PART-II

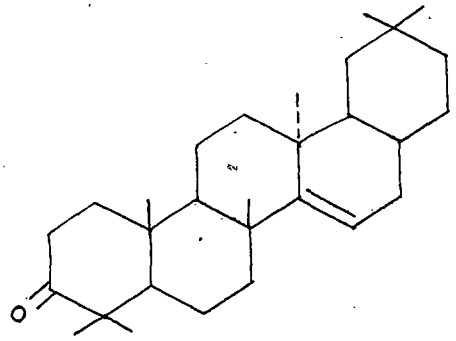
PARTIAL SYNTHESIS OF 2α, 3β - DIACETOXY - 28 - NOR OLEANA - 12, 17 - DIENE : CONFIRMATION OF THE STRUCTURE OF BACCATIN.

PART-II

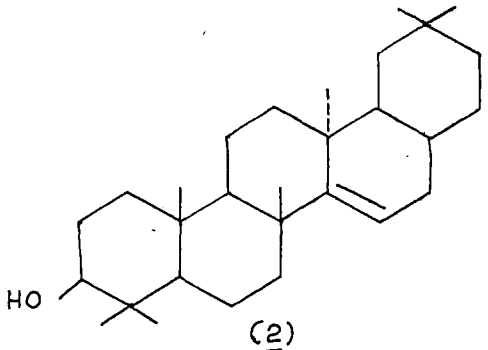
CHAPTER-I

A Short Review on the Structure Elucidation of Baccatin.

Investigations on the benzene extract of the bark of *Sapium baccatum* Roxb in this laboratory led to the isolation of taraxerone (1), taraxerol (2), 1-hexacosanol (3), β-Sitosterol (4), 3,3'-di-O-methyl ellagic acid (5) and 3-acetoxy aleuritolic acid (6)[1-6].



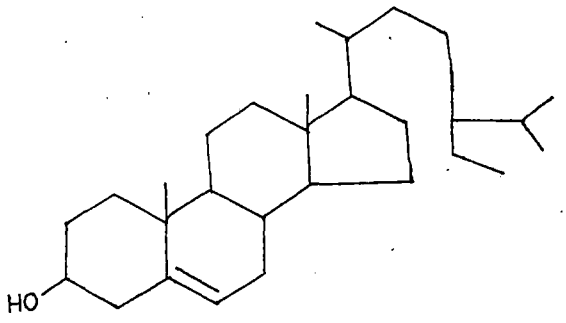
(1)



(2)

$CH_3-(CH_2)_{24}-CH_2OH$

(3)



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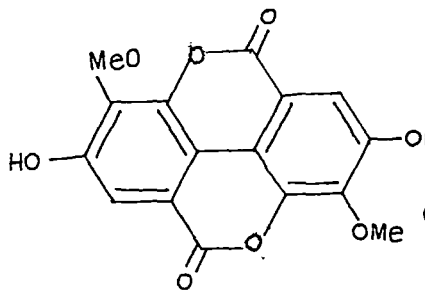

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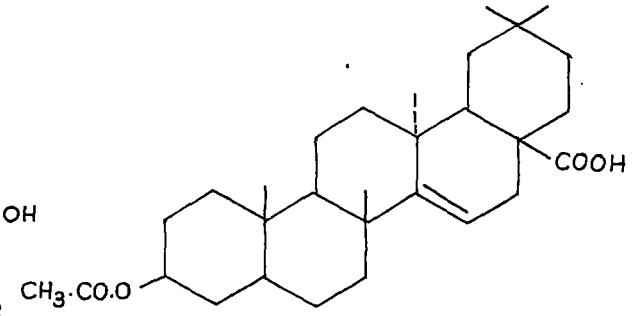
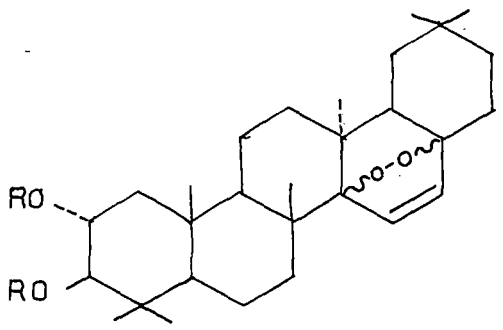


(6)

Further investigations on the more polar fractions of the neutral portion of the same extract afforded a new nor-triterpene peroxide, $C_{29}H_{46}O_4$ which was named baccatin (7a)[7,8].



(7a) R = H

(7b) R = $COCH_3$

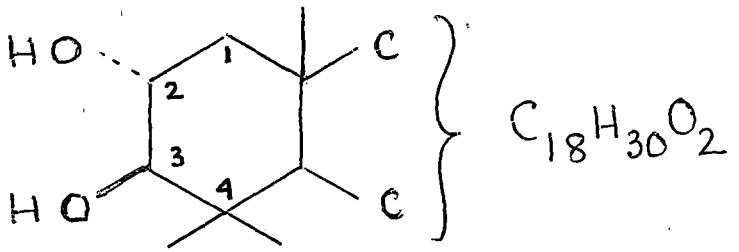
A short review on the structure elucidation[7,8] of baccatin (7a) is given below:

Baccatin (7a), $C_{29}H_{46}O_4$ (M^+458), m.p. 228-29° (d), $(\alpha)_D$ -9.09° showed IR absorption at ν_{max}^{KBr} 3360 (OH), 2970, 1453, 1389, 1369 (gem dimethyl) and 890, 875 cm^{-1} (CH = CH) and did not show any U.V. absorption in the region 220-300 nm. It readily formed an acetate (7b), $C_{33}H_{50}O_6$ (M^+542), m.p. 213-15°, $(\alpha)_D$ 47.5°, IR $\nu_{max}^{CHCl_3}$ 1737 ($OCOCH_3$), 1467, 1453, 1389, 1369 (gem dimethyl), 1245 ($OCOCH_3$) and 895-872 cm^{-1} (-CH = CH-) but no hydroxyl peak in the region 3650-3100 cm^{-1} and also did not show any U.V. absorption in the region 220-300 nm. These data indicated that baccatin (7a) contained two easily acetylable hydroxyl groups.

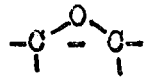
The position and stereochemistry of the two hydroxyl groups were established[7,8] from a study of NMR spectrum of baccatin (7a) and its diacetate (7b). The NMR spectrum (100 MH_Z) of baccatin (7a) showed signals at δ 0.88-1.18 (7 tert methyl groups), two doublets at δ 2.16 and 2.20 and at δ 2.28 and 2.32 (two - OH groups), an unsymmetrical doublet at δ 3.22 and 3.30 (H-C-OH), a quartet of doublets at δ 3.86, 3.95, 4.01 and 4.04 (H-C-OH) and another quartet at 6.42, 6.52, 6.71 and 6.81 (AB quartet, $-\overset{|}{C}-CH = CH-\overset{|}{C}-$). The NMR spectrum (100 MH_Z) of the diacetate (7b), showed peaks at δ 0.88-1.025 (7 tert-methyl groups), 1.99 and 2.055 (6H, 2-O-CO-CH_3), an unsymmetrical

doublet at δ 4.66 and 4.76 (H<u>C</u>-OCOCH$_3$, J = 10 H$_z$), a quartet of doublets at δ 5.01, 5.04, 5.13 and 5.16 (H<u>C</u>-OCOCH$_3$, J = 10 H$_z$ and 10.5 H$_z$) and another quartet at δ 6.40, 6.49, 6.675 and 6.75 (AB quartet, -<u>C</u>-CH = CH-<u>C</u>-). The NMR spectra of baccatin (<u>7a</u>) and its diacetate (<u>7b</u>) were explained[7,8] by assuming that the two hydroxyl groups were present in the diequatorial 2α, 3β-configuration as shown in the partial structure (<u>8</u>). It was argued that the downfield shift in the NMR spectra of the doublet at δ 3.22 and 3.30 and the quartet of doublets in the region δ 3.86-4.01 of baccatin (<u>7a</u>) to δ 4.66 and 4.76 for the doublet and in the region δ 5.01-5.16 for the quartet of doublets, respectively, in its diacetate (<u>7b</u>) was characteristic of protons attached to carbons bearing hydroxyl functions. The doublet at δ 3.22 and 3.30 in the spectrum of baccatin (<u>7a</u>) was thought of as arising from the splitting of the C$_3$-H signal by the proton on C$_2$. In the diacetate (<u>7b</u>) this doublet was shifted to δ 4.66 and 4.76 (J = 10 H$_z$). It was further interpreted that the signal due to C$_2$-H likewise splitted into a doublet by C$_3$-H and this doublet then splitted into a quartet of doublets by coupling with the two geminal protons on C$_1$ and appeared in the region δ 3.86-4.06 in the case of baccatin (<u>7a</u>) and in the region δ 5.01-5.16 in the case of the diacetate (<u>7b</u>). Citing examples from triterpenoid fields[9,10] it was further proposed that this part of the spectrum corresponded to the X-part of the ABXY -type of spectrum

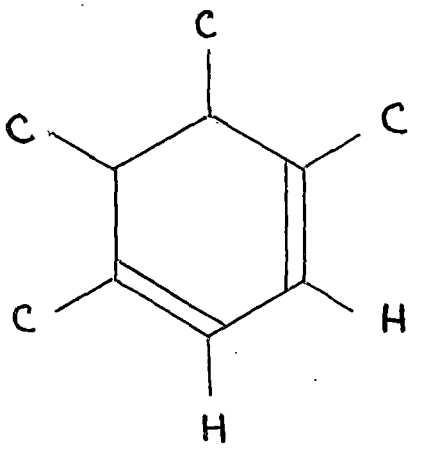
similar to those observed in analogous cases. Moreover, the 10 H_z coupling constant between the C_2 and C_3 protons implied a trans -diaxial arrangement for these two protons and hence a trans-di-equatorial, i.e., a 2α, 3β-configuration for the two hydroxyl groups. These observations established the partial formula (8) for baccatin (7a) corresponding to ring A of the compound with gem-dimethyl groups at C_4.



(8)

The diacetate (7b) liberated one atom of iodine for one atom of oxygen when titrated with potassium iodide in glacial acetic acid. This indicated the presence of two active oxygen atoms either in a peroxide linkage of the type -C-O-O-C- or two epoxide linkage of the type  . The primary argument against the presence of the epoxide linkage was that epoxides were stable

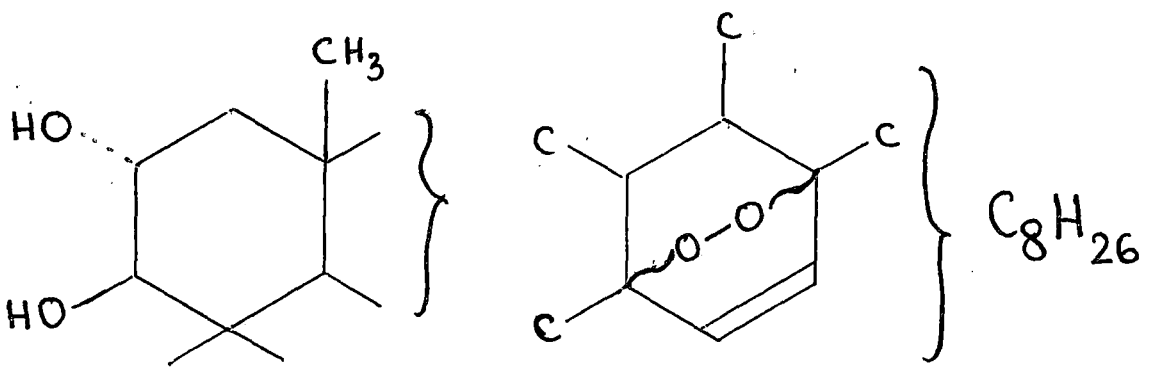
towards alkaline reagents[11,12] whereas baccatin (7a) was found to be very much sensitive to alkali. Thus when baccatin (7a) or its diacetate (7b) was treated with 10% methanolic potash, a new product, $C_{29}H_{46}O_2$ (M^+ 426), m.p. 237-40°, IR ν_{max}^{nujol} 3280 (OH), 1050 and 840 cm^{-1} was obtained. The new compound showed UV absorption at λ_{max}^{MeOH} 282 nm (ϵ, 8300) suggesting that it was a homo-annular conjugated diene as represented by the partial structure (9). The diene, $C_{29}H_{46}O_2$ on acetylation gave a



(9)

diacetate $C_{33}H_{50}O_6$ (M^+ 510), m.p. 226-27°, UV λ_{max}^{MeOH} 282 nm (ϵ, 8030). The NMR spectrum (100 MH_z) of this diacetate showed

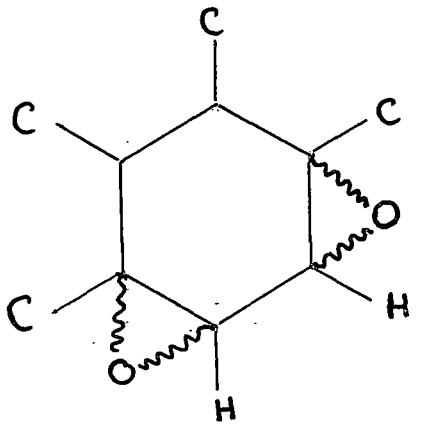
signals at δ 0.9-1.15 (7 tertiary methyl groups), 2.0 and 2.08 (6H, two -O-CO-CH_3 groups), a doublet at 4.7 and 4.8 (H on the acetoxy bearing C-3), a quartet of doublets centred at 5.1 (H on the acetoxy bearing C-2) and a multiplet at 5.58 attributable to two vinyl protons present in the diene system as shown in partial structure (9). From these observations Khastgir et al[7,8] suggested the presence of a perxoy linkage in baccatin (7a). This conclusion was further substantiated by the conversion of an alcoholic solution of the diene diol, $C_{29}H_{46}O_2$ by eosin sensitized photooxidation into a peroxide identical with baccatin (7a). Furthermore they proposed that the presence of abundant peaks at (M^+-32) in the mass spectrum of baccatin (7a) and its diacetate (7b) could also be accounted for by the presence of a peroxide linkage. The peak at (M^+-32) in the peroxide arose from the loss of an oxygen molecule from the parent peak and had been previously reported in case of several peroxides[13-15]. Having thus established the presence of a peroxide linkage in baccatin, they infered that the quartet between δ 6.42 and 6.81 in the NMR spectrum of both baccatin (7a) and its diacetate (7b) arose from a disubstituted double bond of the type $-\overset{|}{C}-CH = CH-\overset{|}{C}-$ present in the same ring as the peroxide moiety. Thus they extended the partial structure of baccatin (7a) to (10).



(10)

Further evidence for the presence of a peroxy linkage in baccatin (7a) was adduced from a study of its expected[12,16,17] rearrangement when a benzene solution of baccatin diacetate (7b) was adsorbed in a column of basic alumina for 48 hours. The rearranged new product, $C_{33}H_{50}O_6$ (M^+ 542), m.p. 263-64°, IR ν_{max}^{nujol} 1720, 1250 ($-OCOCH_3$), 1040 and 920 cm^{-1} showed NMR (100 MH_z) signals at δ 0.9-1.18 (7 tertiary methyl groups), 2.0 and 2.06 (two-$OCOC\underline{H}_3$), symmetrical doublet at 3.00 ($J = 2H_z$, $-C(O)-C\underline{H}-$), symmetrical doublet at 3.5 ($J = 2H_z$, $-C(O)-C\underline{H}-$) unsymmetrical doublet at 4.7, 4.8 (H on the acetoxy bearing C-3) and quartet of doublets at 5.0, 5.03, 5.06 and 5.09 (H on the acetoxy bearing C-2). The significant difference in the NMR spectrum of baccatin

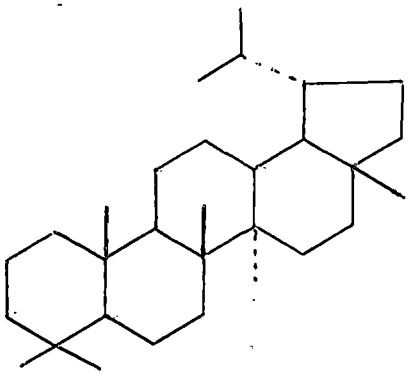
diacetate (7b) and the rearrangement product, $C_{33}H_{50}O_6$ was the absence of the AB type quartet (assigned to $-\overset{|}{C}-CH = CH-\overset{|}{C}-$ group in baccatin) between δ 6.4 - 6.75 in case of the rearranged product. Khastgir et al[7,8] proposed that the double bond and the perxoy linkage had probably been involved in the rearrangement. From a study of the physical data of the rearranged product, $C_{33}H_{50}O_6$ and of its hydrolysis product, $C_{29}H_{46}O_4$, m.p. 242-43°, IR ν_{max}^{nujol} 3340 (OH), 1040 and 920 cm^{-1}; they assigned the partial diepoxide structure (11) to the rearranged product, $C_{33}H_{50}O_6$.



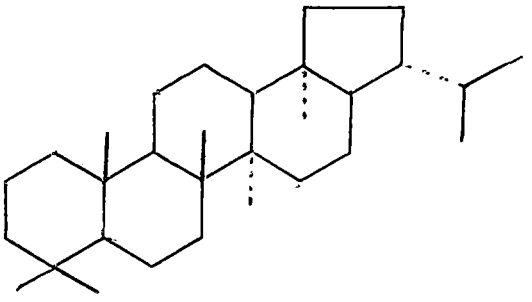
(11)

From the computation of the molecular formula of baccatin (7a) and the diene, $C_{29}H_{46}O_2$, Khastgir et al[7,8] concluded that baccatin (7a) was pentacyclic. The NMR spectrum of baccatin (7a) and its various degradation products showed the presence of seven tertiary methyl groups in each of these compounds. The mass spectrum of these compounds did not show any fragment which could be assigned to the loss of isopropyl or isopropenyl groups. From these observations they suggested that a lupane (12) or hopane (13) type

of nucleus was not possible for baccatin (7a).

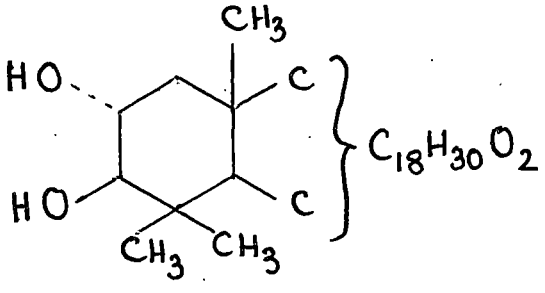


(12)

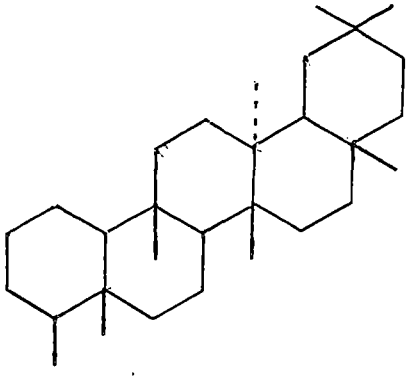


(13)

Ring A of baccatin (7a) was shown from the NMR spectrum to be represented by the partial structure (8). Hence the presence of a friedelane type skeleton (14) was ruled out as it could not explain the ABXY pattern observed in the NMR spectrum of baccatin (7a) and its diacetate (7b).

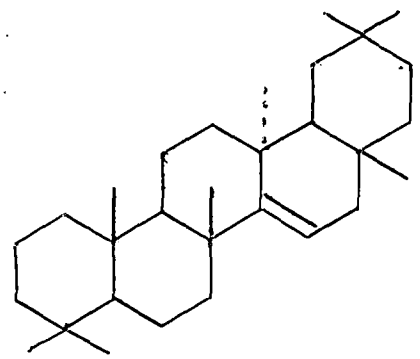


(8)



(14)

Baccatin (7a) was obtained from the benzene extract of the trunk bark and Stem of Sapium baccatum Roxb. along with taraxerone (1), taraxerol (2) and 3-acetoxy aleuritolic acid (6). These three compounds contain the Δ^{14} -taraxerene nucleus (15).



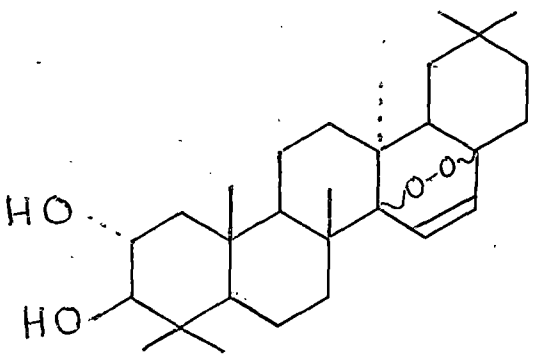
(15)

Therefore, from biogenetic considerations, Khastgir et al[7,8] proposed that the same Δ^{14} - taraxerene type of nucleus (15) might be involved in the formation of the nor-triterpene peroxide, baccatin (7a), in the plant.

From an analysis of the physical data and the partial structure (10) for baccatin (7a) and (9) for the diene, $C_{29}H_{46}O_2$, they concluded that no tertiary methyl groups were present at the peroxide bridge-head.

On the basis of the above considerations, Khastgir et al[7,8] proposed from biogenetic point of view the structure (7a) for

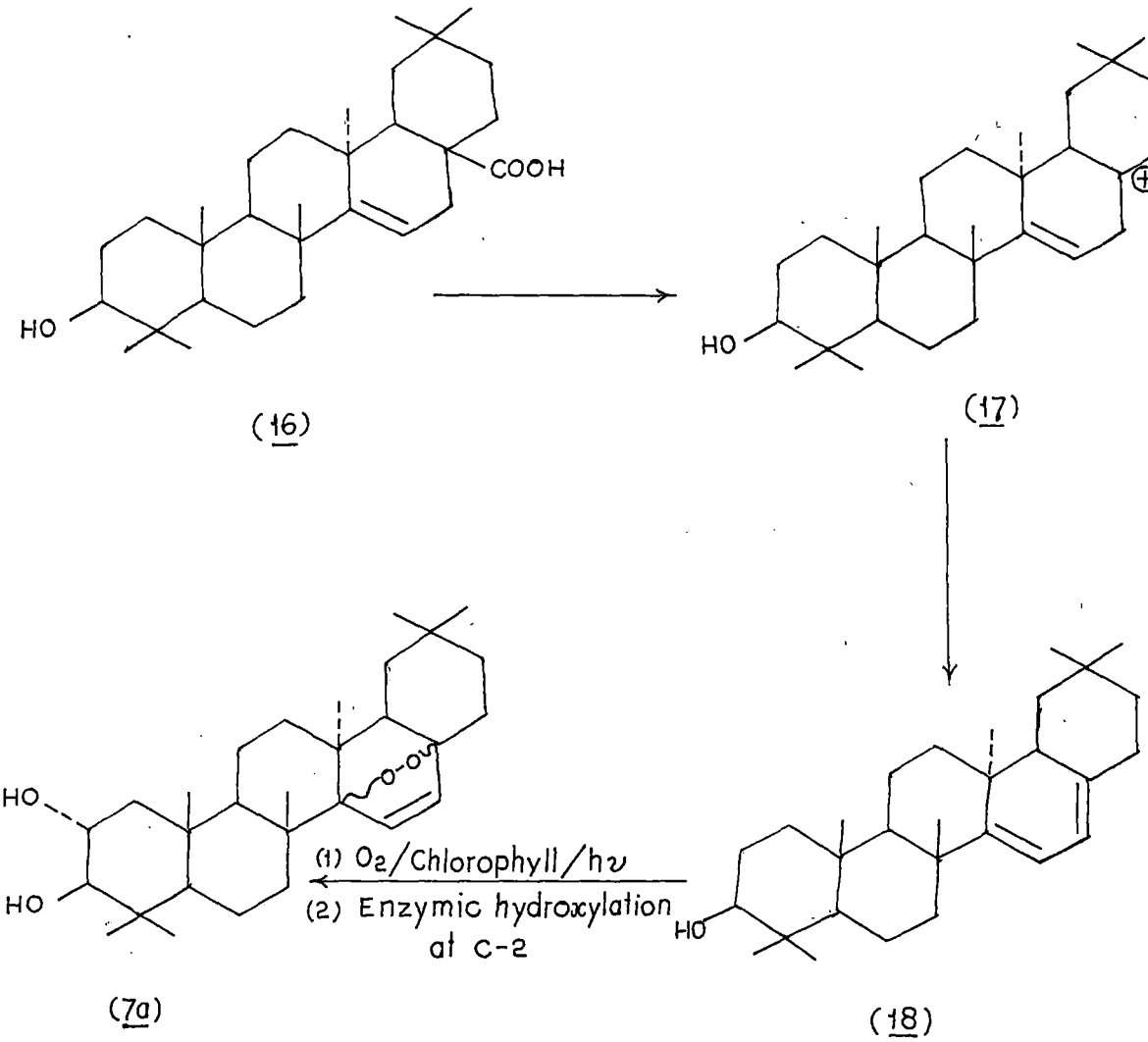
baccatin, which explained all the available chemical and physical evidences in a satisfactory manner.



(7a)

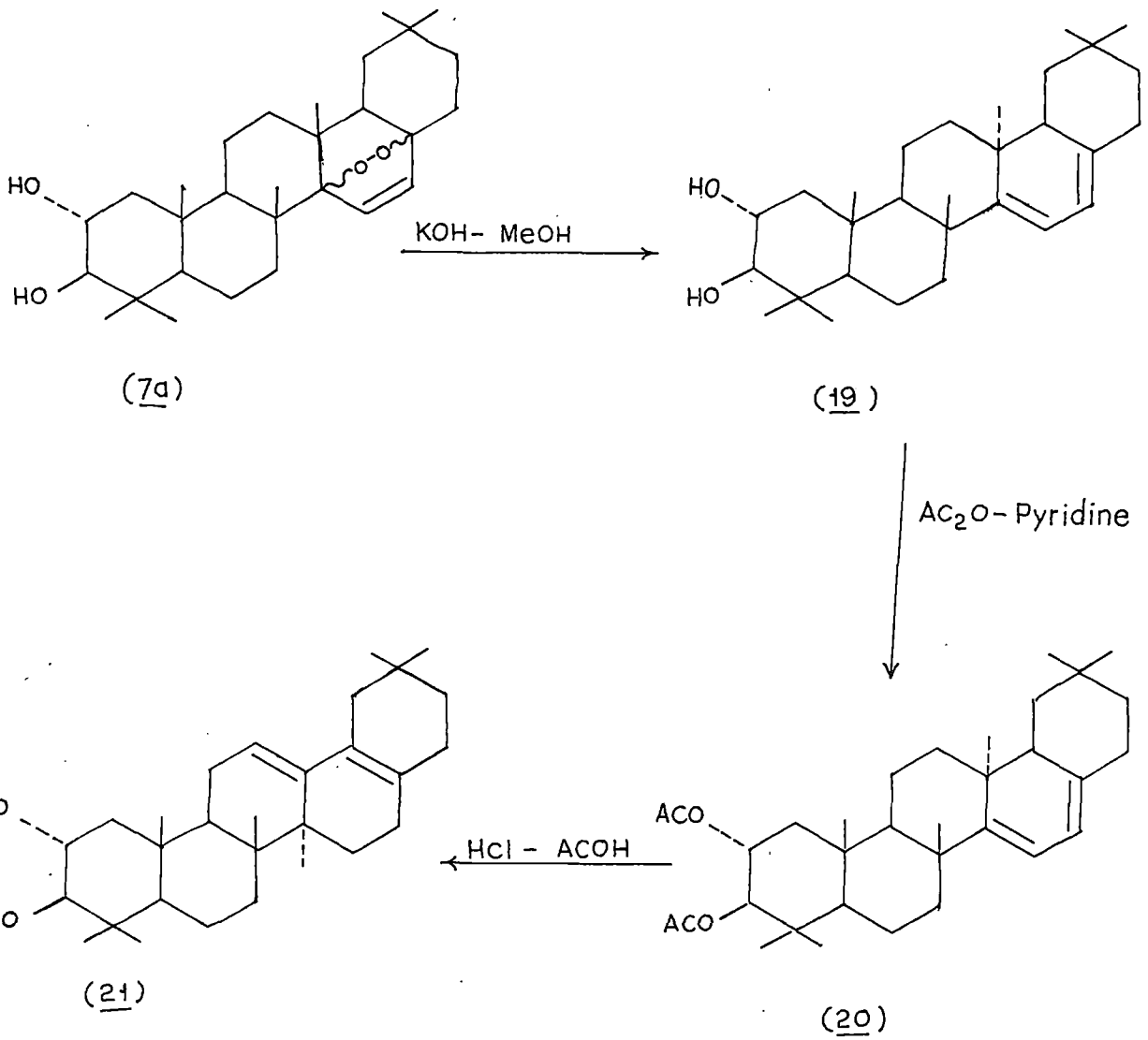
Khastgir et al[7,8] also suggested a biogenetic route for the formation of baccatin (7a) from aleuritolic acid (16) in the plant as shown in Chart-I. According to them decarboxylation of aleuritolic acid (16) in the plant took place generating the carbonium ion (17) which readily eliminated a proton to form the homoannular conjugated diene (18). Photooxidation and enzymic hydroxylation of (18) then gave rise to the nortriterpene peroxide, baccatin (7a).

Chart-I



(16) (17) (18) (7a)

Treatment of baccatin (7a) with methanolic alkali gave the homoannular conjugated diene, $C_{29}H_{46}O_2$, (19) and this on acetylation afforded the diene-diacetate, $C_{33}H_{50}O_4$ (20). Treatment of the latter with a mixture of hydrochloric acid and acetic acid gave a compound, $C_{33}H_{50}O_4$, m.p. 189-90°. The U.V. spectrum of this compound showed absorptions at λ_{max}^{MeOH} 237 (ϵ, 27,000), 244 (ϵ, 28,300) and 252 nm (ϵ, 20,200) thereby suggesting the presence of a heteroannular conjugated diene system in the rearranged product. The NMR spectrum (100 MH_z) of the rearranged product showed signals at δ 0.85 - 1.14 (7 tertiary methyl groups), 1.96, 2.0 (6H, 2-OCOCH_3) unsymmetrical doublet at 4.64, 4.75 (H on the acetoxy bearing C-3), quartet of doublets at 4.95, 5.03, 5.08, 5.2 (H on the acetoxy bearing (C-2) and 5.46 (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 Khastgir et al[7,8] assigned the structure 2α, 3β-diacetoxy-28-nor oleana-12,17-diene, (21) for the rearranged product.



(7a)

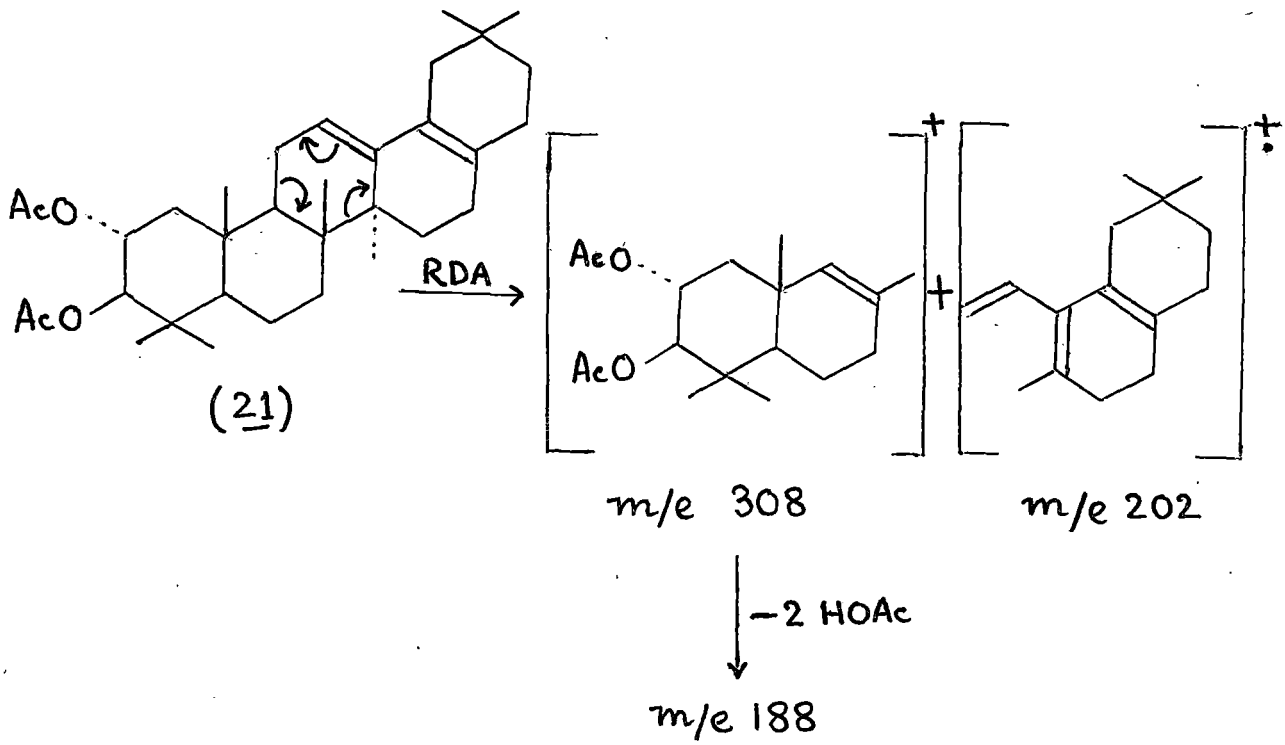
(19)

(21)

(20)

The mass fragmentation pattern of the rearranged product (21) was explained as shown in Chart-II.

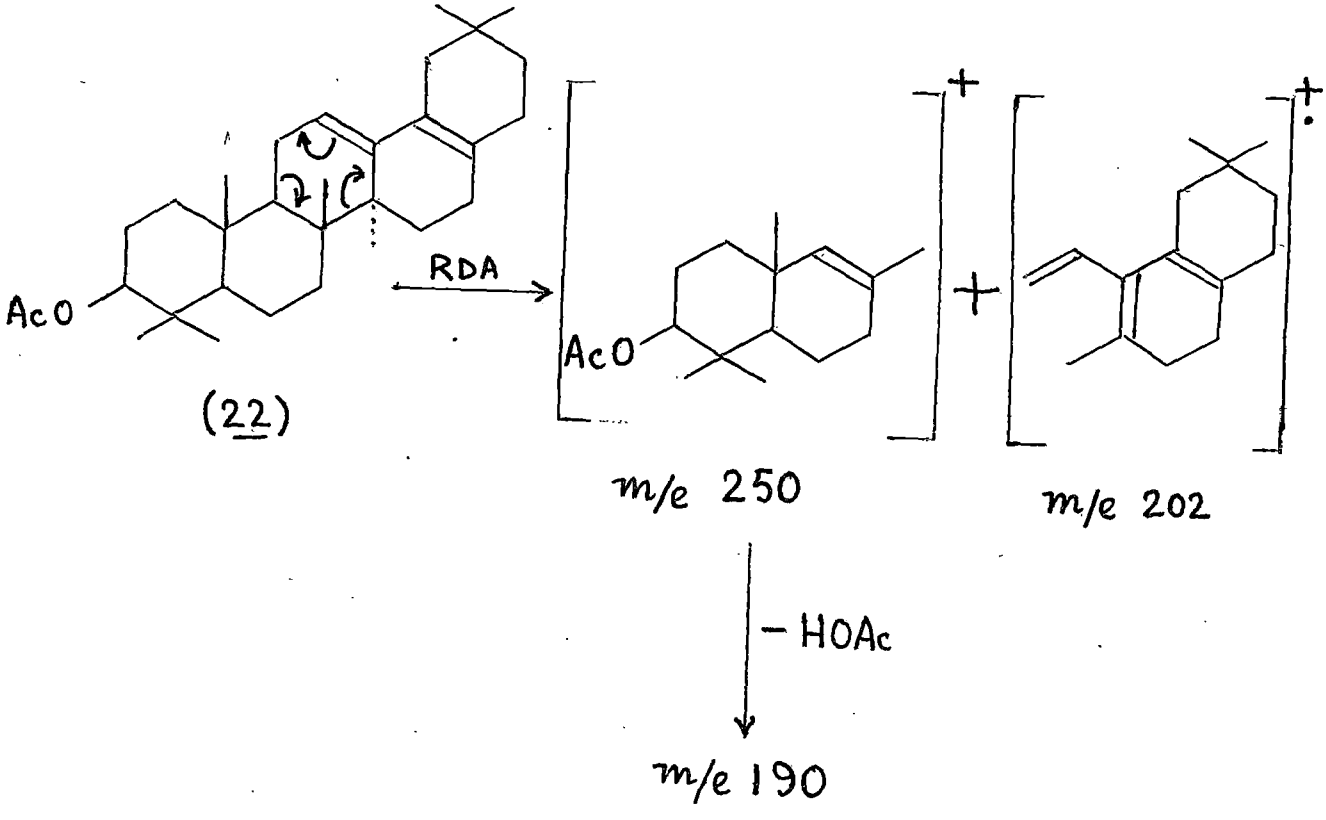
Chart-II



In accordance with the known mass fragmentation pattern of Δ^{12}-oleanenes[18] Khastgir et al[7,8] proposed that the fragments at m/e 308, 202 and 188 were diagnostic of the system shown in

(21). In order to correlate the above observation, they also prepared the compound (22) having a similar heteroannular dienic system starting from acetyl oleanolic acid. They argued that if the peaks at m/e 202 and 188 were indeed diagnostic of structure (21), then the compound (22) should also be expected to exhibit analogous peaks at m/e 202 and 190 corresponding to the fragmentation shown in Chart-III.

Chart-III

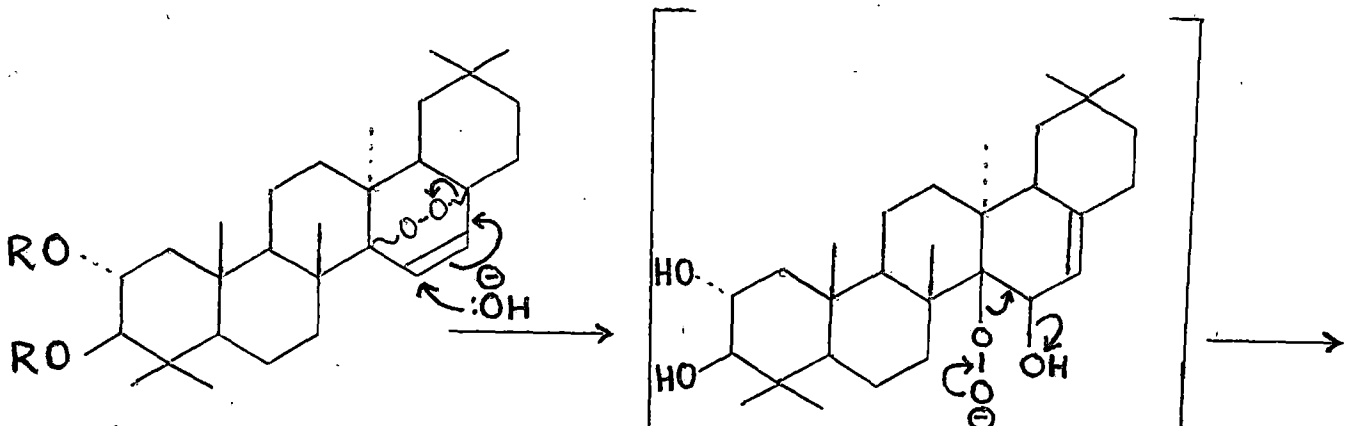


(22)

In accordance with their expectation, the mass spectrum of (22) showed prominent peaks at m/e 452 (M^+), 437 (M^+-15), 392 (M^+-60), 377 (M^+-60-15), 202 and 190. Thus by comparison of the mass spectra of compounds (21) and (22) Khastgir et al[7,8] confirmed the structure (21) for the rearranged diene diacetate. This in turn confirmed the structure (19) for the homoannular diene, $C_{29}H_{46}O_2$ obtained by the alkali treatment of baccatin (7a). Thus they concluded that baccatin must have the structure (7a) and its diacetate (7b).

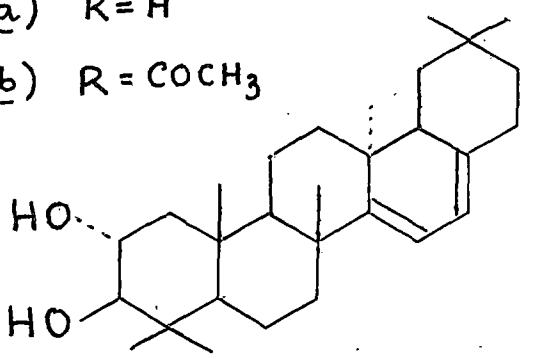
Khastgir et al[7,8] have also proposed a probable mechanism for the transformation of baccatin (7a) or its diacetate (7b) to the homoannular diene (19) by treatment with methanolic alkali as shown in Chart-IV.

Chart-IV



(7a) R = H

(7b) R = $COCH_3$



(19)

An examination of the Dreiding model of (7a) or (7b) with a β-peroxide linkage showed that both rings C and D assumed rigid boat conformation with severe interaction between the hydrogen atoms at C-12 and C-19. In the diene (19) ring C took up half-chair conformation and ring D became almost flat with complete disappearance of the above interactions. They suggested that most probably this relief of strain facilitated the transformation of the peroxide (7a) or (7b) to the homoannular diene (19).