CHAPTER II

Investigation on the neutral part of Sapium Baccatum Roxb.

Isolation of taraxerone, taraxerol, β-sitosterol, 1-hexacosanol and a new nor-triterpene $C_{29}H_{46}O_4$ from the neutral benzene soluble part and 3,3'-di-O-methyl ellagic acid from the benzene insoluble part of trunk bark and stem of Sapium Baccatum Roxb. are described.

Section A : Extraction

Dried and powdered trunk bark and stem of Sapium Baccatum Roxb. was extracted with benzene. On cooling the benzene extract to room temperature, a yellow benzene insoluble solid separated out and was collected by filtration. The chemistry of this benzene insoluble solid is described in Section H. The clear filtrate was concentrated by distilling off benzene when a gummy residue was obtained. The residue was extracted with ether and the ether extract was washed with aqueous sodium hydroxide solution and then with water till it was neutral. The ether solution was dried over anhydrous sodium sulphate and the ether evaporated when a gummy residue was obtained. The gummy residue which constituted the neutral portion was chromatographed and the following fractions were isolated.

Section B : Chromatography of the neutral part

The above gummy neutral part was chromatographed over deactivated alumina and the following fractions were collected.

Table I

Fraction No.	Eluent	Eluate	M.p. of the residue
1	Petroleum ether	Solid with oil	230-6°
2	Petroleum ether: benzene (4:1)	Solid	70-3°
3	Petroleum ether: benzene (3:2)	Solid	265-68°
4	" (1:1)	Solid	128-32°
5	Benzene: ether (1:1)	Solid	210-15°

Section C : Examination of fraction No. 1 (Table I) isoletion and identification of taraxerone ~~152~~

Fraction No. 1 (table I) on rechromatography over alumina and several crystallisations from chloroform and methenol mixture furnished shining crystals which had a constant melting point 238-40°, $(\alpha)_D$ + 10.8°. Elemental analysis and mass spectrum showed the molecular formula of the compound to be $C_{30}H_{48}O$ (M^+ 424). It developed a yellow color with tetranitromethane indicating the presence of a double bond in the compound. It gave a violet coloration in Liebermann Burchardt reaction end gave a positive test in Zimmermann color test showing that the compound is a triterpene ketone, the keto group being at C-3 position.

The compound gave a yellow dinitrophenyl hydrazone derivative, $C_{36}H_{52}O_4N_2$, m.p. 271-3°, showing that the oxygen atom was present as a carbonyl group. The infrared spectra of the compound showed peaks at 1705, 822 cm^{-1} indicating that the carbonyl group is present as a six membered ring ketone. The peaks at 822 cm^{-1} showed the presence of a trisubstituted double bond. The compound showed UV absorption λmax 286 mμ, ϵ = 82.3, showing that the keto group and the double bond were unconjugated.

NMR spectrum of the compound showed the presence of seven methyl groups. The presence of one vinyl proton was indicated by the signal at 5.45 ppm (multiplet). Lithium aluminium hydride reduction of the ketone yielded an alcohol 160, $C_{30}H_{50}O$, m.p. 278-80°, $(\alpha)_D$ + 3.7°, which on acetylation gave the acetate $C_{32}H_{52}O_2$, m.p. 295-7°, $(\alpha)_D$ + 9.16°. The acetate was found to be identical with an authentic sample of taraxeryl acetate 161 (m.m.p., IR and R_f values). Hence the compound isolated from the plant is taraxerone and was found to be identical with an authentic specimen of taraxerone 159 (m.m.p. and IR). Acid isomerisation of the ketone gave a fine needle shaped crystals $C_{30}H_{48}O$, m.p. 175-7°, $(\alpha)_D$ + 105.6° and was found to be identical with an authentic sample of β-amyrone 107.

Section D : Examination of fraction No. 2 (Table I) :
Isolation of 1-hexacosanol

Fraction 2 of the chromatogram on rechromatography and crystallisation from petroleum ether afforded a waxy solid $C_{26}H_{54}O$, m.p. 78-9°, $(\alpha)_D$ ± 0°. Infrared spectrum of the compound showed a broad

peak at 3350 cm^{-1} indicating the presence of a hydroxyl group. It did not show any absorption in the UV spectrum. Acetylation of this alcohol furnished an acetate $C_{28}H_{56}O_2$, 68-69°, $(\alpha)_D \pm 0°$. This was found to be identical with an authentic sample of 1-hexacosanol acetate (m.m.p. and IR comparison). From the above facts, it was evident that the original alcohol was 1-hexacosanol.

Section E : Examination of fraction 3 (Table I) : Isolation and identification of taraxerol ~~160~~

Fraction 3 of the chromatogram on rechromatography and crystallisation from chloroform-methanol mixture afforded an alcohol $C_{30}H_{50}O$, m.p. 278-80°, $(\alpha)_D$ + 73.70. On acetylation with acetic anhydride and pyridine mixture it afforded an acetate $C_{32}H_{52}O_2$, m.p. 295-97°, $(\alpha)_D$ + 9.18°. On oxidation with cromium trioxide-pyridine complex the alcohol afforded a ketone $C_{30}H_{48}O$, m.p. 239-40°, $(\alpha)_D$ + 10.8°. The ketone was identified as taraxerone ~~152~~ by direct comparison with an authentic sample of taraxerone. The acetate was identical with taraxeryl acetate by mixed m.p. and comparison of IR spectra with an authentic sample of taraxeryl acetate. Hence the alcohol m.p. 278-80°, $(\alpha)_D$ + 3.7° was identified as taraxerol.

Section F : Examination of fraction 4 (Table I) : Isolation and identification of β-sitosterol 5

Fraction No. 4 on chromatography and on crystallisation from a mixture of chloroform and methanol mixture gave crystals of m.p. 136-7°, $(\alpha)_D$ - 32°. Elemental analysis corresponded to the molecular

formula $C_{29}H_{50}O$. On treatment with acetic anhydride and pyridine, it afforded an acetate $C_{31}H_{52}O_2$, m.p. 127-29°, $(\alpha)_D$ - 40°. The acetate was identified as β-sitosterol acetate 72 by direct comparison with and authentic specimen of α-sitosterol acetate. Hence the parent alcohol was identified as β-sitosterol 71.

Section G : Examination of fraction 5 : (table I) : Isolation of a new nor-triterpene $C_{29}H_{46}O_4$, m.p. 228-9° $(\alpha)_D$ - 9.09° and investigation on its structure

Fraction No. 5 (Table I) on rechromatography and crystallisation from methanol furnished fine needle-shaped crystals having m.p. 228-29° (decomp.), $(\alpha)_D$ - 9.09°. Elemental analysis and mass spectrometric determination closely corresponded to the molecular formula $C_{29}H_{46}O_4$ (M^+ 458). Infrared spectrum of the compound showed peaks at 3360 (broad, hydroxyl group), 2970 (broad, $-CH_2-$), 1467, 1453, 890, 875 (-CH=CH-), 1389, 1369 (gem dimethyl) cm^{-1}. It did not show any UV absorption in the region 220-300 mμ.

On acetylation with pyridine and acetic anhydride, the compound furnished needle shaped crystals m.p. 213-15°, $(\alpha)_D$ + 47.5°. Elemental analysis and mass spectra of the compound suggested the molecular formula $C_{33}H_{50}O_6$ (M^+ 542). This fact suggested that a diacetate has been formed during acetylation. Infrared spectrum of the acetate had peaks (in chloroform) at 1737 ($-O-COCH_3$), 1467, 1453 (-CH=CH-), 1245 ($-OCOCH_3$), 895-872 (-CH=CH-) cm^{-1} but no hydroxyl peak in the region 3000-3600 cm^{-1}.

NMR spectra of the diol (fig. 20) showed peaks at 0.88, 0.91, 0.95, 1.04, 1.06, 1.14 ppm for seven tertiary methyl groups, two doublets at 2.16 and 2.2 ppm and at 2.28 and 2.32 ppm (two -OH groups), at 3.22 and 3.30 ppm (1H, H-C-OH) and a quartet of doublets at 4.00 ppm (-CH_2-) and another quartet at 6.42, 6.52, 6.72 and 6.80 ppm (AB quartet, -CH=CH-). The diacetate had NMR peaks (fig. 21) at 0.885, 0.93 (6H), 0.96, 0.98, 1.01, 1.025 ppm for seven methyl groups on saturated carbon atoms at 1.99 and 2.055 ppm (6H, 2-O-$COCH_3$), at 4.70 and 4.80 ppm (1H, H-CO-$COCH_3$) at 6.40, 6.49, 6.67, 6.75 ppm (AB quartet, disubstituted double bond, -CH=CH-).

Discussion: Elemental analysis and mass spectral data of the alcohol and its acetate closely corresponded to $C_{29}H_{46}O_4$ and $C_{33}H_{50}O_4$ respectively. Since the compound affords a diacetate at least two oxygen atoms are present as hydroxyl groups which are acetylable. Furthermore, the diacetate does not show any peak in the region 3000-3600 cm^{-1} (fig. 22) in the IR spectrum, which is indicative of the absence of any other hydroxyl group. This has further been proved by the following two chemical reactions (a) chromic anhydride pyridine oxidation and (b) phosphorus oxychloride-pyridine dehydration on the diacetate. Both the reactions gave back the original acetate m.p. 213-15°. Hence the diacetate does not contain any other hydroxyl group. UV spectra of the alcohol and its acetate did not show any absorption in the region 220-300 mμ. From this fact coupled with the IR data (no absorption in carbonyl region) it can be deduced that no carbonyl group is present in the alcohol.

The nature of the hydroxyl groups in the alcohol have been found to be in diequatorial 2α, 3β-configuration, by examination of NMR spectra of its acetate. In the acetate the proton at C-3 gives rise to a signal near 4.80 ppm which is split into an unsymetrical doublet (J, 10Hz) by the proton at C-2. This 10 Hz coupling between these two protons implies a trans diaxial arrangement of the C-2 and C-3 protons[2]. The C-2 proton is further coupled to the methylene at C-1 and the signal for it is discerned as a quartet of doublets at 5 ppm (X part of ABXY). A similar pattern is observed in methyl maslinate diacetate, methyl alphitolate[3] and was recorded for other triterpenoids with identical ring A.

The next problem was to ascertain the nature of the other two oxygen atoms present in the alcohol. In the mass spectrum of the alcohol and its diacetate a prominent peak at 426 (M^+ -32) and at 510 (M^+-32) was observed respectively. This peak might have resulted by the loss of one molecule of methyl alcohol and the presence of the grouping $-CH_2-O-$ might be inferred. This was ruled out as there was no signal in the NMR spectra of the alcohol and the diacetate due to the protons associated with the $-CH_2-O-$ grouping. The absence of any peak due to $-OCH_3$ group precludes the possibility of the presence of a $-OCH_3$ group in the compound. The presence of an oxide linkage of the type $-\overset{O}{\overset{|}{C}}-CH$ was also eliminated by the absence of signals due to proton attached to carbon of the oxide linkage.

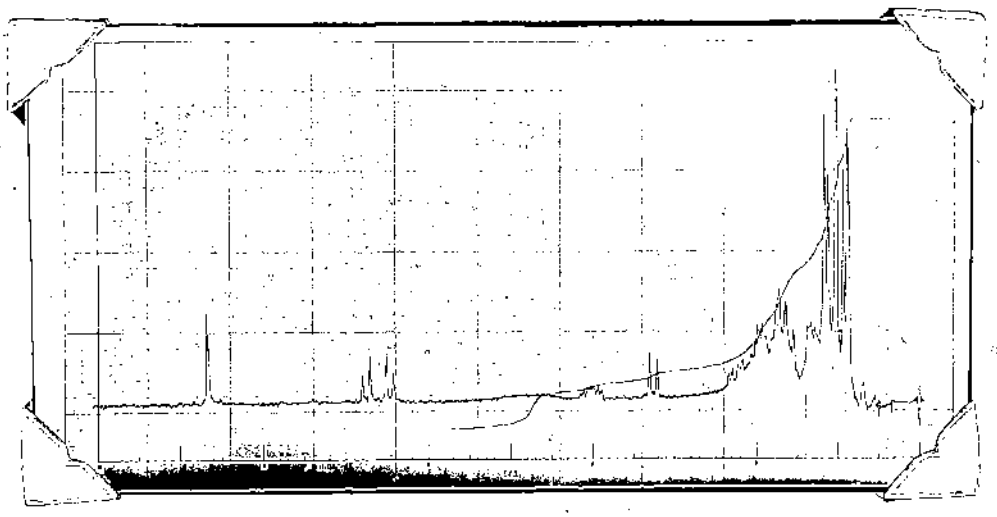


Fig. 2o : NMR spectra of nor-triterpene

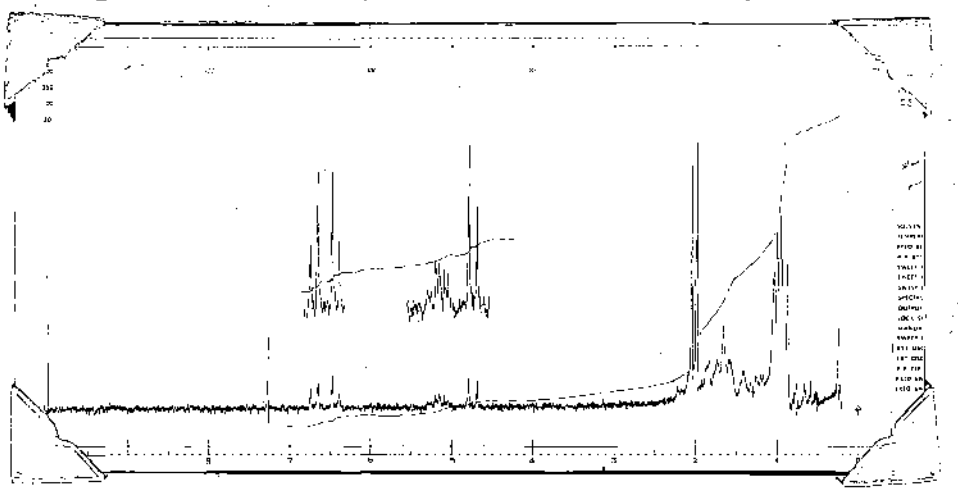


Fig. 21 : NMR spectra of the diacetate of nor-triterpene

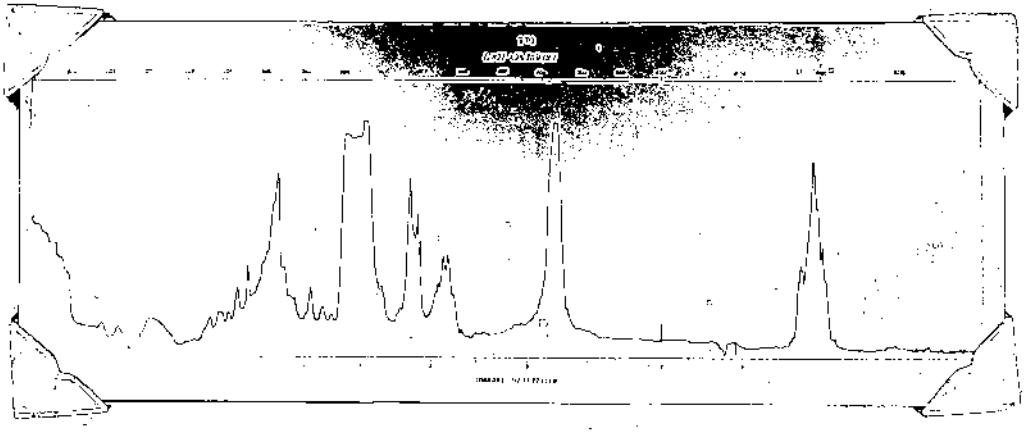
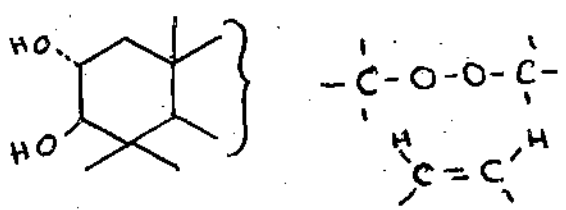


Fig. 22 : IR spectra of nor-triterpene.

The diacetate has been found to liberate one atom of iodine for one atom of oxygen when treated with potassium iodide in glacial acetic acid solution as compared to a similar blank solution. This experimental result seems to be very significant and the presence of a peroxide linkage such as -C-O-O-C- may be envisaged as in the case of ergosterol peroxide[5,6]. The presence of fragments due to loss of mass unit 32 in the spectra of the alcohol and the acetate may be accounted due to loss of two oxygen atoms. Though the NMR spectra of the compound showed the presence of a disubstituted double bond (-CH=CH-) in the molecule, perbenzoic acid titration of tis diacetate did not show any consumption of perbenzoic acid. This anomelous observation might be explained if we assume that one mole of perbenzoic acid is consumed by the compound and an equivalent amount of iodine is liberated during the titration due to peroxide function thus offsetting in the titration value.

On the basis of the foregoing results partial structure 162 may be assigned to the new nor-triterpene.



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Considering the above proposal and the molecular formula $C_{29}H_{46}O_4$ for the original alcohol it may be concluded that the compound is pentacyclic in nature.

Rearrangement during hydrogenation of the diacetate $C_{33}H_{50}O_6$, m.p. 262-63°

The diacetate $C_{33}H_{50}O_6$ on being shaken in an atmosphere of hydrogen in presence of palladium-on-charcoal catalyst gave a compound m.p. 262-63° which in the IR spectrum showed the presence of hydroxyl peak at 3600 cm^{-1} and a peak at 892 cm^{-1} due to trisubstituted double bond. NMR spectrum of the compound (fig. 23) showed peaks at 1.86, 0.885, 0.92 (2 CH_3), 0.96, 0.995 and 1.1 ppm for seven tertiary methyl groups on saturated carbon atoms, peaks at 1.95 and 2.06 ppm (6H, 2-O-CO-$C\underline{H}_3$), at 4.69 and 4.79 ppm (2H, $\underline{H}$-O-$COCH_3$). It also exhibited a peak at 1.9 ppm (-O$\underline{H}$) which disappeared when the spectra was taken after D_2O exchange. The peak at 3.55 ppm sharpening after D_2O exchange was attributed to the proton associated with the hydroxyl group ($\underline{H}$-C-OH). The most significant information provided by the NMR spectra was the disappearance of the symmetrical doublet at 6.40, 6.49, 6.67 and 6.75 ppm (AB, C=C) which was present in the original diacetate. Instead of this a multiplet at 5.9 ppm observed which has been ascribed to one vinylic proton in trisubstituted double bond ($C=C\overset{H}{\diagup}$). The foregoing results clearly indicate that a rearrangement of the molecule had taken place during the process of hydrogenation. Most probably the peroxide linkage in the molecule first opens up to give a compound with two tertiary alcohol functions as depicted below. The latter subsequently

$$-C-O-O-C- \xrightarrow{H_2} -\overset{|}{C}-OH \qquad HO-\overset{|}{C}-$$

undergoes rearrangement with shifting of one of the hydroxyl groups

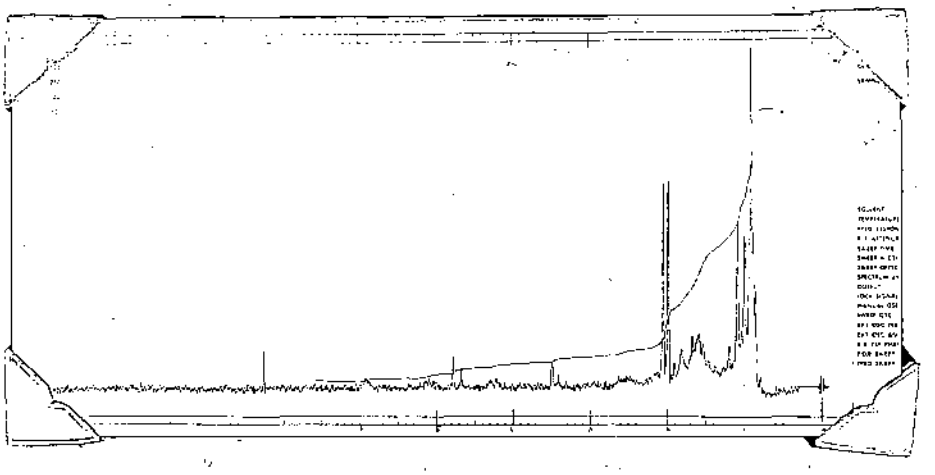


Fig. 23 : N.M.R spectra of the hydrogenated product of the diacetate of nor-triterpene.

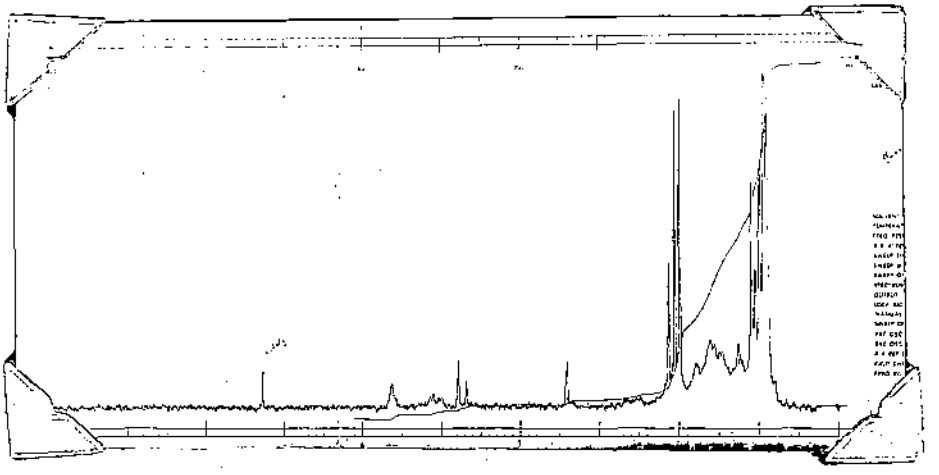


Fig. 24 : NMR spectra of the acetylated product obtained after hydrogenation of diacetate of nor-triterpene.

and the double bond producing a secondary alcohol function and a tri-substituted double bond. It is evident that this double bond is most probably located in a hindered position as it did not get reduced further even after prolonged hydrogenation. Although it is clear that a rearrangement has taken place, further work is necessary to understand the correct nature of this unique type of change.

Acetylation of the rearranged product m.p. 262-63° with acetic anhydride-pyride afforded an acetate derivative m.p. 170°. The latter still showed the presence of hydroxyl group in the IR spectrum ($\nu_{max}^{chloroform}$ 3600 cm^{-1}). NMR spectrum of the compound (fig. 24) showed peaks at 0.88, 0.9, 0.96, 0.98, 0.93 and 1.08 ppm accounting for seven tertiary methyl groups on saturated carbon atoms. Furthermore, it exhibited three peaks at 1.98, 2.08 and 2.12 ppm indicating the presence of three acetyl groups (3-$OCOCH_3$) and peaks at 3.5, 4.68 ppm for protons associated with acetyl groups (3H, H-C-$OCOCH_3$). Formation of this new triacetyl derivative proved that a secondary hydroxyl group was present in the rearranged product. A peak at 5.64 ppm (multiplet) was also observed due to one vinyl proton (tri-substituted double bond).

Lithium aluminium hydride reduction of the nor-triterpene alcohol, $C_{29}H_{46}O_4$

LAH reduction of the alcohol in dioxan afforded a crystalline compound m.p. 302-3° . Acetylation of the latter yielded a compound m.p. 301° , different from the triacetate m.p. 170° described above.

Further work is being pursued actively in these laboratories towards elucidation of the structure of this nor-triterpene.

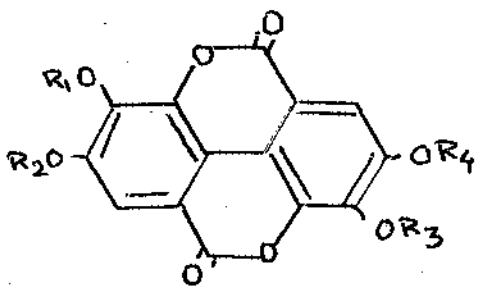
Section H: Investigation on the benzene insoluble part of the benzene extract of Sapium baccatum Roxb. : Isolation and identification of 3,3'-di-O-methylellagic acid

The insoluble solid (Part IV, Chapter I, Section A) that separated out during the benzene extract of Sapium Baccatum Roxb., was dissolved in 10% aqueous sodium hydroxide solution and filtered. The clear filtrate was acidified with dil. hydrochloric acid and kept in frigidaire for three days. The precipitated solid was collected by filtration, dried and crystallised from dimethyl formamide to afford fine needle shaped crystals having m.p. 322-24°. The compound was very sparingly soluble in ethanol, ethyl acetate, acetone but was soluble in dimethylformamide, pyridine and dioxan.

Elemental analysis and high resolution mass spectrometry established the molecular formula as $C_{16}H_{10}O_8$ (M^+ 330). It showed a mass fragmentation pattern as m/e 330 (M^+), 315 ($M^+ - CH_3$), 300 ($M^+ - 2CH_3$). The methoxy group determination showed the presence of two methoxy groups in the molecule. It showed IR peaks at 3300 (broad, -OH), 1722 (lactone carbonyl), 1610, 1590 (aromatic-CH) cm^{-1}. From IR spectra it was evident that the compound contained aromatic nucleus with phenolic hydroxyl group and lactone groups. UV spectra showed peaks at 250, 270 mμ, no shift was observed in UV spectrum when mixed with 0.1M sodium acetate solution.

Methylation of the solid by refluxing with dimethylsulphate and anhydrous potassium carbonate in acetone gave a solid which after crystallisation from dioxan-methanol gave needle shaped crystals m.p. 338-41°, which was identified as tetra-O-methyl ellagic acid 165, by comparison with an authentic sample (m.m.p. and IR comparison) kindly supplied by Prof. T.R. Seshadri. Acetylation of the solid either by refluxing with anhydrous sodium acetate in acetic anhydride or by heating with pyridine-acetic anhydride gave the same diacetate of m.p. 300-302° and this has been identified as 3,3'-di-O-methyl-4,4'-diacetate derivative of ellagic acid 166. This fact established that the compound of m.p. 322-24° isolated from the bark of the plant was 3,3'-di-O-methyl ellagic acid 164. This has been confirmed by direct comparison of the solid with an authentic sample of 3,3'-di-o-methyl ellagic acid (no depression in m.m.p. and superimposable IR).

The compound 164 isolated from the plant gave a light brown coloration with ferric chloride solution[7]. It is reported, that synthetic 3,3'-di-O-methyl ether of ellagic acid[8] does not give a positive ferric chloride coloration[9-11]. The probable reason put forward for this is that the partial methyl ethers of ellagic acid may



163, $R_1 = R_2 = R_3 = R_4 = H$

164, $R_1 = R_3 = Me$; $R_2 = R_4 = H$

165, $R_1 = R_2 = R_3 = R_4 = Me$

166, $R_1 = R_3 = Me$; $R_2 = R_4 = Ac$.

frequently occur along with the acid 163 itself[11], the purification of which is difficult[12]. In this connection, however, it is necessary to mention that no free ellagic acid could be isolated from the plant.

It has long been known that gallic acid undergoes C-C coupling to form hexahydroxy diphenic acid which occurs in nature as complex esters of glucose known as 'ellagetanins'[13]. The hexahydroxy diphenic acid can also undergo internal lactonisation to yield ellagic acid, which is astringent and widely distributed in nature in a number of plants. Besides ellagic acid, its various partial methyl ethers have been isolated from plants e.g. 3,3'-di-O-methyl ellagic acid occurs in Euphorbia Formosona[12], Terminalia Paniculate[14] and Celtis maria[11] 3,3',4'-tri-o-methyl ellagic acid in Eugenia maria[13], 2-O-methyl ellagic acid in Leptospemum scolarium[15] and 3,4-di-O-methyl ellagic acid from Sapium sebiferum Roxb[16]. The recent isolation of gentiobioside of ellagic acid named amritoside from the stem bark of Psedium guava and the ∝-arabinose ester of hexahydroxy diphenic acid from the half ripe fruits of guava along with some gallic acid shows that ellagic acid derivatives are formed from gallic acid precursors[17]. The oxidative coupling of gallic acid to diphenic acid derivatives is very facile chemically as well as enzymatically[18,19]. Three compounds named nasutinus A, B, C have recently been isolated from the hymolymph of the termite, Nasutitermes exitioses[20]. Nasutins B and C are ellagic acid derivatives, the former being 3,3',4-tri-O-methyl ellagic acid and the latter being 3,3'-di-O-methyl ellagic acid.