CHAPTER - VI

Detection and structure determination of a new triterpene - a cyclolaudenol homologue, $C_{32}H_{54}O$ by mass - spectrometry.

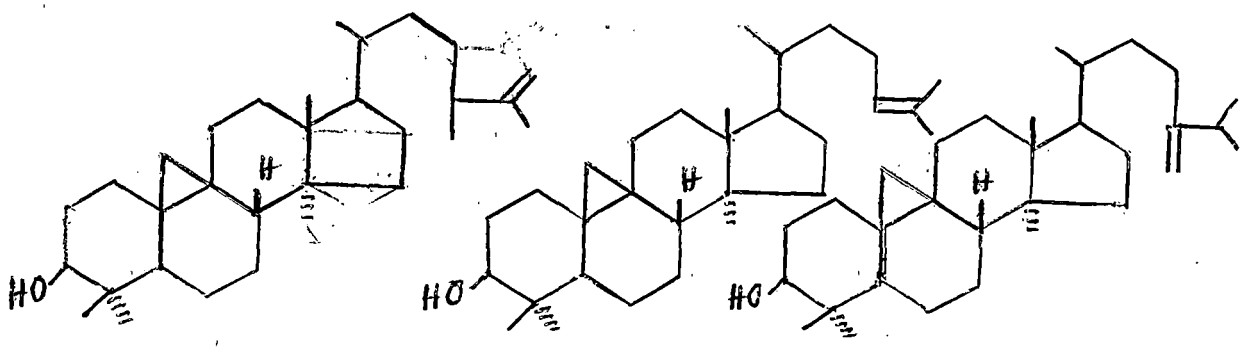
Fraction No. 2 (Chapter - III, Page -187, TableI) was rechromatographed and the separation of Polypodinol A, Polypodinol B, a compound m.p. 148-50° has been described in Chapter - IV, Page -204, Table - II. The next fraction from the petroleum - benzene (3:2) eluate was found to be a mixture of cyclolaudenol and cyclolaudenol homologue (A). Isolation of cyclolaudenol and its homologue has also been described in Chapter - IV (Page -204, Table -II, fractions 18-20).

(IR: Fig-26)

The above mixture (A), was acetylated with acetic anhydride and pyridine when an acetate (B), m.p. 107-8°, $(\alpha)_D$ 55.17° was obtained. Hydrogenation of the original mixture in presence of Adam's catalyst in alcohol gave a compound (C), m.p. 132-33°, which on acetylation gave an acetate (D), m.p. 127-28°. NMR spectrum of the original mixture (A, Fig - 27) and its acetate (B, Fig - 28) both showed distinct peaks at δ4.65 (= CH_2) and δ 4.65 (3H, = CH_2 and -C<u>H</u>-OAc) respectively but in the spectrum of the hydro - genated product (C, Fig-29) the peak due to vinylidene group was no longer present indicating thereby that both the

compounds in the mixture probably contained a $=CH_2$ group. Oxidation of the original mixture (A) with Jone's reagent gave a mixture of ketones (E), m.p. 117-18°. We have measured the mass spectra of the original mixture (A, Fig-30) its acetate (B, Fig-31), the dihydrocompound (C, Fig-32) and its acetate (D, Fig-33) and it was observed that along with cycloläudenol M^+ 440, a second component M^+ 454, that is a new C-32 triterpene alcohol, $C_{32}H_{54}O$, was present in the original mixture.

The mass spectrum of this new compound was found to correspond very closely to that of cycloläudenol 36. The



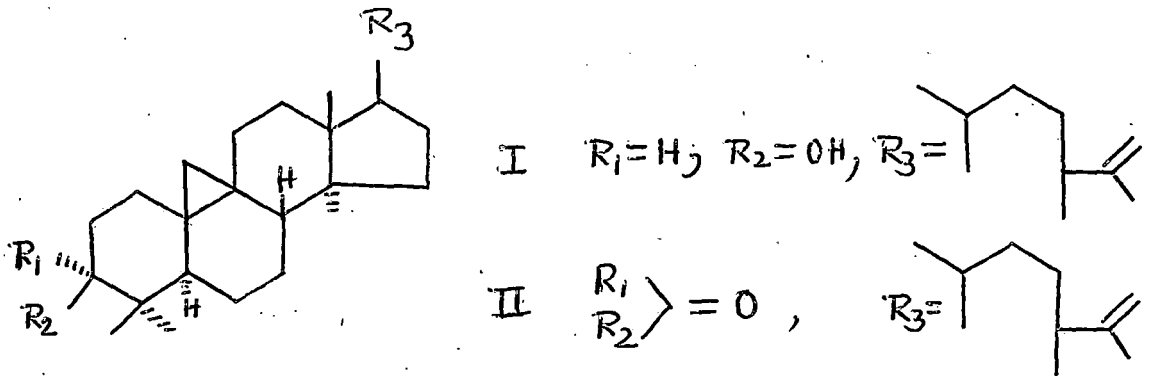
36 37 38

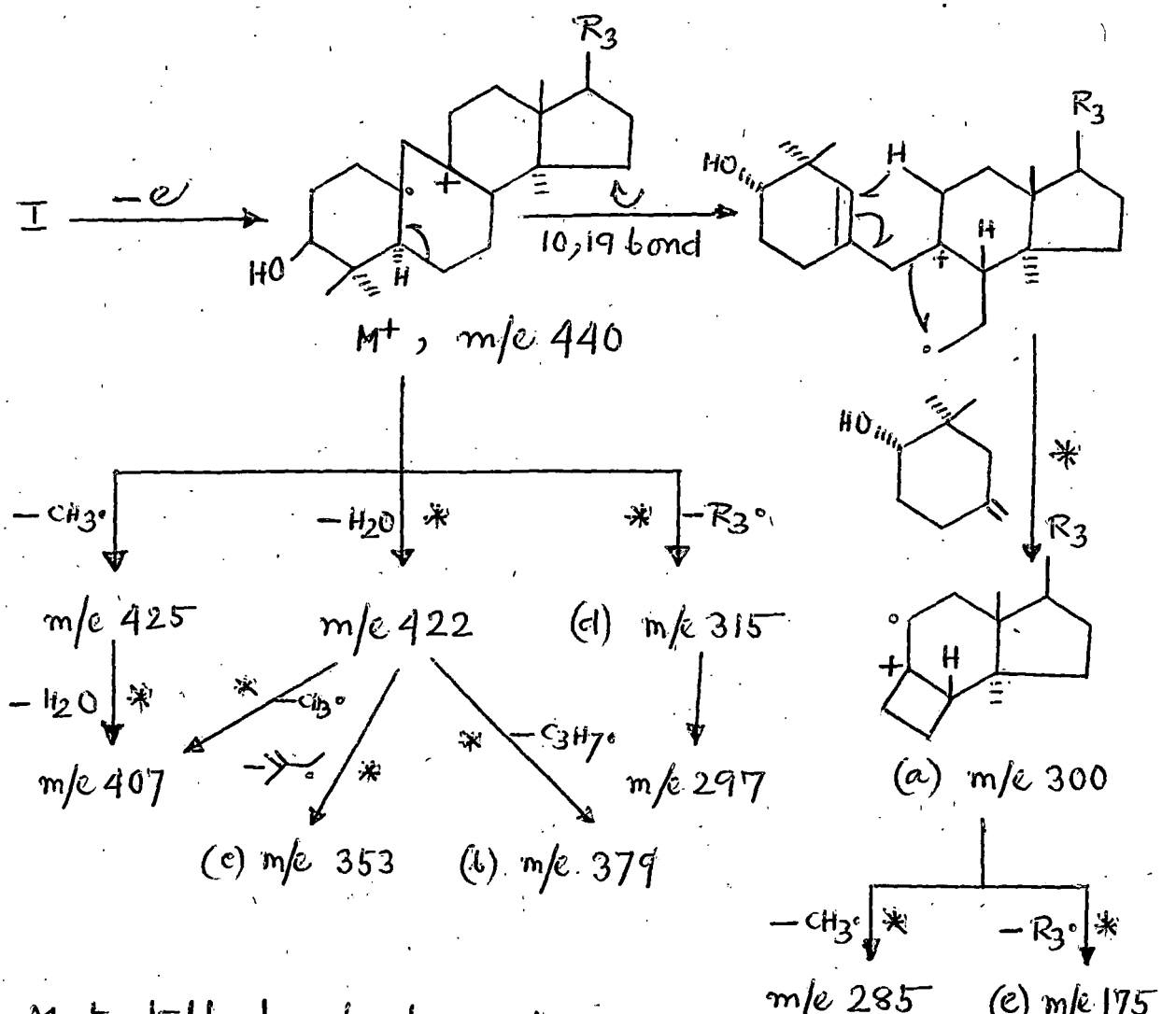
mass spectra of 9,19-cycloartenol 37 and methylene - 24 -

cycloartenol 38 derivatives has been discussed by Benveniste, Hirth and Ourisson[39] who have mentioned that the compounds showed strong peaks at m/e 286 and m/e 300 respectively corresponding to loss of ring A. Audier, Bengelmans and Das[40] have also discussed the mass spectra of some 9,19 - cyclolanostane derivatives and indicated four types of fragmentation peaks. Aplin and coworker[41] have also des - cribed in detail about application of mass spectrometry to the structural investigation of 9, 19 - cyclosterols and triterpenes and have recorded detailed mass spectral fragmentation of various triterpenes containing 9,19 - cyclofunction including cyclolaudenol. It will be evident from the discussions which follow that our data are in agreement with the earlier findings[39-41].

The unusual feature of the spectrum of cyclolaudenol I[41] (Chart - IV) is the intense fragment (a) at m/e 300 and have the composition $C_{22}H_{36}$ and corresponds to loss of ring A plus one hydrogen atom, since it remains at m/e 300 in the spectrum of the corresponding ketone II. This is formed from the molecular ion m/e 440 by a one step process. The other fragments, besides the loss of a methyl group (m/e 425), water (m/e 422) and methyl-plus-water (m/e 407), arise from M-18 fragment and involve cleavage of the side chain - fragment (b) m/e 379 (M-18-C_3H_7·) and (C) m/e 353 (M-18-C_5H_9·). A further fragment (e) m/e 175, common to all spectra,

CHART - IV





* Metastable peak observed

corresponds to the loss of both ring A and the side chain. Another significant peak (d) at m/e 315 ($M-R_3\cdot$) is common to all its side chain derivatives which arises by elimination of R_3 group from M. The most plausible mechanism[42] for the formation of these fragments is depicted in Chart - IV where the initial ionisation of 9-10 bond relieves the strain imposed on ring B, fission of the activated 5-6 bond followed by transfer of one of the C_{11} hydrogen via a "Mc Lafferty" type of rearrangement.

Aplin et al[41] have also stated in their paper that in the spectra of the corresponding ketone II the intensity of the fragment (a) m/e 300 is greatly reduced, presumably owing to competitive ionisation at the carbonyl group. However, fragments corresponding to the well established preferential fragmentation in the vicinity of the carbonyl groups[43a] were not apparent.

Discussions on the mass spectrum of the original mixture (Chart - V) :

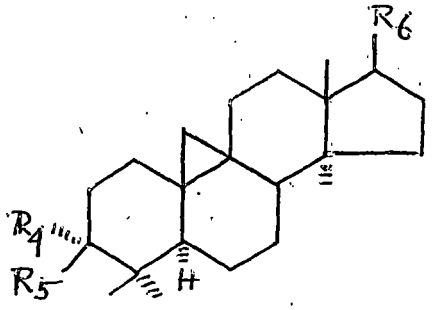
The mass spectrum of the original mixture (III A + III B ; Chart - V) was measured and it has been observed that it contained all the peaks characteristic of cycloläudenol and a cycloläudenol homologue, $C_{32}H_{54}O$ having the molecular ion peak at M^+ 454. It will be observed from Chart - V that in addition to the peak at m/e 300 corresponding to fragment (a)

in Chart -IV for cycloläudenol there is an intense peak at m/e 314 (III B, fragment a') indicating the presence of an extra carbon atom in the side chain of the new triterpene. Moreover, the prominent peak (d) at m/e 315 for fragment $M-R_6$. also indicated the presence of an extra carbon atom in the side chain. A further single peak at m/e 175 due to fragment(e) is also indicative of the presence of the extra carbon atom in the side chain of the cycloläudenol nucleus. The other major fragment (b), besides the loss of methyl radical at m/e 439 (III B) water at M/e 436 (III B) and methyl-plus-water at m/e 421 (III B) is from the M-18 fragment (m/e 436) and appears at m/e 393 (III B) and involve cleavage of the side chain (M-18-C_3H_7.) The appearance of all these mass peaks can be explained by the same mechanism as that for cycloläudenol as is given in Chart - IV. We, therefore, conclude from the above studies that the mixture contains a new triterpene having the same cycloläudenol nucleus but having a extra $-CH_2-$ in the cycloläudenol side chain.

Similarly, the mass fragmentation of the acetate (IV A + IV B ; Chart - V) also leads us to the same conclusion that the new cycloläudenol homologue contains an extra - CH_2- in the side chain.

Examination of the mass spectra of the dihydroalcohol mixture (V A + V B ; Chart - VI) also indicates the presence

CHART - V



III A $R_4=H, R_5=OH, R_6 = C_9H_{17}$

III B $R_4=H, R_5=OH, R_6 = C_{10}H_{19}$

IV A $R_4=H, R_5=OAc, R_6 = C_9H_{17}$

IV B $R_4=H, R_5=OAc, R_6 = C_{10}H_{19}$

Mixture of alcohols III A (M^+ 440) and III B (M^+ 454)

$-CH_3^\bullet$ * → m/e 425 (III A); m/e 439 (III B)

$-H_2O$ * → m/e 422 (III A); m/e 436 (III B)

$-R_6^\bullet$ * → (d) m/e 315 (for both III A and III B)

(a) m/e 300 (III A); (a') m/e 314 (III B)

m/e 436 (III B): $-H_2O$ * → m/e 407 (III A); m/e 421 (III B)

m/e 436 (III B): $-CH_3^\bullet$ * → (c) m/e 353

m/e 436 (III B): $-C_3H_7^\bullet$ * → (b) m/e 379 (III A); (b') m/e 393 (III B)

m/e 314 (III B): $-CH_3^\bullet$ * → m/e 285 (III A); m/e 299 (III B)

m/e 314 (III B): $-R_6^\bullet$ * → (e) m/e 175 (for both III A and III B)

Mixture of acetates IV A (M^+ 482) and IV B (M^+ 496)

$-CH_3^\bullet$ * → m/e 467 (IV A); m/e 481 (IV B)

$-HOAc$ * → m/e 422 (IV A); m/e 436 (IV B)

$-R_6^\bullet$ * → m/e 357 (for both IV A and IV B)

(a) m/e 300 (IV A); (a') m/e 314 (IV B)

m/e 357: $-HOAc$ * → (d) m/e 297 (for both IV A and IV B)

m/e 314 (IV B): $-R_6^\bullet$ * → (e) m/e 175 (for both IV A and IV B)

m/e 436 (IV B): $-CH_3^\bullet$ * → (c) m/e 353 (IV A); (c') m/e 367 (IV B)

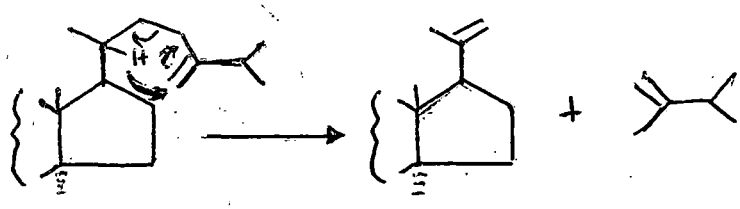
m/e 436 (IV B): $-C_3H_7^\bullet$ * → (b) m/e 379 (IV A); (b') m/e 393 (IV B)

m/e 422 (IV A): $-HOAc$ * → m/e 407 (IV A); m/e 421 (IV B)

of the extra carbon atom in the side chain cyclolaudenol nucleus. The presence of the fragment (a') at m/e 316 (VB) and the fragment (d) ($M-R_9\cdot$) at m/e 315 (single peak) of VB again established beyond doubt that the extra carbon atom is present in the side chain R_9 of dihydrocyclolaudenol homologue. The other fragments depicted in the Chart - VI also bear strong evidence for the presence of a extra - CH_2 - in the side chain of the dihydrocyclolaudenol nucleus.

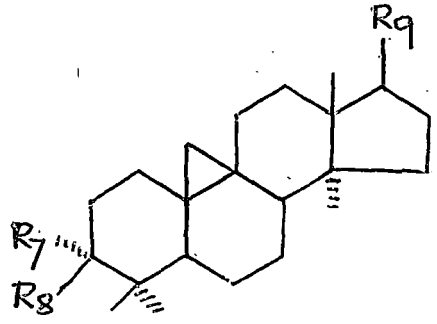
Similar examination on mass fragmentation pattern of the dihydroacetate mixture (VI A + VI B; Chart - VI) also corroborates the presence of the extra - CH_2 - in the side chain of cyclolaudenol nucleus.

The position of the double bond (= CH_2. NMR ; Fig-27) in the side chain at 25(26) position may be tentatively assigned from the mass spectra of (III A + III B), (IV A + IV B), (VA + VB) and (VI A + VIB) as the M-84 fragment observed in the "McLafferty" rearrangement[176, 436] involving 24-28 double bond and the C-20 hydrogen has been found to be absent in their spectra.



M-84

CHART - VI



V A $R_7=H$, $R_8=OH$, $R_9=C_9H_{19}$

V B $R_7=H$, $R_8=OH$, $R_9=C_{10}H_{21}$

VI A $R_7=H$, $R_8=OAc$, $R_9=C_9H_{19}$

VI B $R_7=H$, $R_8=OAc$, $R_9=C_{10}H_{21}$

Mixture of Dihydroalcohols V A (M^+ 442) and V B (M^+ 456)

$-CH_3^\circ$ * → m/e 427 (V A), m/e 441 (V B)

$-H_2O$ * → m/e 424 (V A), m/e 438 (V B)

$-R_9^\circ$ * → (d) m/e 315 (for both V A and V B)

(d) m/e 315 $\xrightarrow[*]{-H_2O}$ m/e 297 (for both V A and V B)

(a) m/e 302 (V A), (a') m/e 316 (V B) → $*\ -CH_3^\circ$ → m/e 287 (V A), m/e 301 (V B)

(a) m/e 302 (V A), (a') m/e 316 (V B) → * → (e) m/e 175 (for both V A and V B)

m/e 424 (V A), m/e 438 (V B) → $-CH_3^\circ$ * → m/e 409 (V A), m/e 423 (V B)

m/e 424 (V A), m/e 438 (V B) → * → (e) m/e 355 (V A), (e') m/e 369 (V B)

m/e 424 (V A), m/e 438 (V B) → $-C_3H_7^\circ$ * → (b) m/e 381 (V A), (b') m/e 395 (V B)

Mixture of the corresponding acetates VI A (M^+ 484) and VI B (M^+ 498)

$-CH_3^\circ$ * → m/e 469 (VI A), m/e 483 (VI B)

$-HOAc$ * → m/e 424 (VI A), m/e 438 (VI B)

$-R_9^\circ$ * → (d) m/e 357 (for both VI A and VI B)

(d) m/e 357 $\xrightarrow[*]{-HOAc}$ m/e 297 (for both VI A and VI B)

(a) m/e 302 (VI A), (a') m/e 316 (VI B) → * → $-R_9^\circ$ → (e) m/e 175 (for both VI A and VI B)

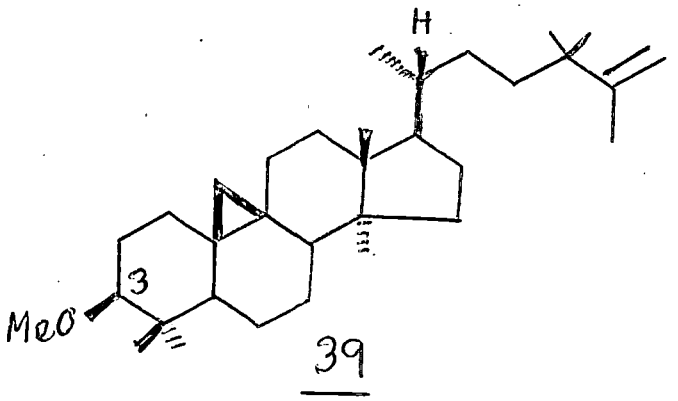
m/e 424 (VI A), m/e 438 (VI B) → * $-HOAc$ → m/e 364 (VI A), m/e 378 (VI B)

m/e 424 (VI A), m/e 438 (VI B) → $-CH_3^\circ$ * → m/e 409 (VI A), m/e 423 (VI B)

m/e 424 (VI A), m/e 438 (VI B) → * → (e) m/e 355 (VI A), (e') m/e 369 (VI B)

m/e 424 (VI A), m/e 438 (VI B) → $-C_3H_7^\circ$ * → (b) m/e 381 (VI A), (b') m/e 395 (VI B)

From the foregoing evidence, it may be reasonably concluded that the new triterpene is a cycloläudenol derivative with a extra carbon ($-CH_2-$) in the side chain and a probable structure can be arrived at by merely placing the extra carbon atom in the side chain of cycloläudenol. In the known triterpenes any additional carbon atom or atoms are always attached to C-24 as either methyl, methylene, ethyl or ethylidene groups[44]. Recently, Ritchie and coworkers[45] have isolated a new triterpene, $C_{33}H_{56}O$, cycloneolitsin from Neolitsea dealbate R.Br.Merr. for which a 24,24 - dimethyl structure 39 has been assigned on the basis of chemical and physical evidence. This is, also the first example of 24,24 - dimethylation on the



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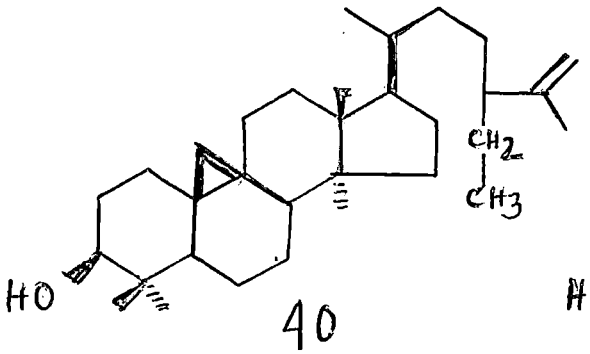
side chain of known triterpenes. Accordingly, two probable structures 40 and 41 can be assigned to the new cycloläudenol derivative.



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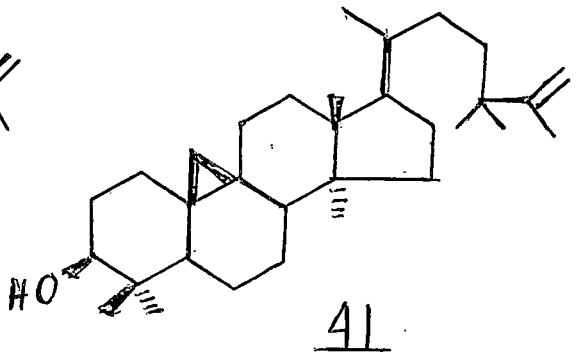

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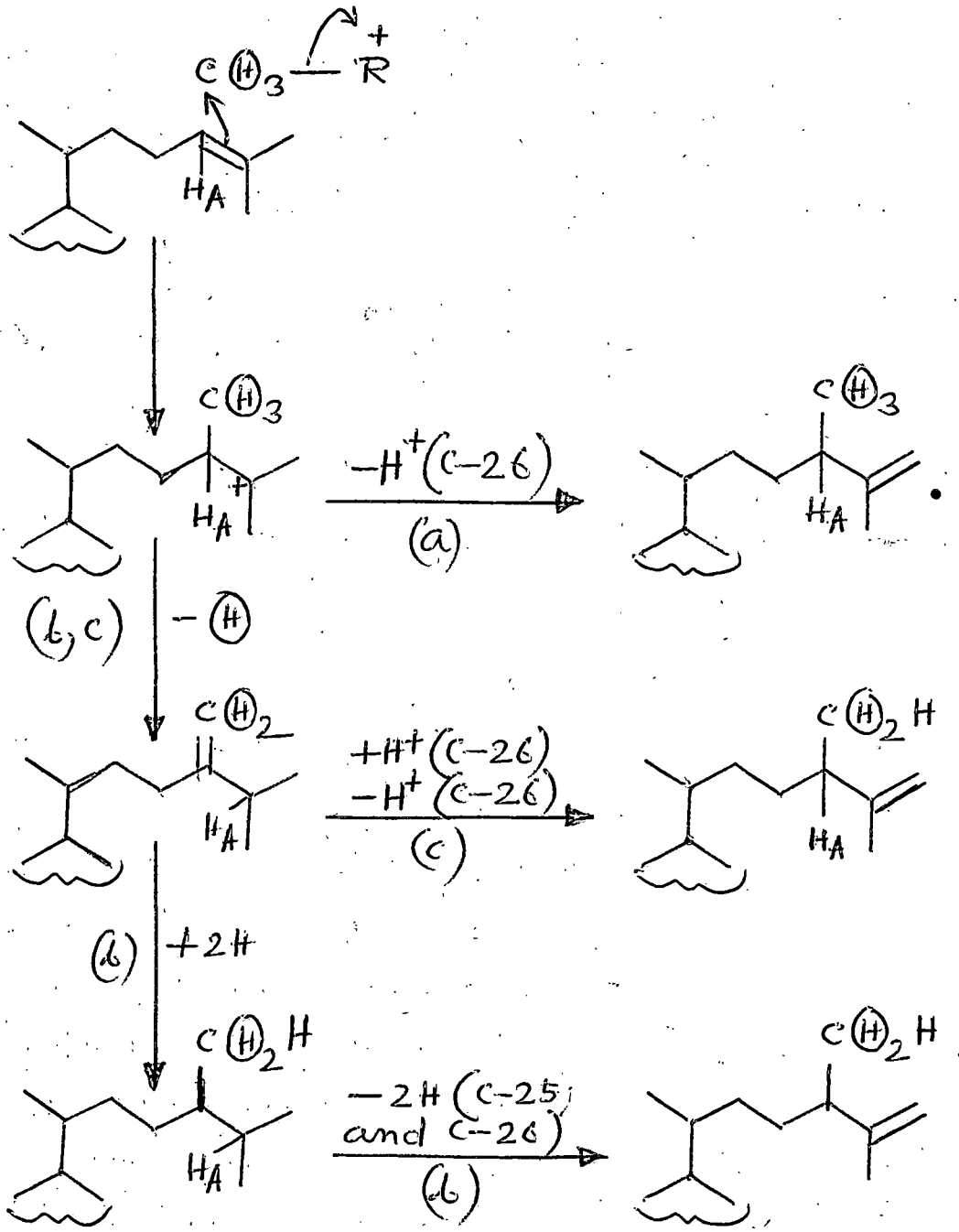
Chart - VII



Possible mechanism of methylation to produce cyclolaudenol. The circled H is derived from the methyl group of methionine, and H_A arises from the 4-pro-R hydrogen of mevalonic acid.

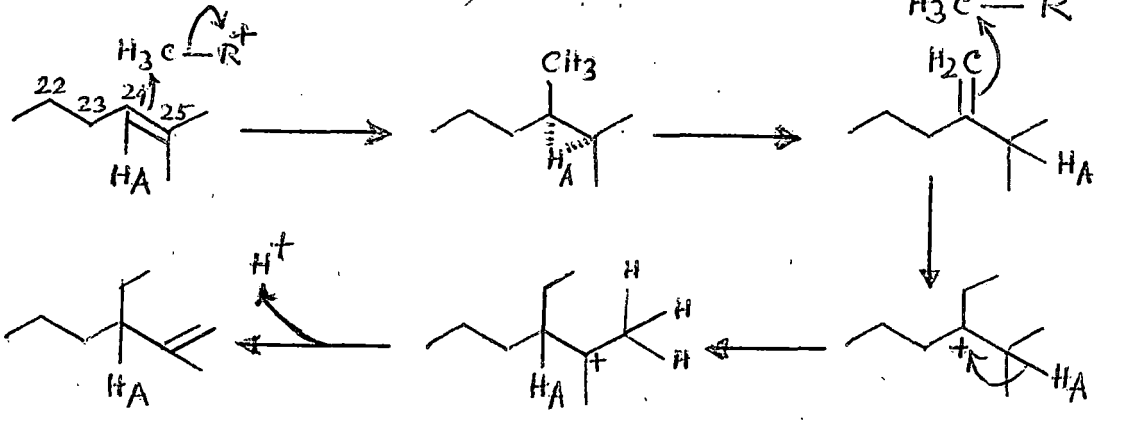
The outlines of the biosynthetic pathways involved in the formation of C-24 alkylated derivatives of tetracyclic triterpenes are now established[45]. The alkylation mechanism linked with the formation of Δ^{25-26} bond, as in the formation of cycloläudenol I and 24S-24-ethylcholesta- 5,22,25 - trien-3β-ol is now well known. Three possibilities exist for the methylation and Δ^{25-26} double bond formation in cycloläudenol (Chart - VII). Route (a) involves elimination of a proton from C-26 from the intermediate cation indicated, which would give the cycloläudenol side chain directly with retention of all three hydrogens of the C-24 methyl group and hydrogen (H_A) of a precursor (e.g. cycloartenol) remaining at C-24. Routes (b) and (c) both involve a C-24 methylene intermediate and thus the retention of only two of the hydrogens of the incoming methyl group; however, route (c) would result in retention of H_A at C-24 and route (b) would result in its elimination. Experiments with [2-^{14}C-(4R)-4-$^{3}H_1$] MVA showed that cycloläudenol fromed by rhizomes of Polypodium vulgare retained H_A at C-24 and that the methylene group at C-25 arose stereospecifically from C-2 of MVA. Thus route (c) was eliminated; evidence which favours route (a) was obtained by comparing the ^{3}H ; ^{14}C ratio in cycloläudenol and 24 - methylene cycloartanol synthesized from [^{14}C ; $^{3}H_3$] methionine by P. Vulgare in the

same experiment. The ratio in cyclolaudenol (17.4:1) was very close to that of starting methionine (16.5:1) whereas in 24-methylene cycloartanol was significantly lower[47] (14.1 :1). However, this result needs confirmation by use of [C^2H_3] methionine, in order to eliminate any possible isotopic effect.

Since biosynthesis of triterpenes and sterols having ethyl group at C-24 is known and has been established, we prefer, tentatively for "biogenetic prejudice" the structure 40 for the new triterpene - cyclolaudenol homologue, $C_{32}H_{54}O$. The mechanism for its biosynthesis in Polypodium juglandifolium is indicated in Chart - VIII and is probably operating in producing a simultaneous alkylation and desaturation at C-24[48].

However, the final structure determination of this new triterpene must await its isolation in pure form from its mixture with cyclolaudenol. Further work in this direction is in progress in our laboratories.

Chart - VIII



Mechanism of alkylation to produce 24-ethyl-cyclolaudenol

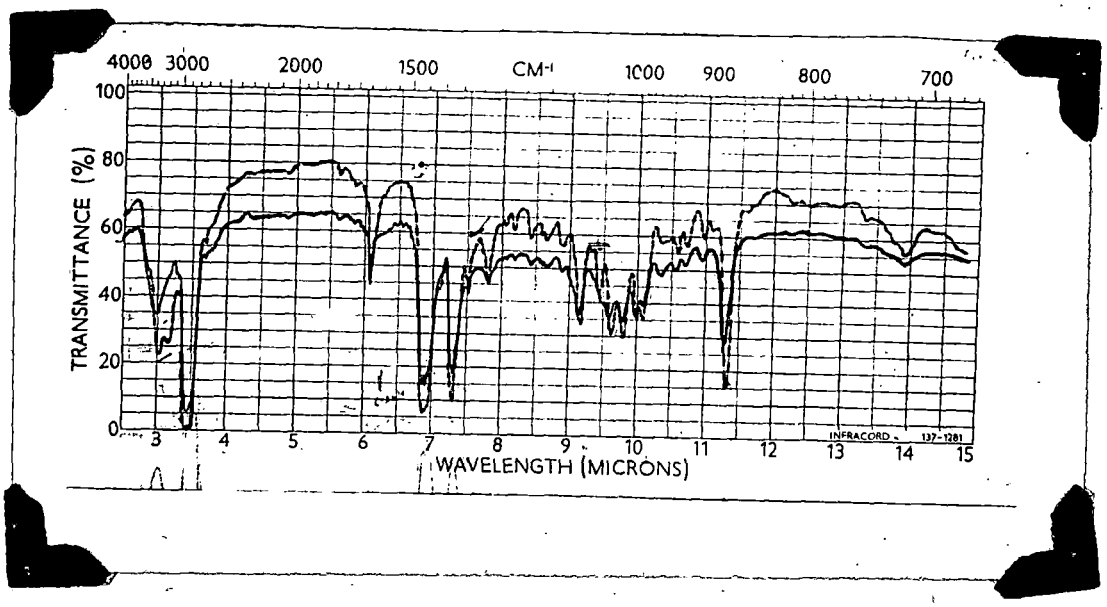


Fig. 26 : IR spectrum of the cyclolaudenol-cyclodaudenol homologue mixture A(solid line) and authentic cyclolaudenol (Dotted line)

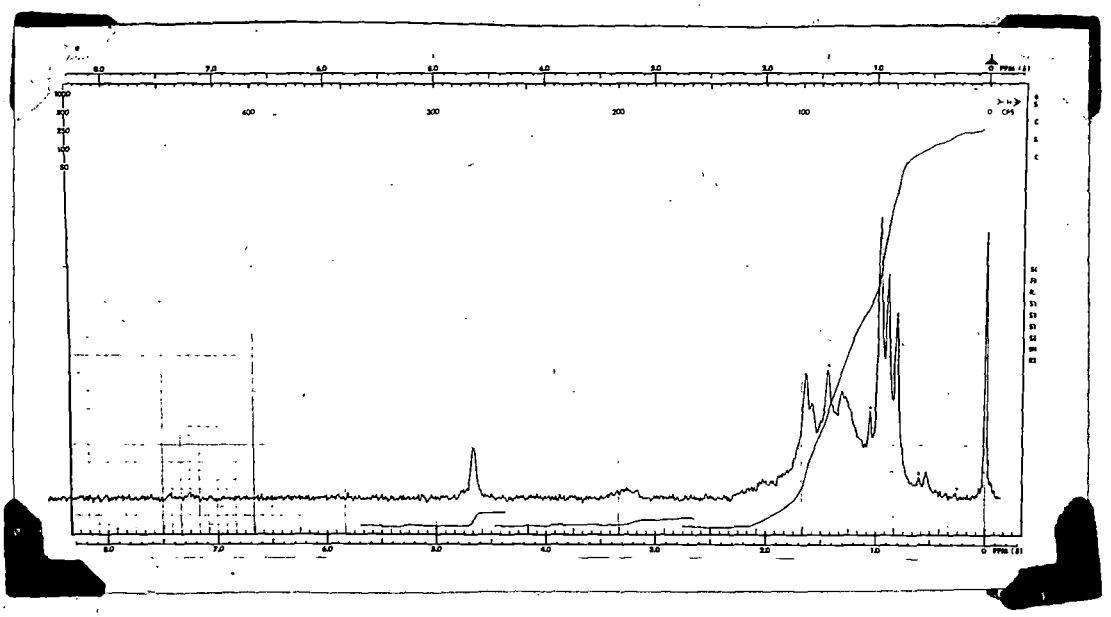


Fig. 27 : NMR spectrum of the mixture of cyclolaudenol and its homologue A

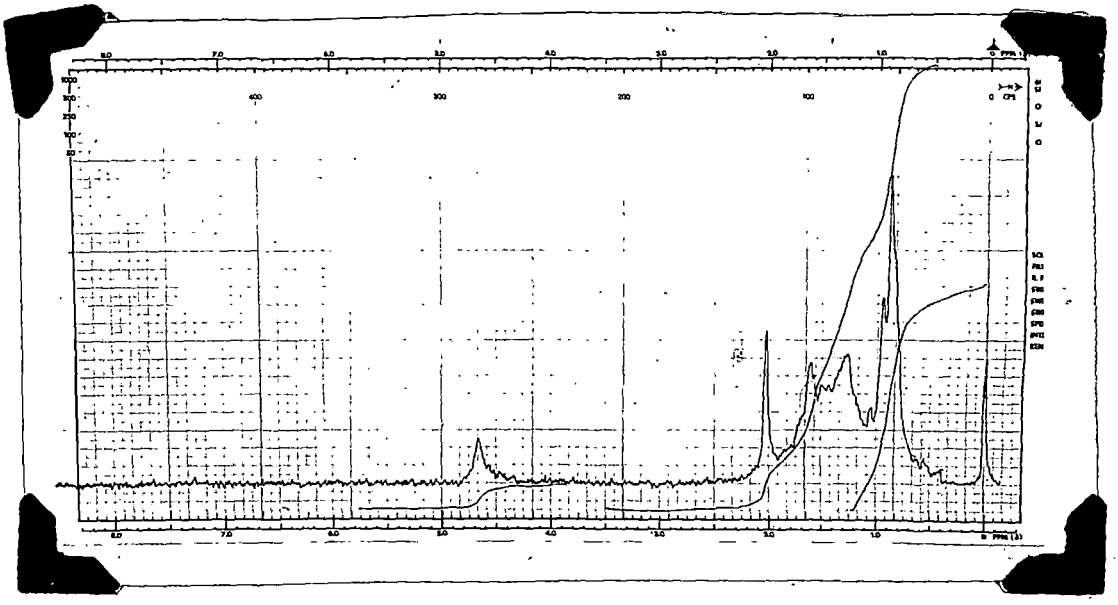


Fig. 28 : NMR spectrum of the mixture of acetates of cyclolaudenol and its homologue B

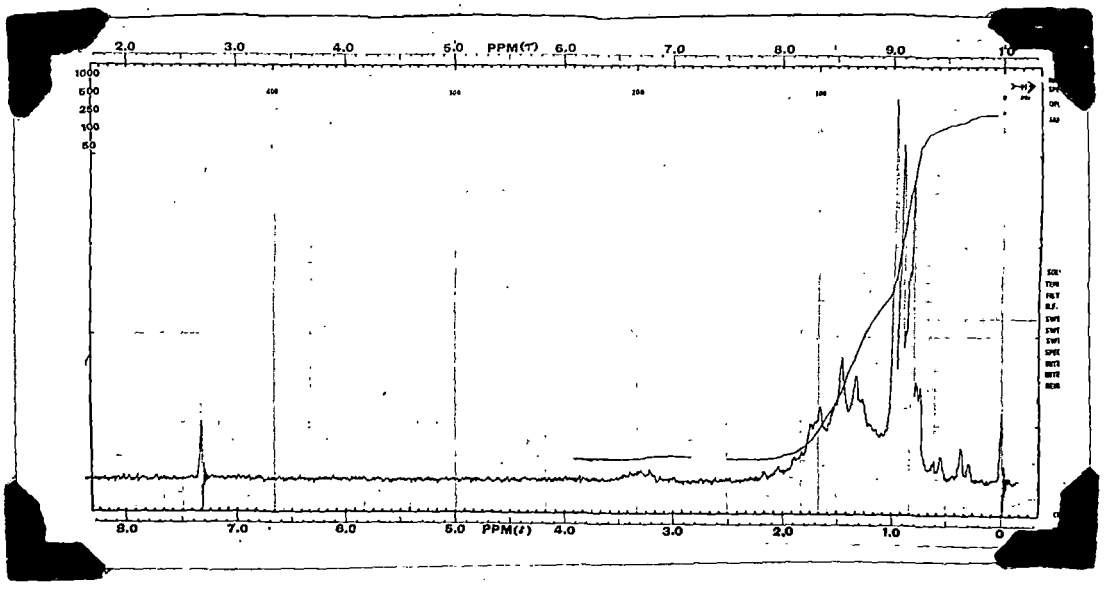


Fig. 29 : NMR spectrum of the mixture of dihydro-derivatives of cyclolaudenol and its homologue C

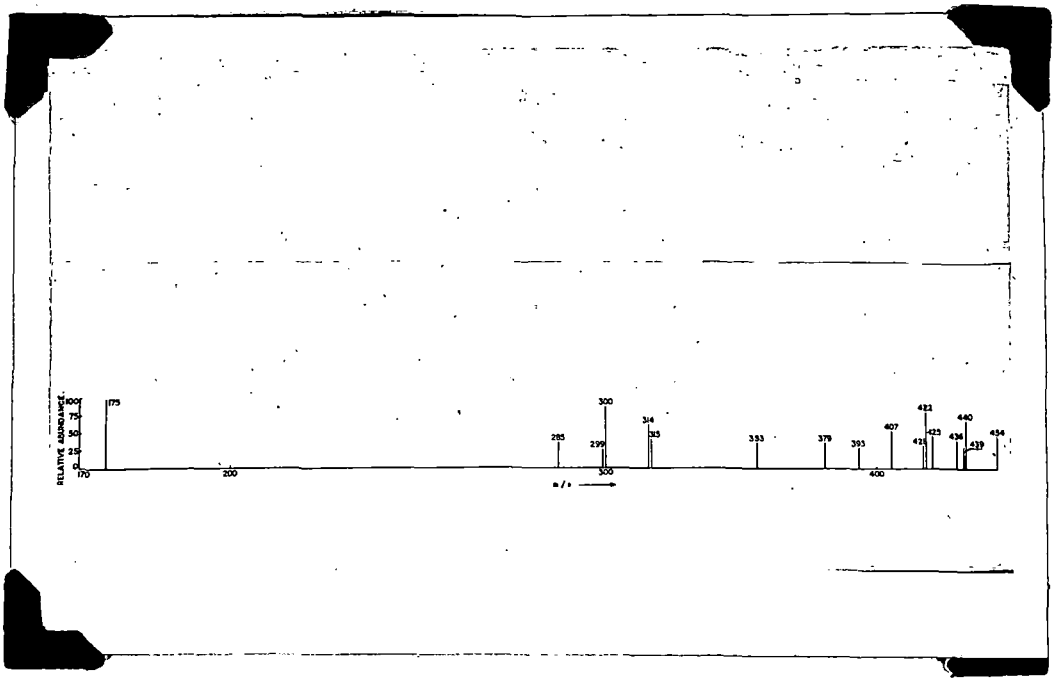


Fig. 30 : Mass spectrum of the mixture of cyclolaudenol and its homologue A

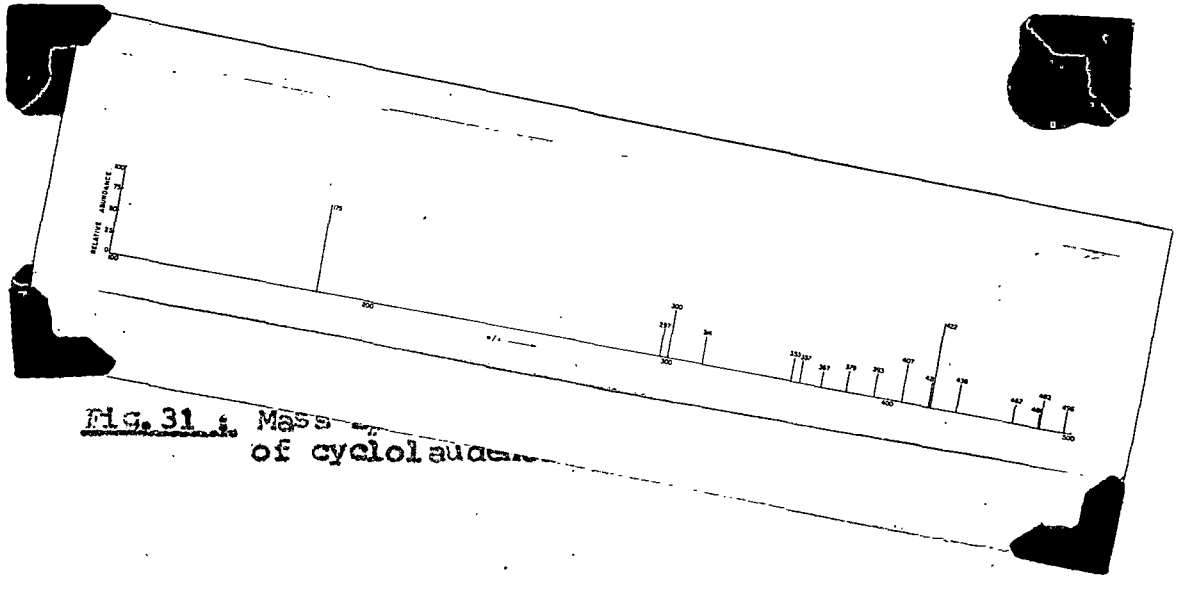


Fig. 31 : Mass [illegible] of cyclolaude[illegible]

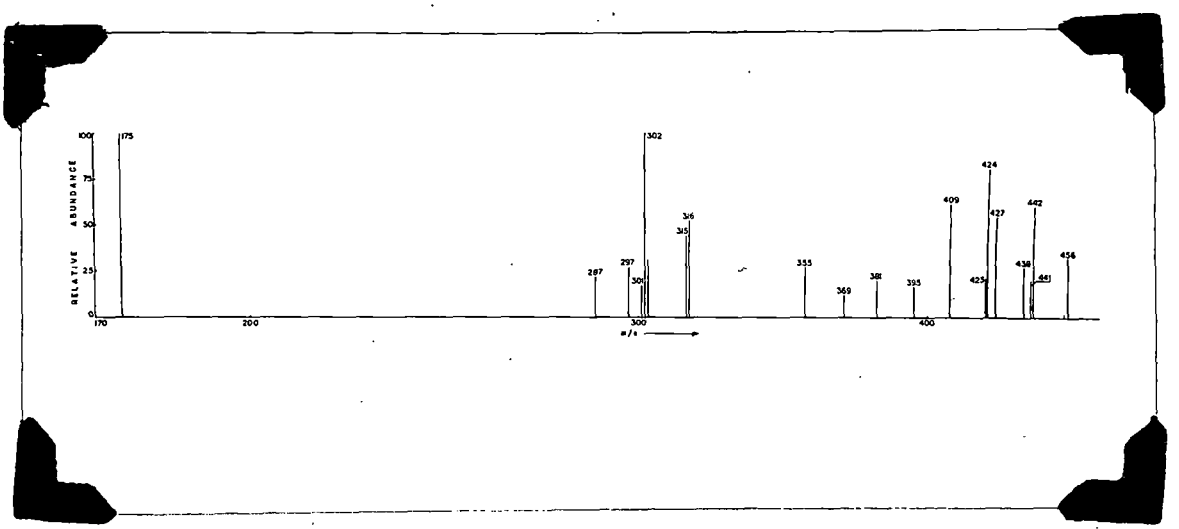


Fig. 32 : Mass spectrum of the mixture of dihydro-derivatives of cyclolaudenol and its homologue C

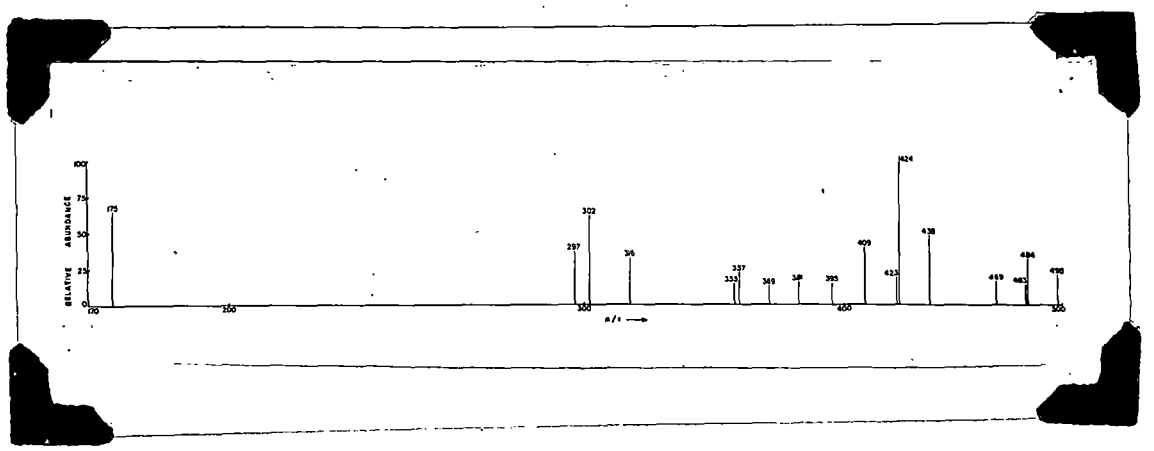


Fig. 33 : Mass spectrum of the mixture of acetates of dihydro-derivatives of cyclolaudenol and its homologue D

EXPERIMENTAL

Rechromatography of fraction no. 2 (Chapter - III, Page - 187, Table - I) :

Rechromatography of fraction no. 2 (Chapter - III, Page - 187, Table - I) has been described (Chapter - IV, Page - 204, Table - II). Fractions 18-20 were combined and on crystallisation from methanol gave a solid (A), m.p. 121-22°. This product (A) was found to be a mixture of two compounds (Two spots in TLC and mass fragmentation pattern)

IR : 3340 cm^{-1} (OH) Fig - 26

Mass spectrum : M^+ 440 and M^+ 454 Fig - 30

NMR spectrum (60 Mc) : δ 4.65 ($=CH_2$) Fig - 27

Acetylation of the above mixture (A) : Preparation of the mixture of acetates (B) :

The above alcohol mixture (A, 200 mg) was dissolved in pyridine (2 ml) and acetylated with acetic anhydride (2 ml) by heating on a water bath for 5-hours. After working up in the usual manner it gave a solid residue (185 mg). The residue dissolved in benzene (3 ml) was placed over a column of alumina (15 gm, deactivated with 0.6 ml of 10% aqueous acetic acid) developed with petroleum and eluted with the following solvents (Table - XVI).

Table - XVI

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum	1 - 3	Solid, m.p. 105-7° (170 mg)

Further elution with more polar solvent did not yield any solid material.

Fractions 1-3 were combined and on crystallisation from a mixture of methanol and acetone furnished crystals (B), m.p. 107-8°, $(\alpha)_D$ 55.17° which was found to be a mixture of two compounds (TLC and mass fragmentation pattern)

NMR spectrum (60 Mc) : δ 4.65 (= CH_2) Fig-28

Mass spectrum : M^+ 482 and M^+ 496 Fig-31

Hydrogenation of the original mixture (A) ;
Preparation of the mixture of dihydrocompounds (C) :

The original mixture (A, 500 mg) dissolved in alcohol (50 ml) was shaken in an atmosphere of hydrogen in presence of PtO_2 catalyst (50 mg) until absorption of hydrogen ceased.

The solid obtained (450 mg) after usual working up was subjected to chromatography over alumina (30 gm, deacetivated with 1.2 ml of 10% aqueous acetic acid) developed with petroleum and eluted with the following solvents (Table -XVII).

Table - XVII

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum	1 - 3	Nil
Petroleum : Benzene (4:1)	4 - 7	Solid (400 mg) m.p. 128-30°.

Further elution with more polar solvent did not yield any solid material.

Fractions 4-7 were combined and on crystallisation from acetone gave (C), m.p. 132-33°. The product (C) was found to be a mixture of two compounds (TLC and mass fragmentation pattern)

NMR spectrum (60 Mc) : Fig-29

Mass spectrum : M^+ 442, M^+ 456 Fig-32

Acetylation of the dihydro mixture (C) ; Preparation of the corresponding acetate mixture (D) ;

The dihydro mixture (C, 200 mg) was acetylated with pyridine (2ml) and acetic anhydride (2 ml) by heating on a water bath for 5-hours. After working up in the usual manner it gave a solid residue (180 mg). This product dissolved in benzene (3 ml) was placed over a column of alumina (15 gm, deactivated with 0.6 ml of 10% aqueous acetic acid) developed with petroleum and eluted with the following solvents (Table - XVIII).

Table - XVIII

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum	1 - 3	Solid (160 mg) m.p. 125-27°.

Further elution with more polar solvent did not yield any solid material.

Fractions 1 - 3 were combined and on crystallisation from a mixture of chloroform and methanol gave a solid (D), m.p. 127-28°. This product (D) was found to be a mixture of two compounds (TLC and mass fragmentation pattern)

Mass spectrum : M^+ 484 and M^+ 498 Fig-33

Oxidation of the original mixture (A) with Jone's reagent : Preparation of the mixture of ketones (E) :

To a solution of the original mixture (A, 200 mg) in pure acetone (100 ml) was added Jone's reagent dropwise until a faint orange colour persisted. The mixture was kept at room temperature for 10 minutes, diluted with water and extracted with ether. The ether layer was washed thoroughly with water, dried (Na_2SO_4) and the ether evaporated. The residue (180 mg) dissolved in benzene (3 ml) was chromatographed over a column of alumina (15 gm, deactivated with 0.6 ml of 10% aqueous acetic acid) developed with petroleum and eluted with the following solvents (Table - XIX).

Table - XIX

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum	1 - 3	Solid (160 mg) m.p. 115-18°.

Further elution with more polar solvent did not yield any solid material.

Fractions 1-3 were combined and on crystallisation from a mixture of chloroform and methanol furnished shining crystals (E), m.p. 117-18°. This compound again, was found to be a mixture of two compounds (TLC).