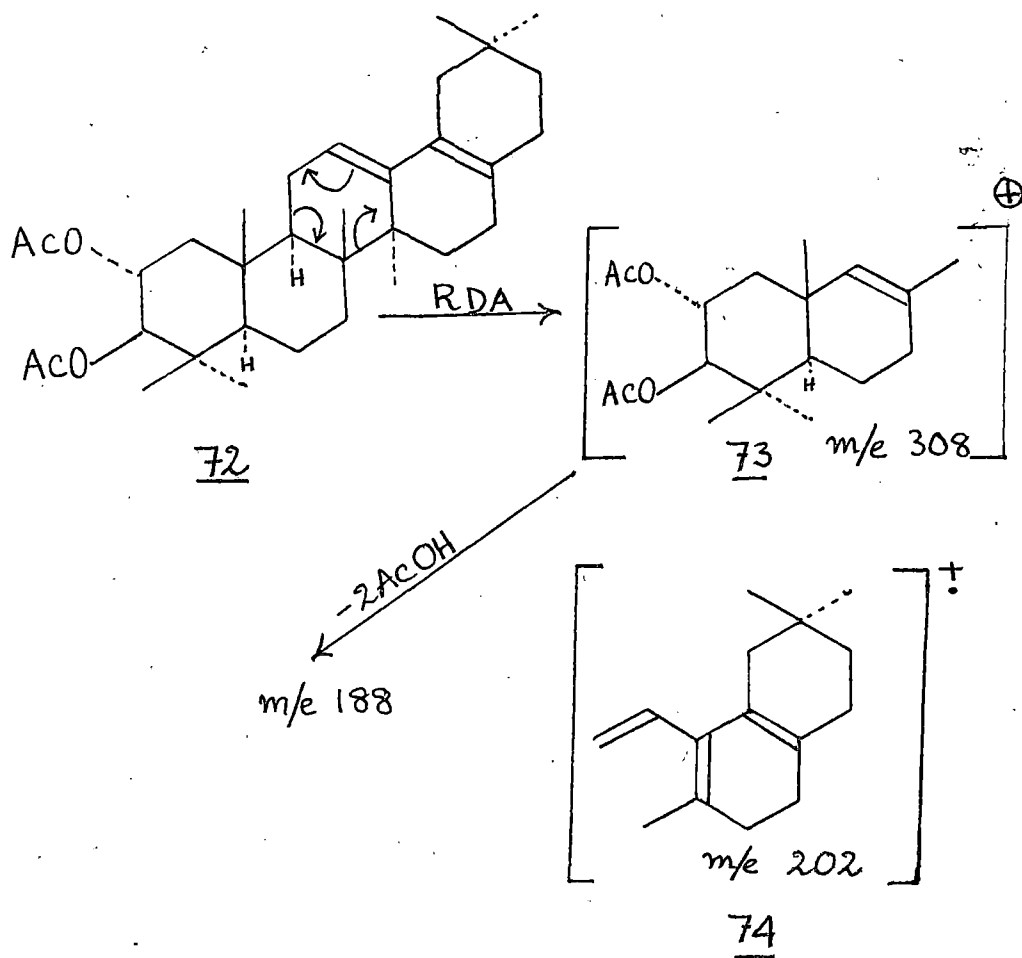
Treatment of baccatin³⁷ 69, m.p. 228-229°, $[\alpha]_D -9.09^\circ$ with methanolic alkali gave the homoannular conjugated diene, $C_{29}H_{46}O_2$, 70 and this on acetylation afforded the diene diacetate, $C_{33}H_{50}O_4$, 71. Treatment of the latter with a mixture of hydrochloric acid and acetic acid gave a compound 72, $C_{33}H_{50}O_4$, m.p. 189-190°. The UV spectrum of this compound showed absorption at $\lambda_{\text{max}}^{\text{MeOH}}$ 237 (ϵ , 27,000), 244 (ϵ , 28,300) and 252 nm (ϵ , 20,200) thereby suggesting the presence of a heteroannular conjugated system of the rearranged product. The NMR spectrum (100 MHz) of the rearranged product showed signals at 0.85-1.14 ppm (7 tertiary methyl groups), 1.96, 2.0 (6H, 2-OCOCH₃) ppm, unsymmetrical doublet at 4.64, 4.75 (H on the acetoxy bearing C-3) ppm, quartet of doublets at 4.95, 5.03, 5.08, 5.2 (H on acetoxy bearing C-2) ppm and 5.46 ppm (1H, vinyl proton). The mass spectrum of the rearranged product showed significant peaks at m/e 510 (M^+), 495 ($M^+ - 15$), 450 ($M^+ - 60$), 435 ($M^+ - 60 - 15$), 390 ($M^+ - 60 - 60$), 375 ($M^+ - 60 - 60 - 15$), 308, 202, 188. On the basis of these data, the structure of the rearranged product was assigned

to be 2α , 3β -diacetoxy-28-nor oleana-12,17 diene, 72
by Khastgir et al^{38,39}.



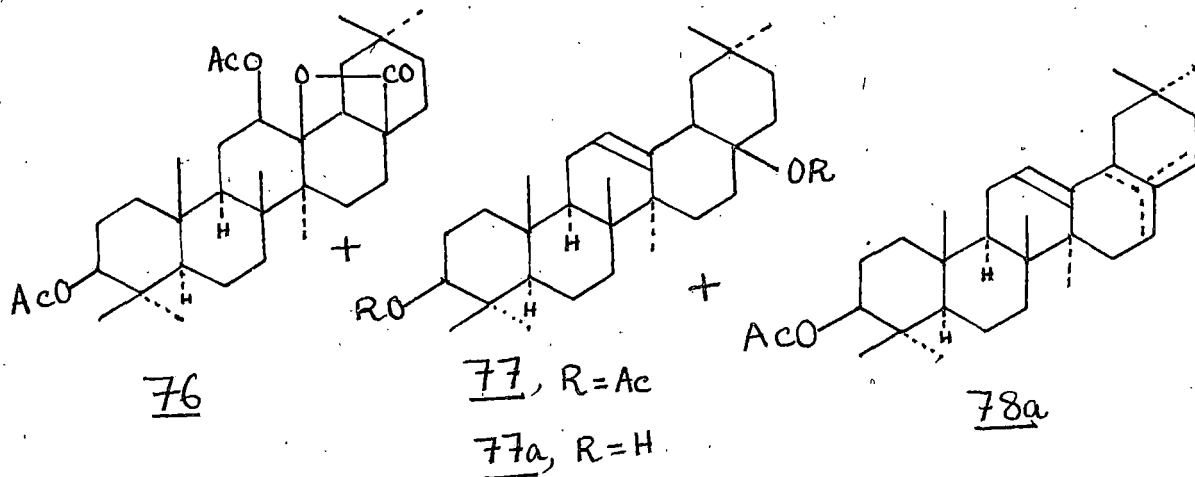
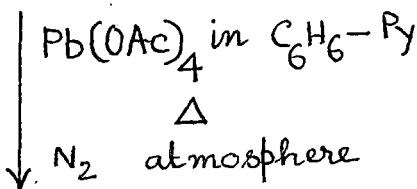
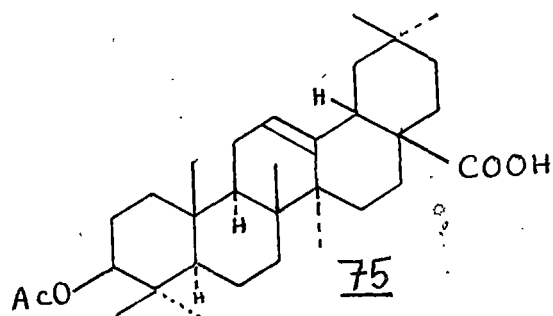
The mass fragmentation pattern of the rearranged product 72 was explained as shown in chart II.

Chart-II



In order to correlate the above observation, Khastgir et al^{38,39} prepared a similar system starting from acetyl oleanolic acid.

Oleanolic acid m.p. 308-310°, $[\alpha]_D^{20}$ 80° was isolated from the seeds of Achyranthes aspera Linn⁴⁰. Acetylation with acetic anhydride pyridine gave acetyl oleanolic acid 75, m.p. 261-263°. The I.R. spectrum of this compound is shown in Fig. 11. Oxidative decarboxylation of acetyl oleanolic acid 75 with lead tetraacetate by the method of Cambie et al⁴¹ could give a mixture of products, the lactone diacetate 76, the diacetate 77 and the mixture of mono acetates represented by 78a.



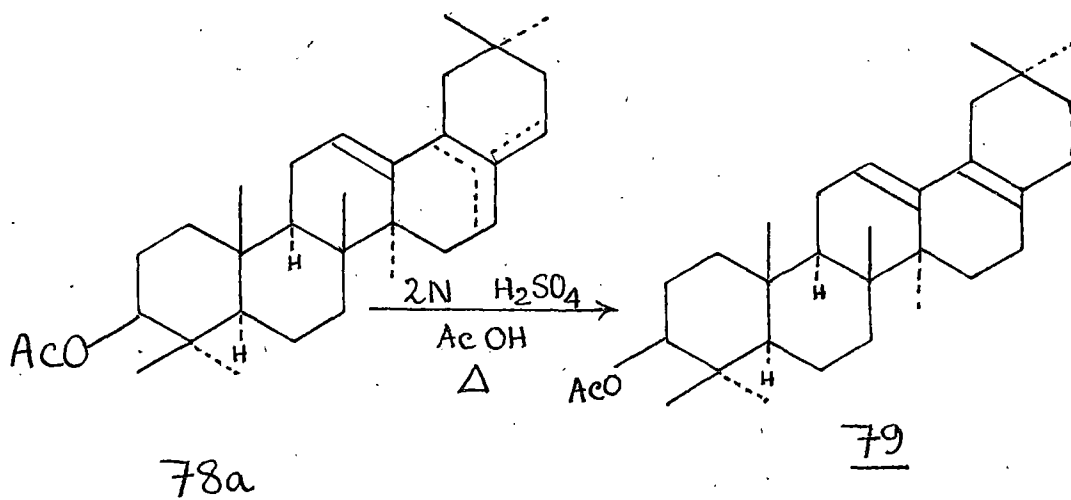
The reaction product was chromatographed over alumina. Elution with petrol afforded a solid mass which on crystallisation from chloroform-methanol mixture gave crystals m.p. 167-69°. TLC examination showed that it was indeed a mixture of three compounds. The IR spectrum showed bands at 1730, 1240 cm^{-1} [Fig. 12].

Elemental analysis closely corresponded to the molecular formula $\text{C}_{31}\text{H}_{48}\text{O}_2$. This solid was therefore, a mixture of the monoacetates represented by the three diene monoacetates 78a. The UV spectrum of the mixture 78a showed peaks at $\lambda_{\text{max}}^{\text{MeOH}}$ 238 (ϵ , 9450), 244 (ϵ , 10,070), 252 (ϵ , 7560) indicating that some amount of the hetero annular conjugated diene system was present in the mixture. Elution with a mixture of petrol and benzene (4:1) afforded a solid which was crystallised from a mixture of chloroform and methanol. Elemental analysis corresponded to the formula $\text{C}_{34}\text{H}_{52}\text{O}_6$, m.p. 288-90°. The UV spectrum showed no absorption in the range 220-270 nm. The IR spectrum showed bands at 1760 (ν -lactone), 1720, 1240 (acetate) cm^{-1} . These physical evidences clearly indicated it to be a diacetate lactone 76.

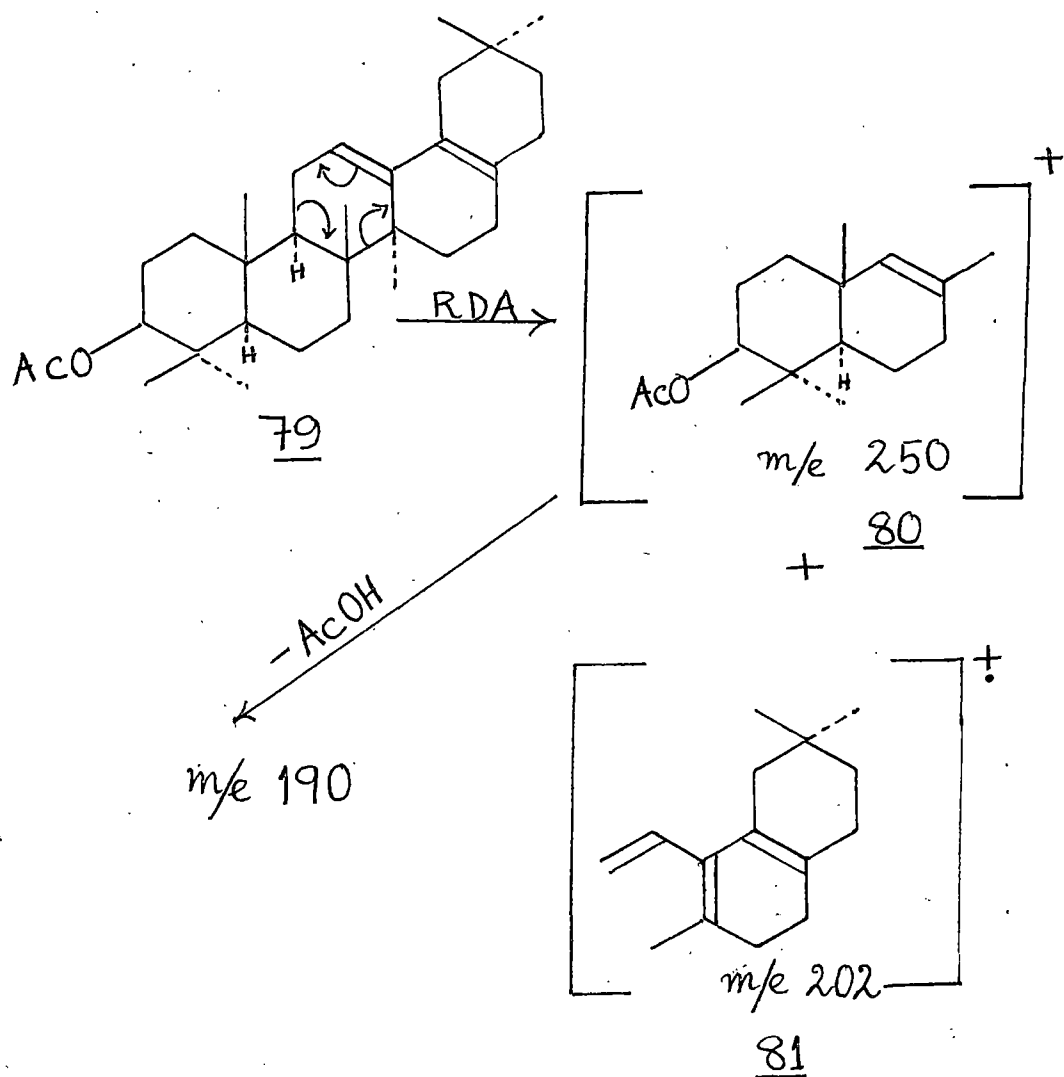
When the mixture of monoacetates was heated with 2(N) H_2SO_4 in acetic acid it rearranged to the single thermodynamically stable heteroannular conjugated diene monoacetate

79 which on chromatography followed by crystallisation from a mixture of chloroform and methanol afforded needle shaped crystals of 3β -acetoxy-28-nor-oleana-12,17-diene 79, $C_{31}H_{48}O_2$, m.p. $177-178^\circ$ (TLC - single spot), IR, $\nu_{\text{max}}^{\text{nujol}}$ 1710, 1245 (acetate), UV, $\lambda_{\text{max}}^{\text{MeOH}}$ 237 (ϵ , 27,090), 243 (ϵ 29, 180), 252 (ϵ 25, 530) nm.

The NMR spectrum of this compound 79 showed signals at 0.82, 0.92, 1.15 (21H, seven tertiary methyl groups); 2.01 (3H, $-\text{OCOCH}_3$); doublet at 4.74, 4.82 ($\text{H}-\text{C}_3-\text{OCOCH}_3$) and 5.45 (1H, vinyly proton) ppm. On this basis the structure 79 was assigned to the rearranged product by Khastgir et al.³⁹.

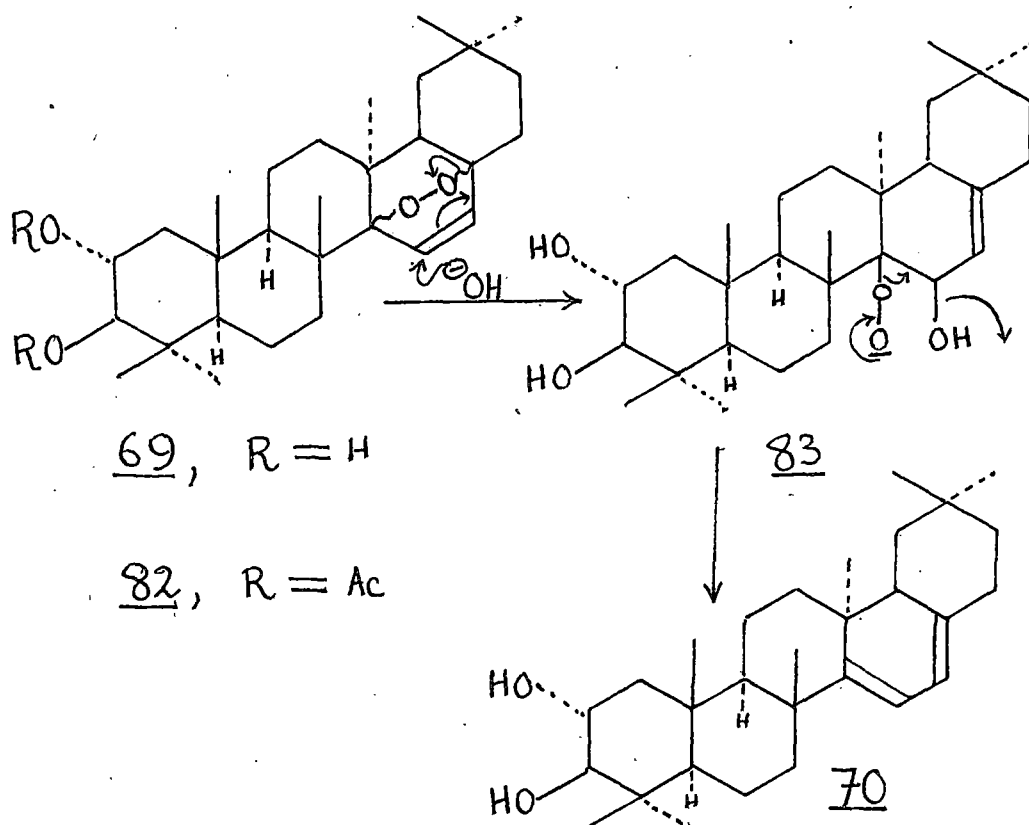


If the peaks at m/e 202 and 188 are indeed the diagnostic of structure 72 it is expected that 79 which has the same skeleton as that of 72 should exhibit peaks at m/e 202 and 190 corresponding to the following fragmentation.

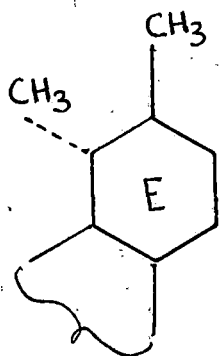


In accordance with the above expectation Khastgir *et al*³⁹ found that the mass spectrum of 79 showed prominent peaks at m/e 452 (M^+), 437 ($M^+ - 15$), 392 ($M^+ - 60$), 377 ($M^+ - 60 - 15$), 202 and 190. Hence the structure 72 for the rearranged diene diacetate was confirmed which in turn confirms the structure 70 for the homoannular conjugated diene obtained by the alkali treatment of baccatin which must have structure 69. Khastgir *et al*³⁹ have also proposed a probable mechanism for the transformation of baccatin 69 or its diacetate 82 to the homoannular conjugated diene 70 by the treatment with methanolic alkali as shown in chart III.

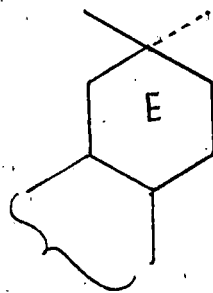
Chart - III



Though the structure of baccatin 69 had been correlated by comparing the mass fragmentation pattern of its degraded product i.e. 2α , 3β -diacetoxy-28-nor-oleana-12,17 diene 72 with that of 3β -acetoxy-28-nor-oleana-12, 17 diene 79 a degraded product from acetyl oleanolic acid 75, it was felt pertinent to correlate further by chemical evidence. Because of the fact that the presence of an ursane-type E ring such as shown in 84 in the heteroannular diene 72 (and consequently in baccatin) could also explain the mass fragmentation pattern and other physical data to a fair degree of accuracy. It was therefore, thought that it was necessary to prove that 72 (and consequently baccatin) indeed contained an oleanane-type E ring as shown in 85.



84



85

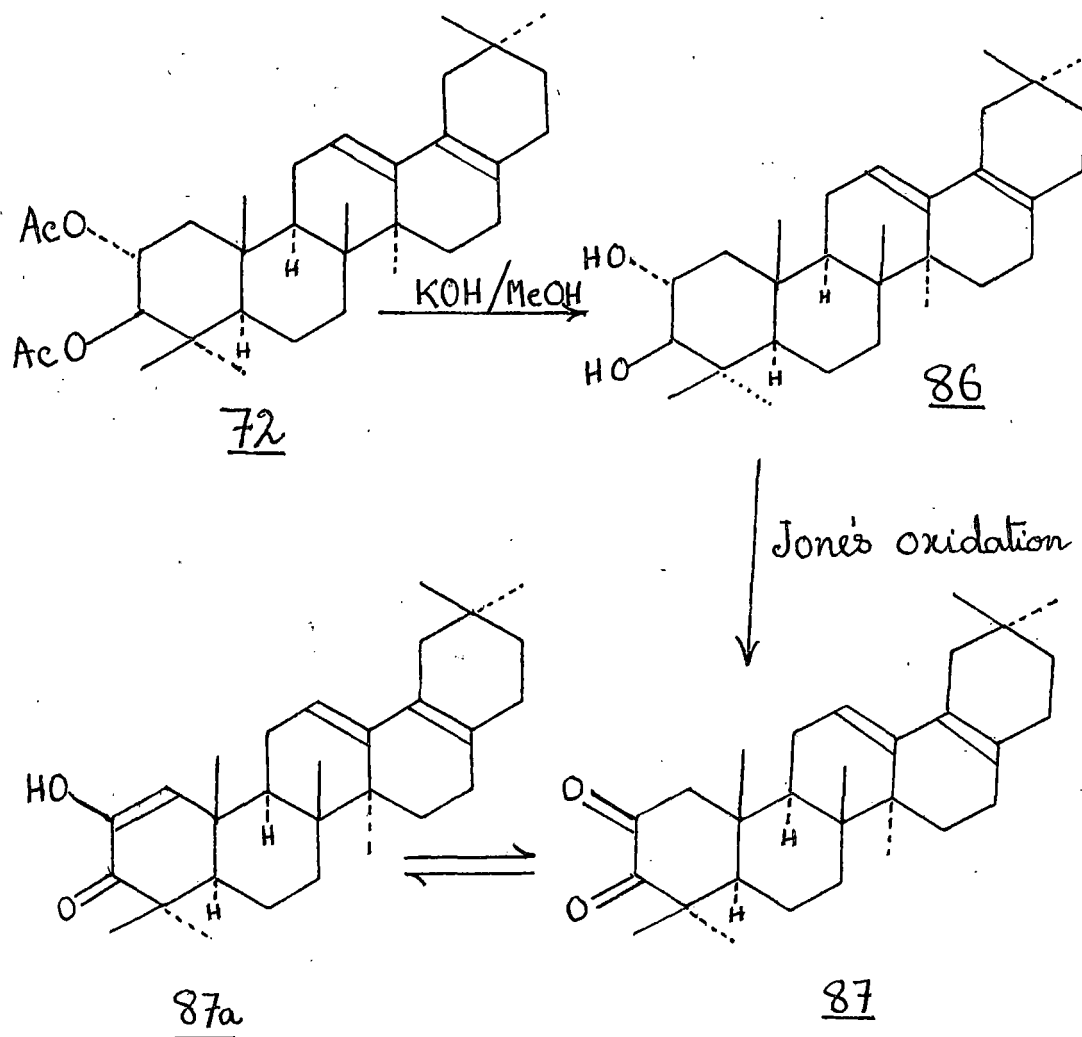
In order to establish the structure of baccatin by chemical evidence, the rearranged heteroannular diene diacetate 72 obtained from degradation of baccatin had been degraded further to 2,3-dioxo-28-nor-oleana-12,17 diene and this had been found to be identical with 2,3-dioxo-28-nor-oleana-12, 17 diene which had been prepared by partial synthesis from the rearranged heteroannular diene acetate i.e. 3 β -acetoxy-28-nor oleana-12, 17-diene 79 obtained from acetyl oleanolic acid.

2 α , 3 β diacetoxy-28-nor-oleana-12, 17 diene 72 obtained by degradation of baccatin (as discussed earlier) was further hydrolysed by treatment with methanolic alkali to 2 α , 3 β , dihydroxy-28-nor-oleana-12, 17 diene 86, C₂₉H₄₆O₂, m.p. 192-195° (TLC single spot). The IR spectrum indicated significant bands at 3240, 1055 and 830 cm⁻¹ [Fig. 13]. The UV spectrum showed absorptions at $\lambda_{\text{max}}^{\text{MeOH}}$ 237 (ϵ , 25,000), 244 (ϵ 26,200) and 252 nm (ϵ , 20,100).

This on oxidation with Jones's reagent^{43a,b,c} gave 2,3-dioxo-28-nor-oleana-12,17 diene 87 C₂₉H₄₄O₂, m.p. 205-206°. The UV spectrum showed absorption maxima at $\lambda_{\text{max}}^{\text{MeOH}}$ 237, 244, 252 and 271 nm and an alkali shift at 326 nm. The IR spectrum indicated significant band at 3400, 1700 and 1650 cm⁻¹ [Fig. 17]. It developed intense violet colouration with neutral ferric chloride

solution. All these data prove the presence of keto 87 and diosphenol 87a system present as tautomeric mixture as shown in chart IV. TLC showed two spots (solvent benzene) $R_f = .56$ and $.52$ for the two respectively.

Chart - IV



The heteroannular diene monoacetate 79 that might contain minor amount of 77 (obtained from acetyl oleanolic acid 75 as discussed earlier) was then hydrolysed with potassium tertiary butoxide and dimethyl sulfoxide following the method of Chang and Wood⁴² so as to hydrolyse both C-3 and C-17 acetoxy groups for easy separation which was then chromatographed and crystallised to give 3 β -hydroxy-28-nor-oleana-12, 17 diene 88 as the major product,

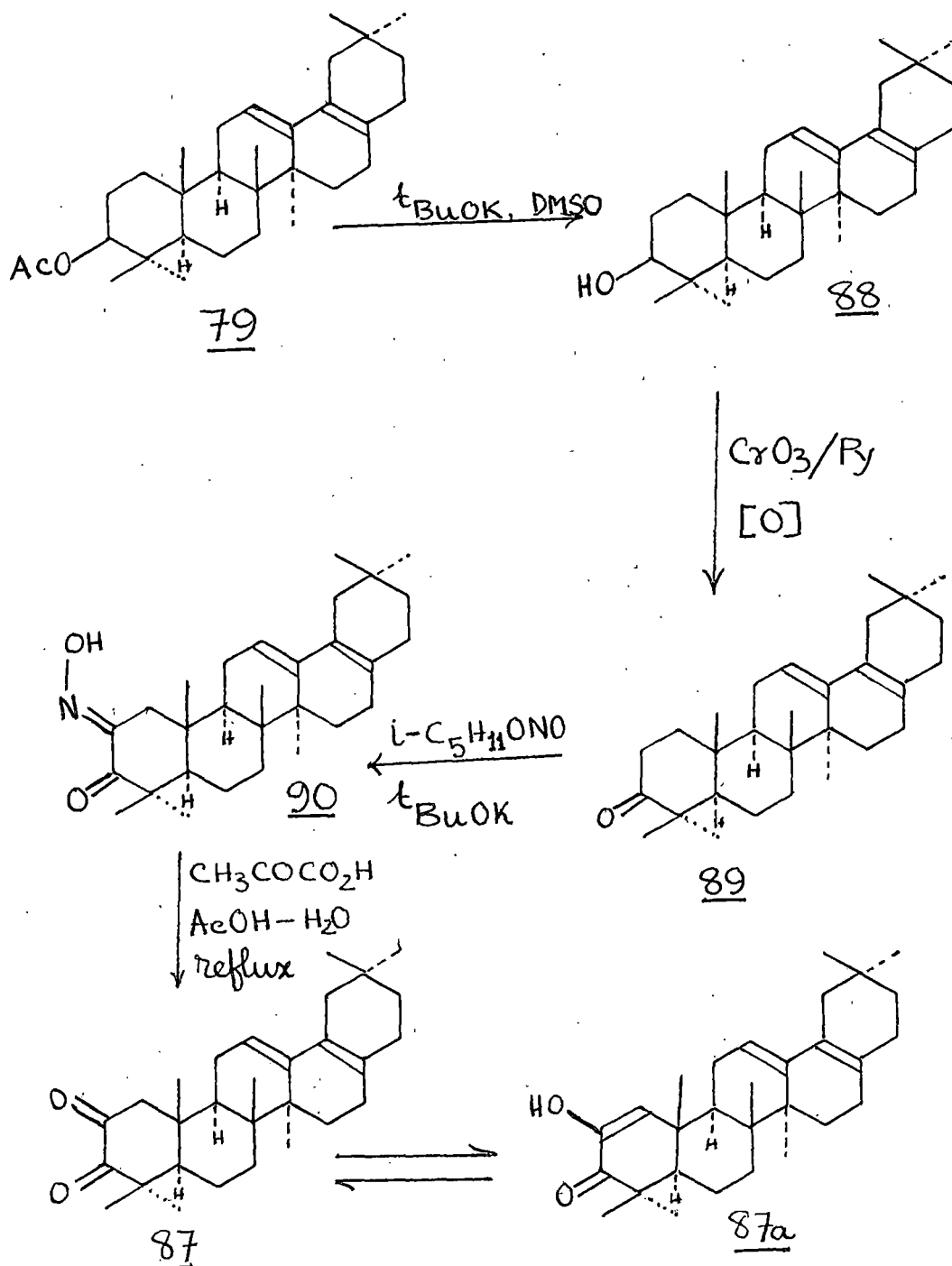
C₂₉H₅₀O, m.p. 182-183^o (TLC showed a single spot). The UV spectrum of the compound showed absorption at $\lambda_{\text{max}}^{\text{MeOH}}$ 237 (ε 8150), 244 (ε 8340), 252 (ε 8020) nm. The IR spectrum exhibited significant peak at $\nu_{\text{max}}^{\text{nujol}}$ 3380 cm⁻¹ (-OH),

[Fig. 14]. This was oxidised by chromium trioxide-pyridine complex⁴⁵ to 3-oxo-28-nor-oleana-12,17 diene 89 C₂₉H₄₈O, m.p. 110-112^o. Infrared spectrum exhibited a significant band at $\nu_{\text{max}}^{\text{nujol}}$ 1700 cm⁻¹ (C=O) [Fig. 15].

This compound on treatment with isoamyl nitrite in presence of potassium tertiary butoxide³² afforded 2-oximino-3-oxo-28-nor-oleana-12, 17-diene 90, m.p. 220-225^o which could not be crystallised from any usual solvent.

Infrared spectrum of this crude compound indicated bands at $\nu_{\text{max}}^{\text{nujol}}$ 3200 (N-OH), 1700 (C=O), 1635 (C=N) cm⁻¹ [Fig. 16]. This compound was hydrolysed by refluxing with freshly distilled pyruvic acid¹⁸ in presence of dilute acetic acid to give 2,3-dioxo-nor-oleana-12, 17-diene, m.p. 205-206^o which had been separated by chromatography over silicagel by elution with petrol:benzene (2:3) and crystallised from acetone-methanol mixture. This had been

found to identical in all respects throughout the IR region [Fig. 17] with that of the degraded product 87 from baccatin. The mixed melting point showed no depression of melting point as well as the co-tle was same.



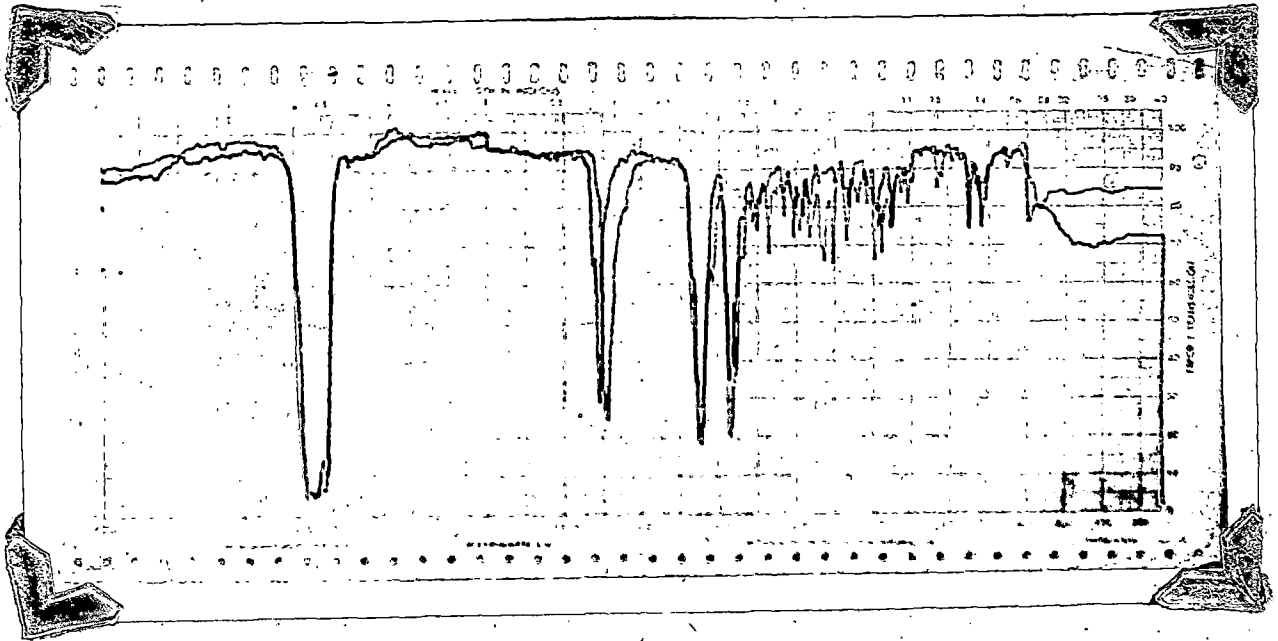


Fig. 1 IR spectra of
 (Upper band) lupanone
 (Lower band) 2-bromo lupanone
intense line

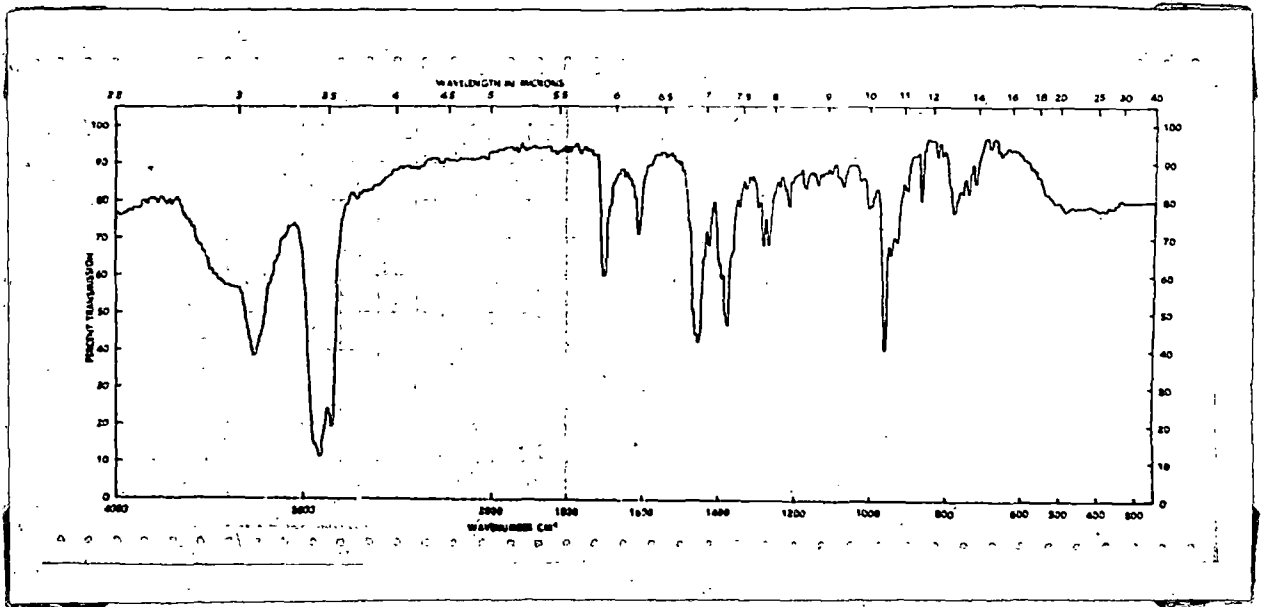


Fig. 2 IR spectrum of 2-oximino lupanone

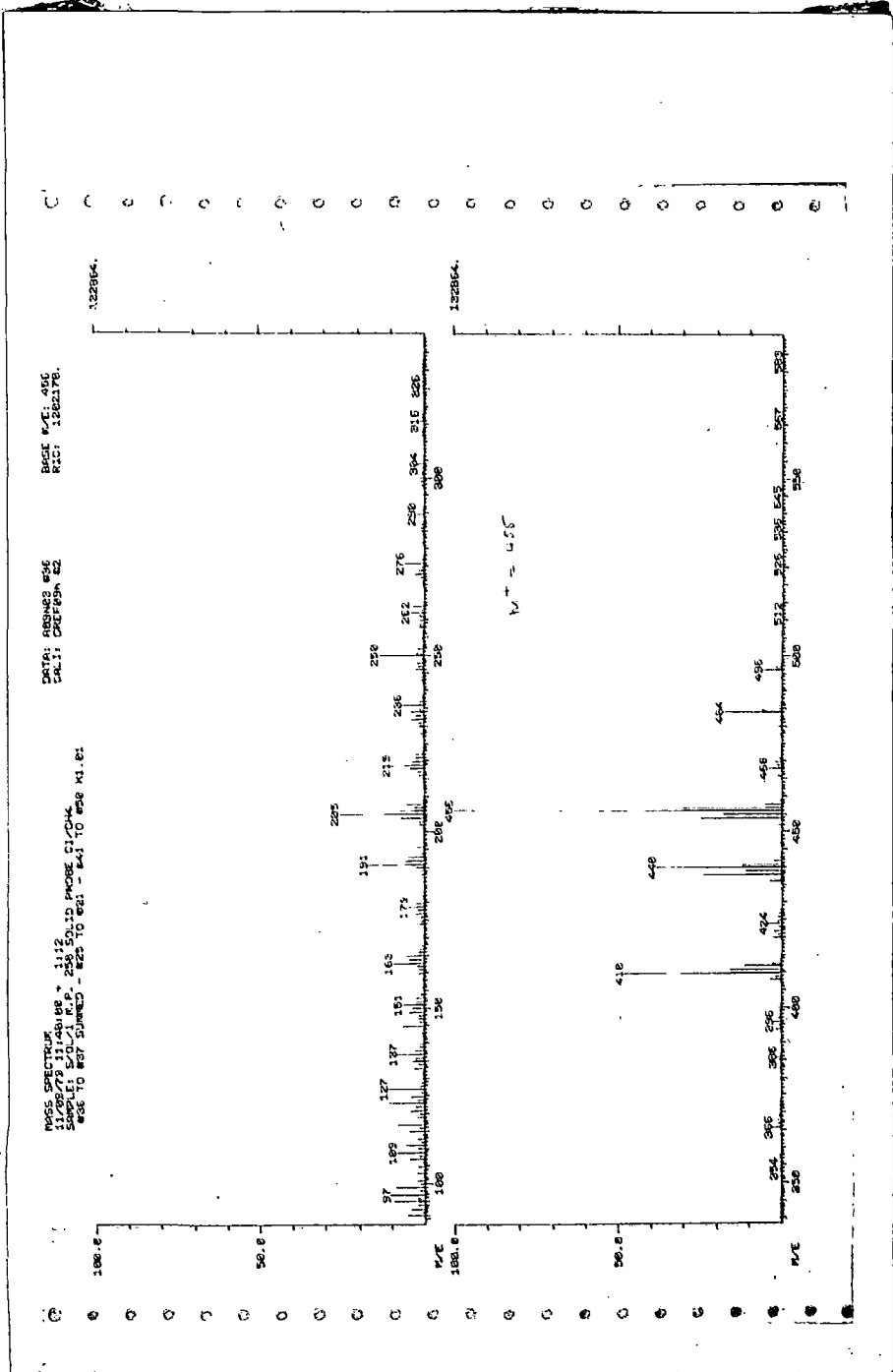


Fig. 3 Mass spectrum of 2-oximino lupanone

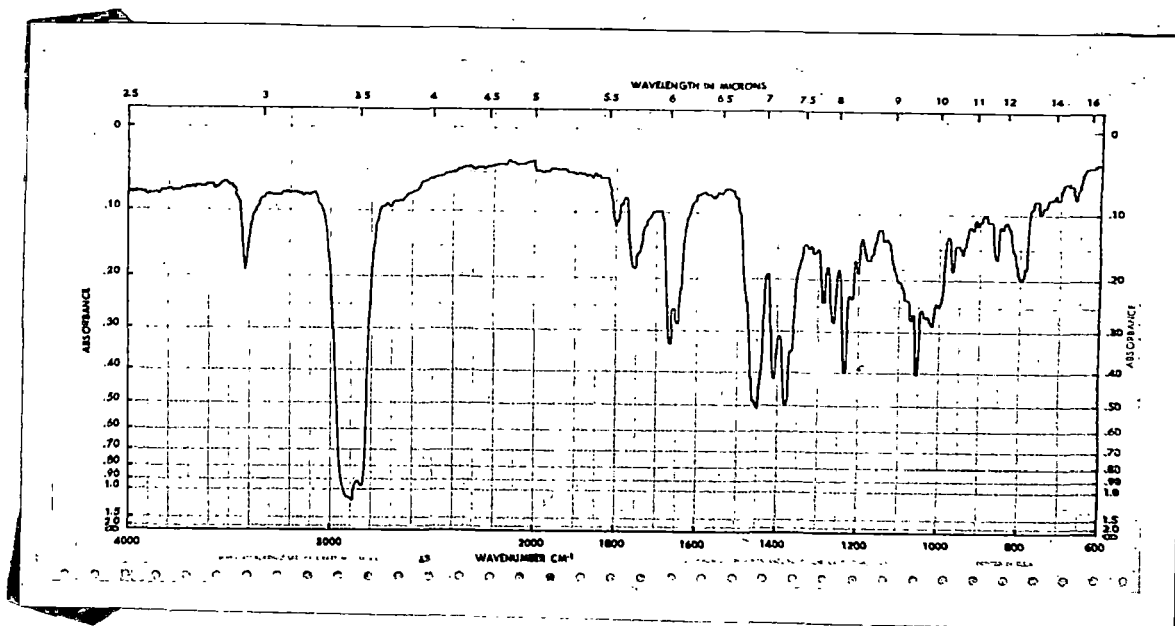


Fig. 4 IR spectrum of diosphenol of lupanone

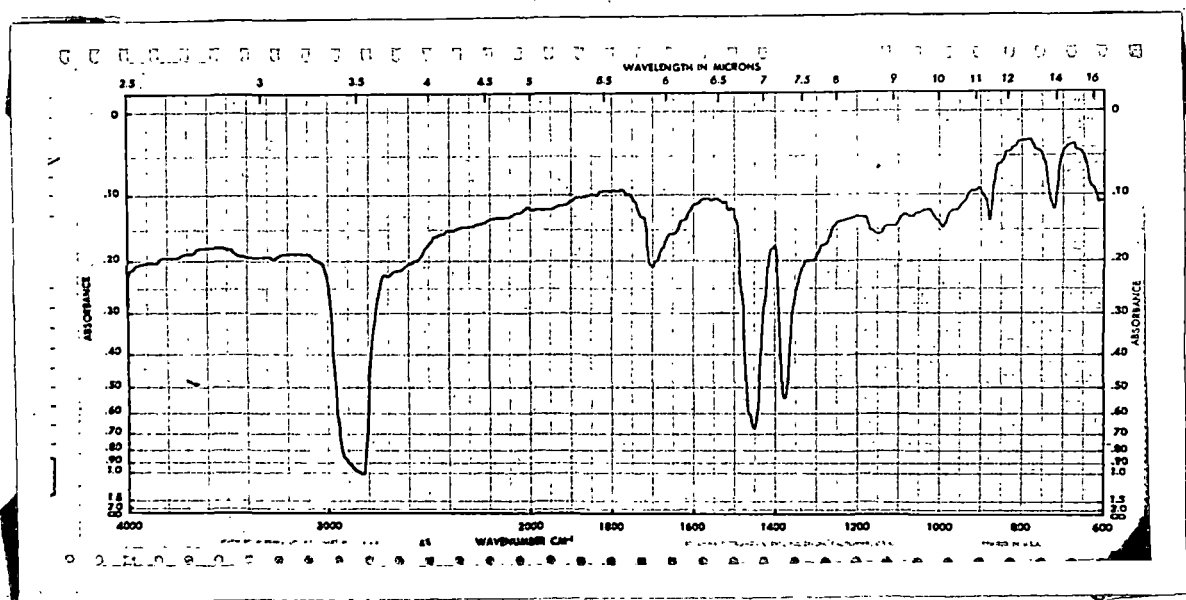


Fig. 5 IR spectrum of moretenone.

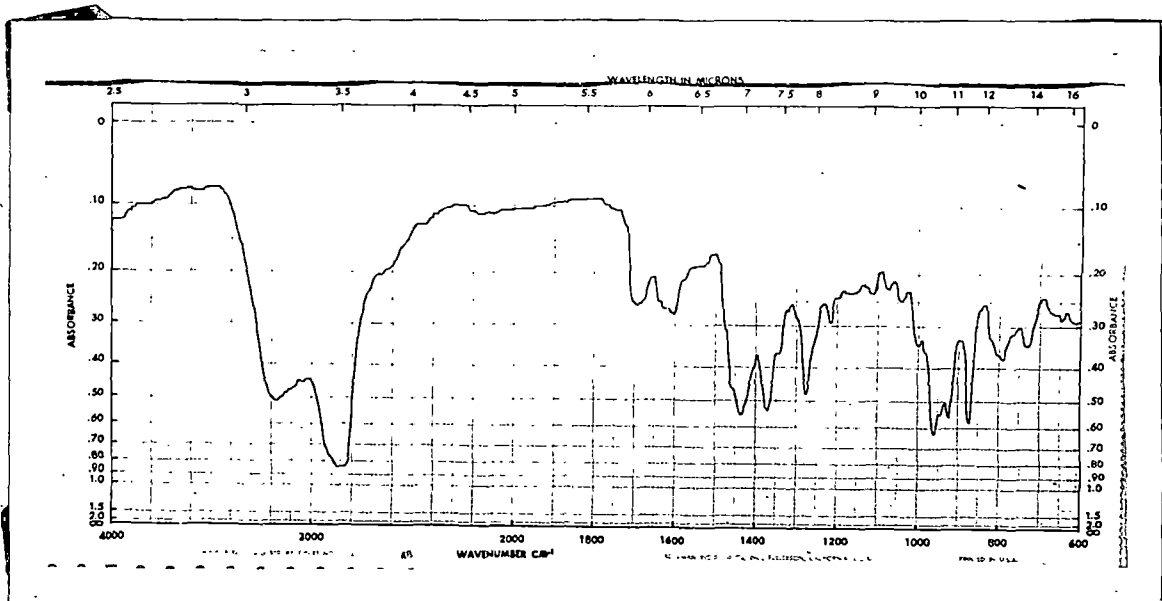


Fig. 6 IR spectrum of 2-oximino moretenone

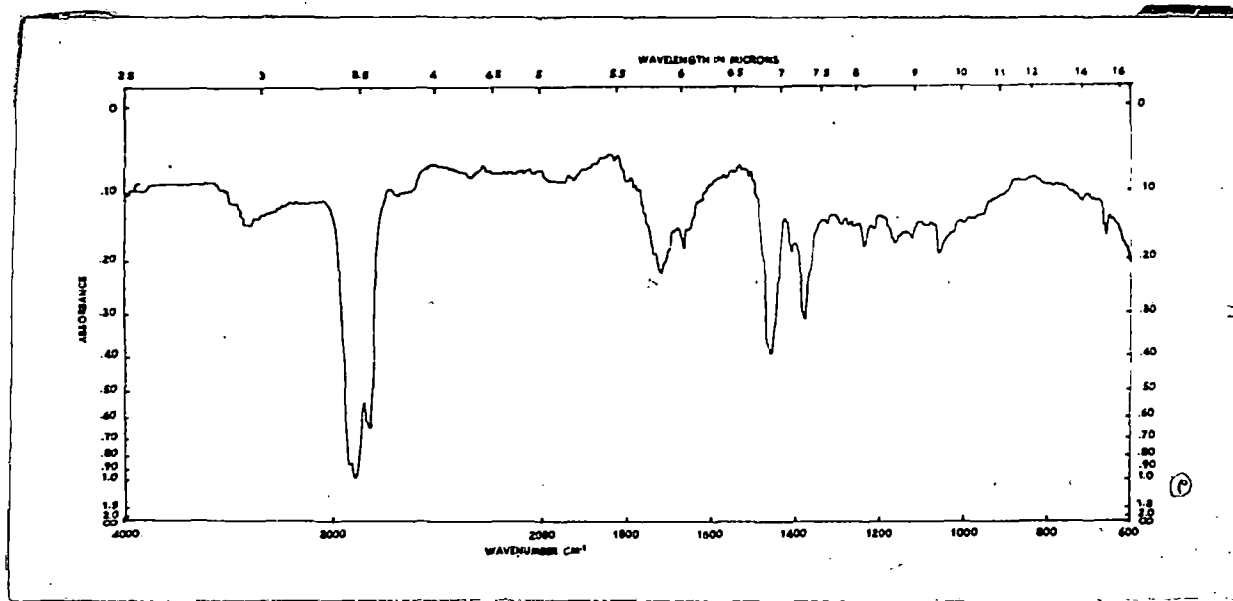


Fig. 7 IR spectrum of diosphenol of moretenone

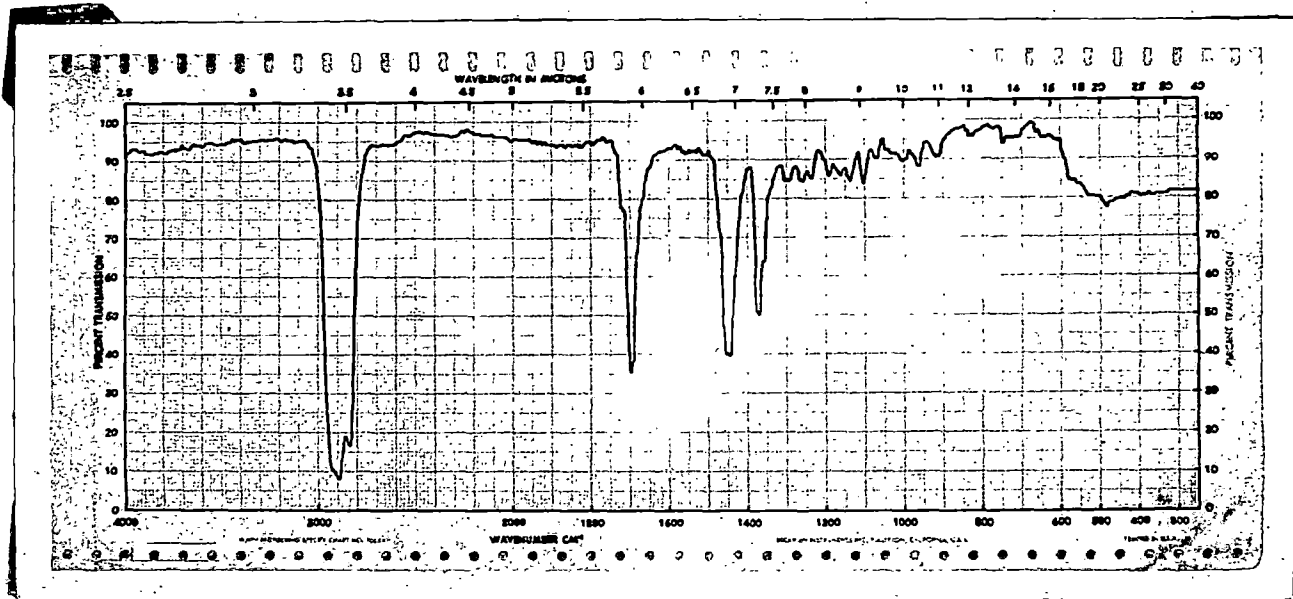


Fig. 8 IR spectrum of β -amyrone

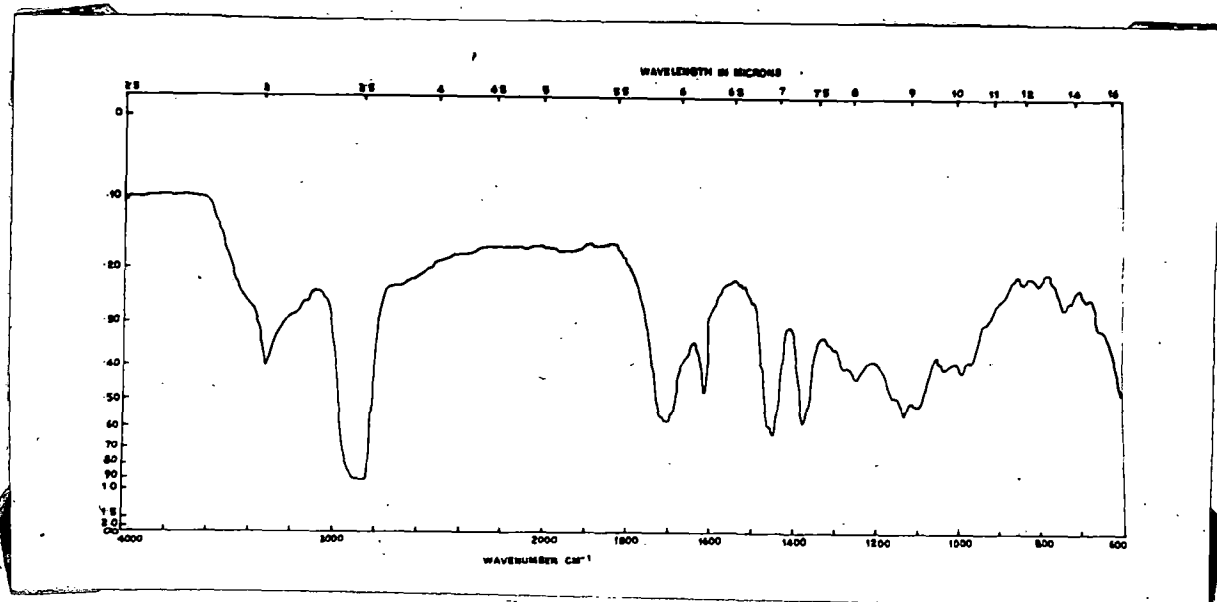


Fig. 9 IR spectrum of 2-oximino β -amyrone

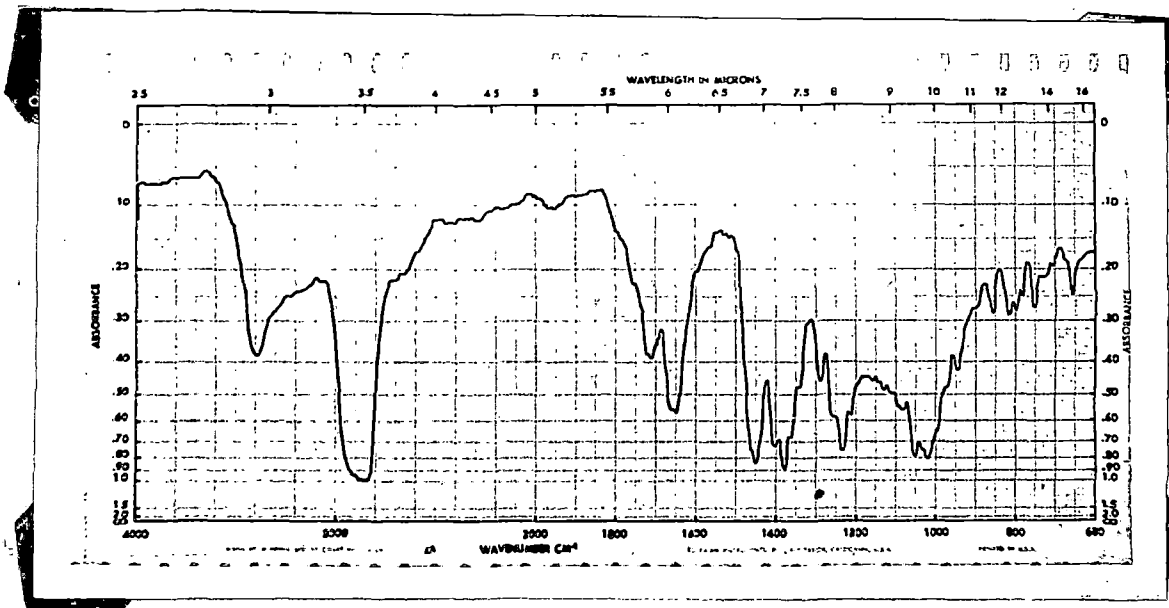


Fig. 10 IR spectrum of diosphenol of β -amyrone

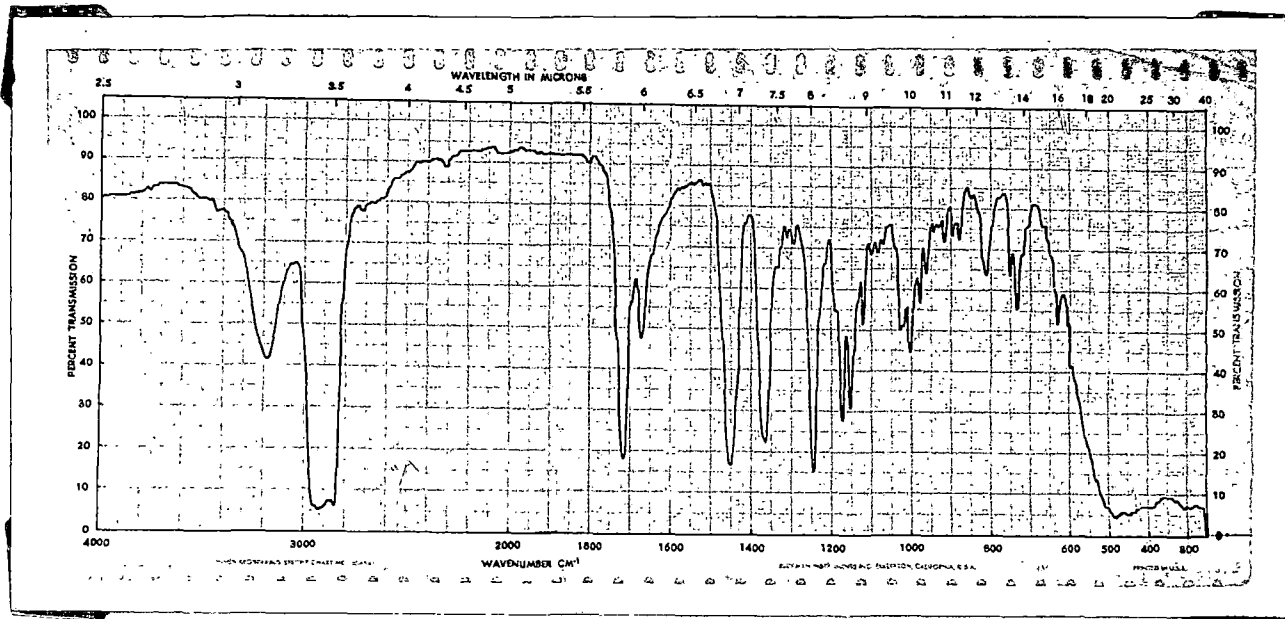


Fig. 11 IR spectrum of acetyl oleanolic acid

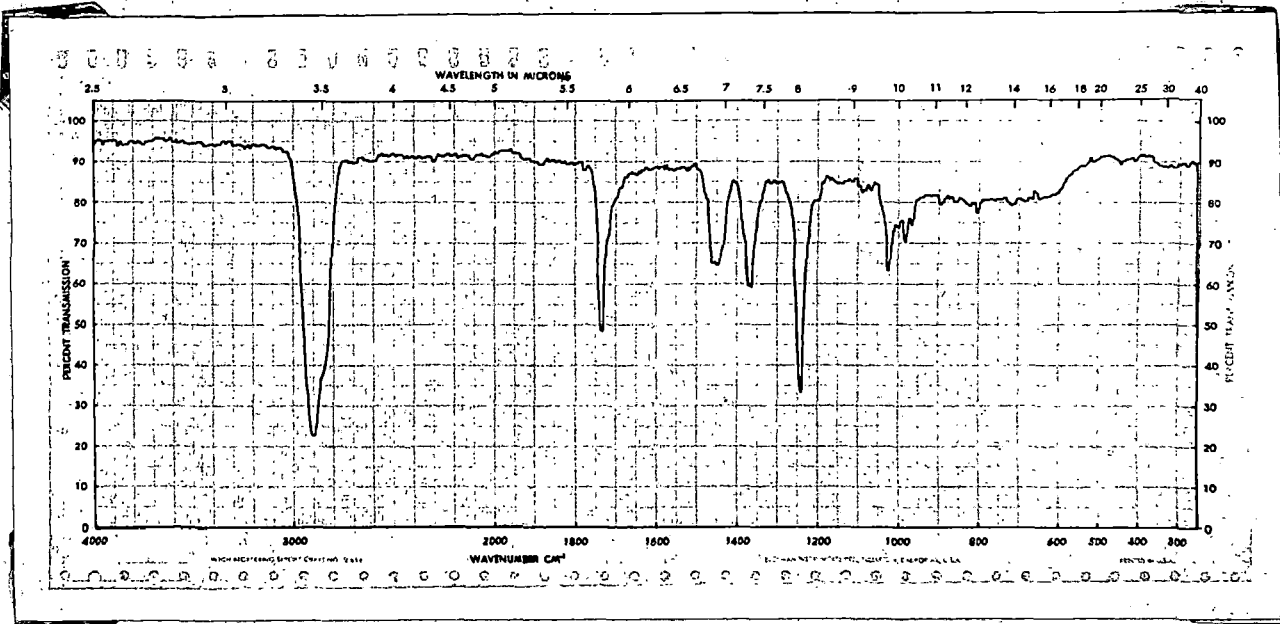


Fig. 12 IR spectra of mixture of monoacetates obtained after lead tetraacetate decarboxylation of acetyl oleanolic acid.

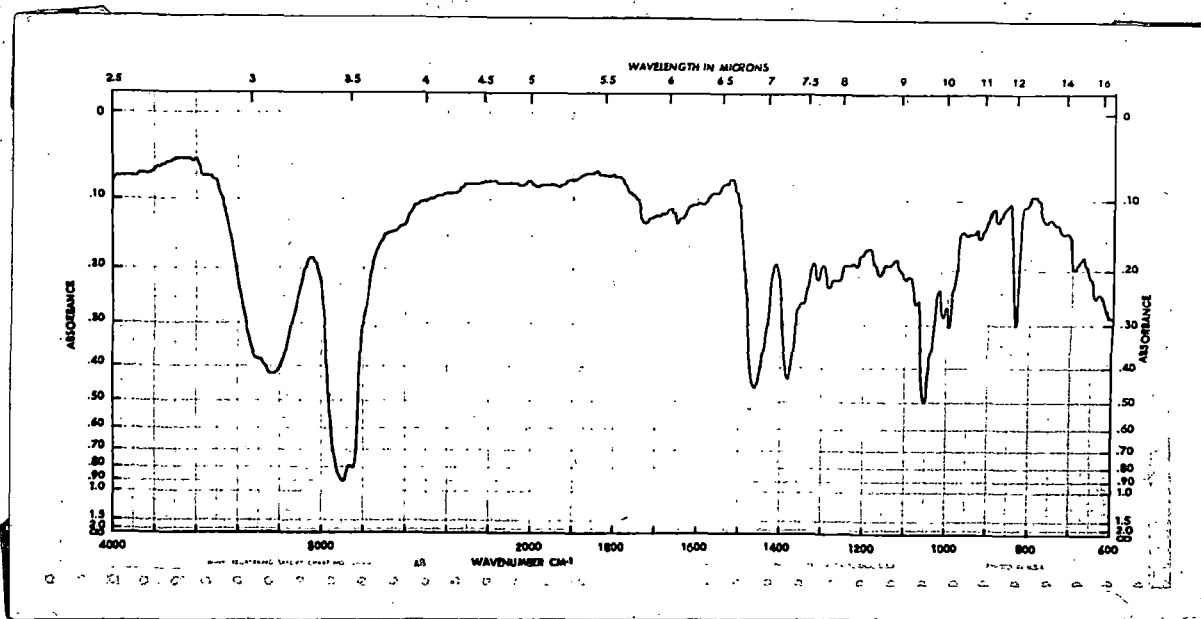


Fig. 13 IR spectrum of $2\alpha, 3\beta$ dihydroxy-28-nor-oleana-12, 17 diene.

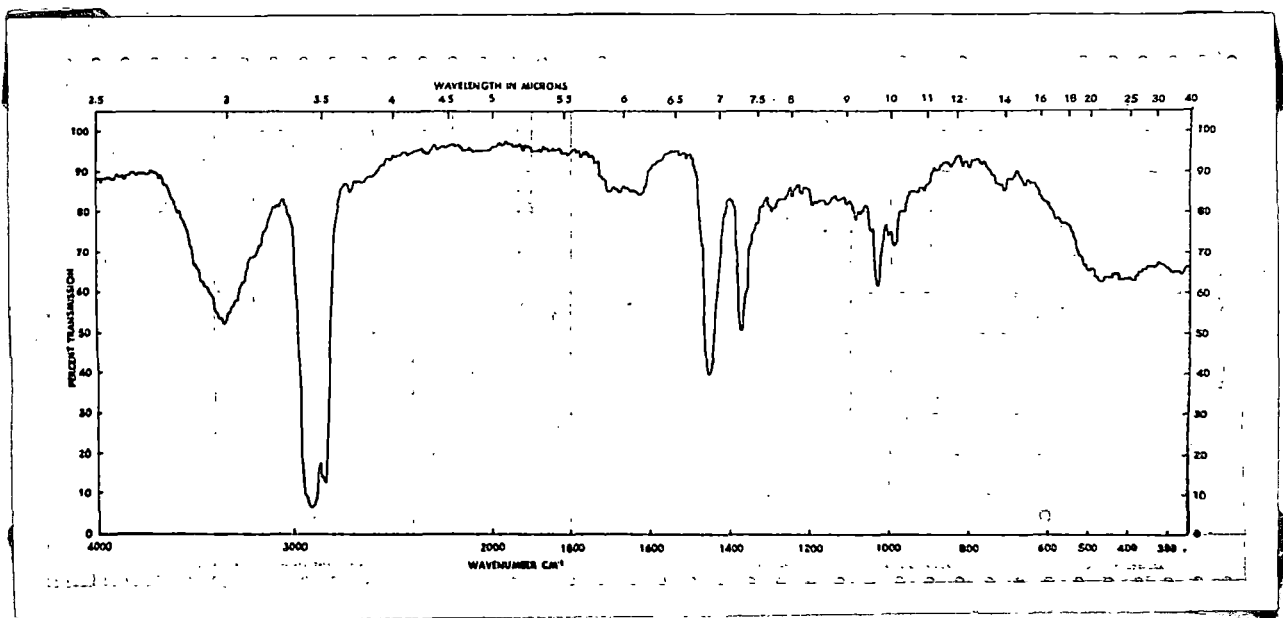


Fig. 14 IR spectrum of 3 β -hydroxy-28-nor-oleana-12, 17 diene

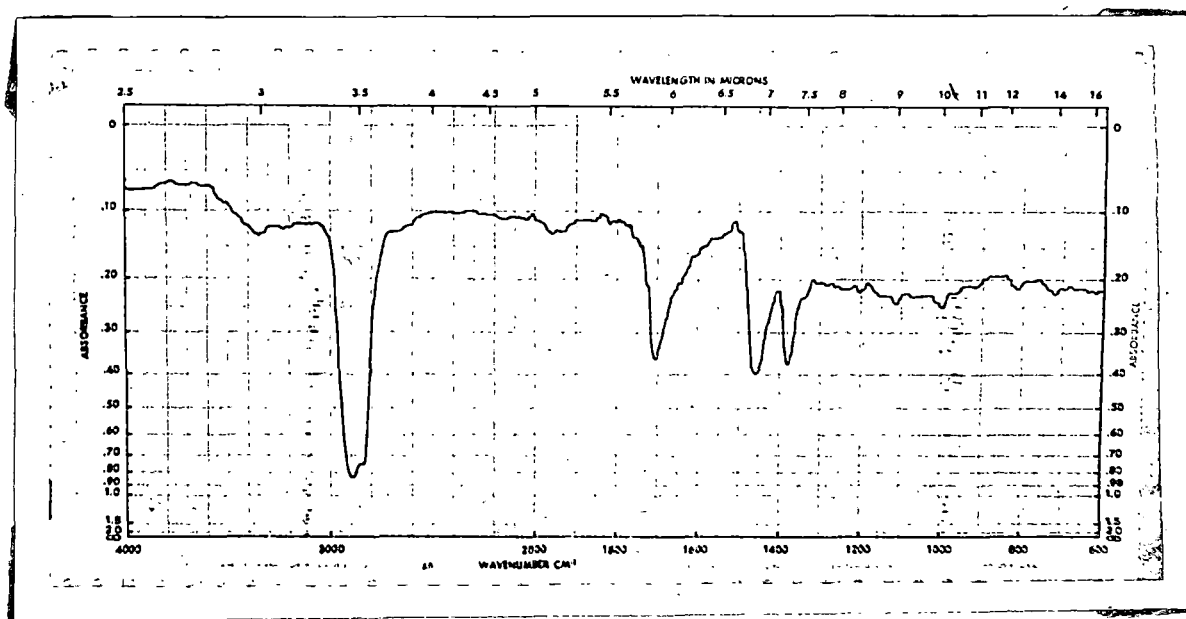


Fig. 15 IR spectrum of 3-oxo-28-nor-oleana-12, 17 diene

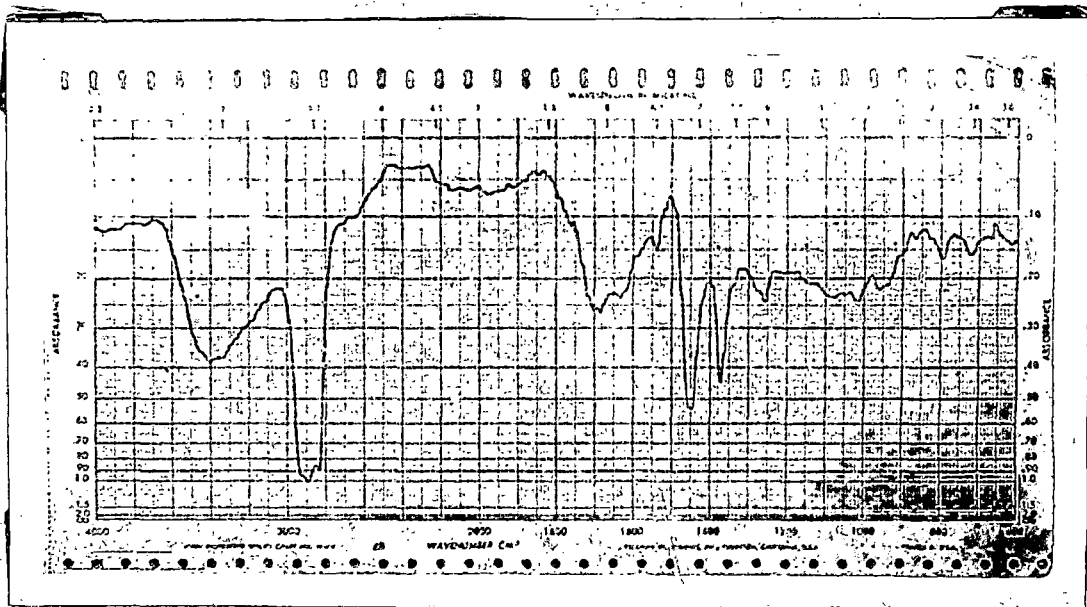


Fig. 16 IR spectrum of 2-oximino-3-oxo-28-nor-oleana-12, 17 diene

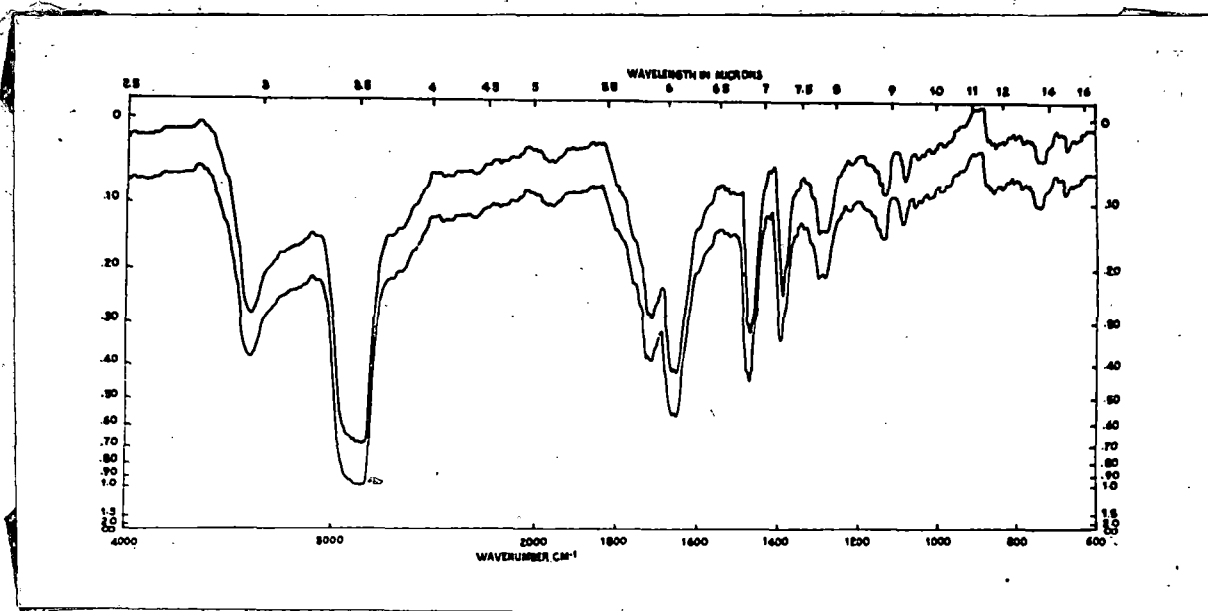


Fig. 17 IR comparison of
 (Upper band) 2,3-dioxo-28-nor-oleana-12, 17 diene
 (synthesised from oleanolic acid)
 (Lower band) 2,3-dioxo-28-nor-oleana-12, 17 diene
 (degraded product from baccatin)