

PART-III

INVESTIGATION ON THE BENZENE INSOLUBLE PART FROM SAPIUM SEBIFERUM
ROXB. AND ELUCIDATION OF ITS STRUCTURE AS 3,4-DI-O-METHYL ELLAGIC
ACID

CHAPTER-I

ELUCIDATION OF THE STRUCTURE OF THE BENZENE INSOLUBLE SOLID C (PART-I,

CHAPTER-III, P.13) : 3,4-DI-O-METHYL ELLAGIC ACID 1

Section A : Introduction

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 'ellagitanins'¹. 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. For example, 3,3'-di-O-methyl ellagic acid occurs in Euphorbia Formosana², Terminalia paniculata³ and Celtis australis⁴, 3,3',4'-tri-O-methyl ellagic acid in Eugenia maria¹, 3-O-methyl ellagic acid in Leptospermum Scolarium⁵. The recent isolation of gentiobioside of ellagic acid named amritoside from the stem bark of Psidium guava and the α -arabinose ester of hexahydroxy diphenic acid from the half ripe fruits of guava along with some gallic acid shows the ellagic acid derivatives are formed from gallic acid precursor⁶. The oxidative coupling of gallic acid to diphenic acid derivatives is very facile chemically as well as enzymatically^{7,8}. Three compounds named nasutinus A, B, C have recently been isolated from the hymenolymph of the termite, Nasutitermes exitiosus⁹. 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'-dimethyl ellagic acid.

The present author has isolated a new derivative of ellagic acid namely 3,4-di-O-methyl ellagic acid from the bark of Sapium sebiferum Roxb. The details of the work is described below.

Section B : Purification of the insoluble solid C (Part-I, Chapter-III)

The insoluble solid C (Part-I, Chapter-III, p.13) that separated out during the benzene extract of Sapium sebiferum Roxb., was dissolved in 10% aqueous sodium hydroxide solution and filtered. The clear filtrate was acidified with dil. hydrochloric acid and kept in the frigidair^e for seven days. The precipitated solid was collected by filtration, dried and crystallised from dimethylformamide to afford fine needle shaped crystals having melting point above 360°. The compound was very sparingly soluble in ethanol, ethyl acetate, acetone but was soluble in dimethylformamide and dioxan.

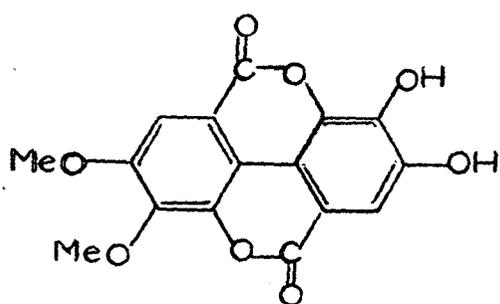
Section C : Structure of 3,4-di-O-methyl ellagic acid

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 3395-3180 (OH, broad), 2990, 1735, 1715 (lactone carbonyl), 1685, 1617, 1608, 1595 (aromatic -CH), 1495, 1437, 1365-1345, 1280, 1210, 1170, 1110, 1070, 990, 985, 920, 870, 790 and 753 cm^{-1} . From the IR spectra it was evident that the compound contained aromatic nucleus with phenolic hydroxyl group and lactone groups. UV spectra showed peaks at 259 (ϵ , 35,582), 350 (inflection) and 410 (ϵ , 10,187) $m\mu$ (Fig.13).

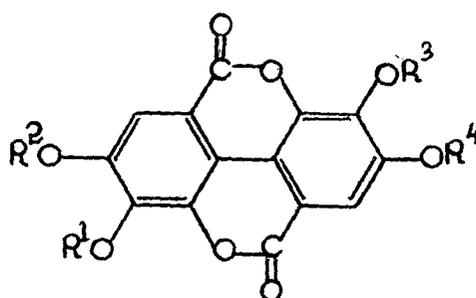
The compound developed a brown colouration with ferric chloride solution characteristic of phenolic compounds. It produced a yellow colour with aqueous sodium hydroxide and sodium carbonate solutions which changed to violet on heating.

On acetylation with pyridine and acetic anhydride method or by sodium acetate and acetic anhydride method it yielded a diacetate, m.p. 302-4°. Elemental analysis showed the molecular formula $C_{20}H_{14}O_{10}$ and acetyl group estimation showed the presence of two acetyl groups in the acetate. It showed UV absorption peaks at 255, 378, 405 m μ and gave no colour with ferric chloride solution. The diacetate was insoluble in alkali but produced a yellow colour on keeping for a long time. On heating, the colour changed to violet possibly due to hydrolysis of the acetate groups. Benzylation of the compound, with benzyl chloride, in presence of potassium carbonate and potassium iodide in acetone furnished a dibenzyl derivative $C_{30}H_{22}O_8$, m.p. 284-5°, λ_{max} 250, 348, 360 m μ . On methylation with dimethyl sulphate in dry acetone in presence of anhydrous potassium carbonate the compound afforded a crystalline solid, m.p. 336-8°. Analytical data indicated molecular formula as $C_{18}H_{14}O_8$. Methoxy estimation showed the presence of four methoxy groups. It was neither soluble in sodium hydroxide solution nor did it show any colouration with ferric chloride solution. It was found to be identical with an authentic specimen of tetra-O-methyl ellagic acid kindly supplied by Prof.T.R.Seshadri. Since the melting point and its UV spectra were completely different from the previously known di-O-methyl ellagic acid derivatives - viz. 3,3'-di-O-methyl ellagic acid 2¹⁰ and 4,4'-di-O-methyl ellagic acid 3¹⁰, it was inferred that the only possible structure for the compound is 3,4-di-O-methyl ellagic acid 1 which has

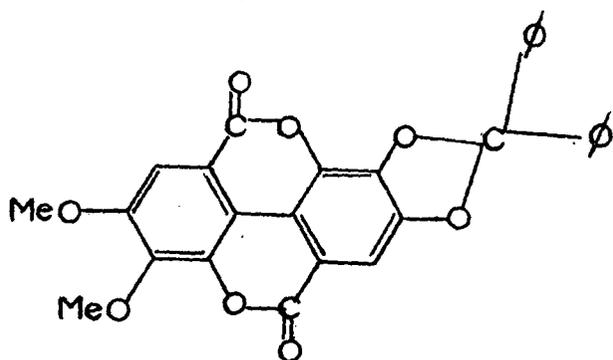
hitherto not been isolated from any plant source nor has it been synthesised. In order to obtain a definite chemical evidence regarding the presence of orthodihydroxy group in the compound we prepared the diphenyl methylene acetal derivative by the method described by O.T.Schmidt¹¹ and L. Jurd¹⁰. The compound when heated with α,α -dichlorodiphenyl methane to 175-8° formed a crystalline solid 6 of m.p. above 360° which depressed the melting point when mixed with the original compound 1. This analysed for $C_{29}H_{18}O_8$ and showed UV absorption peaks at 257, 350 and 362 m μ . From these data the presence of orthodihydroxy group was confirmed and the structure 1 is proposed of the compound.



1



- 2 ($R^1=R^3=Me, R^2=R^4=H$)
- 3 ($R^1=R^3=H, R^2=R^4=Me$)
- 4 ($R^1=R^2=R^3=R^4=H$)
- 5 ($R^1=R^2=R^3=R^4=Me$)



6

The compound 1 also gave a dibenzyl derivative $C_{30}H_{22}O_8$, m.p. 285-6°, UV absorption at 250, 348, 360 m μ .

Ultraviolet absorption data of the compound 1 and the previously known ellagic acid derivatives are given in Table-I below for a comparative study:

TABLE-I

No.	Name	$\lambda_{m\mu}^{max}$	$\lambda_{m\mu}^{max}$
<u>4</u>	Ellagic acid ¹⁰ + NaOAc	255 256, 278	352 ^a , 366 355
<u>3</u>	4,4'-Di-O-methyl ellagic acid ¹⁰ + NaOAc	253 255, 280	246 ^a , 261 356
<u>2</u>	3,3'-Di-O-methyl ellagic acid ¹⁰ + NaOAc	248 No change	359 ^a , 372 No change
<u>1</u>	3,4 - Di-O-methyl ellagic acid + NaOAc + NaOEt + AlCl ₃ + H ₃ BO ₃	259 271 270 247 248	359 ^a , 410 415 435 372 372
	4,4'-Di-O-methyl ellagic acid diacetate	239	354, 368
	3,3'-Di-O-methyl ellagic acid diacetate	248	259, 372
	3,4 -Di-O-methyl ellagic acid diacetate	255	378, 405

^ainflexion

Section D : Discussion of UV spectra

It will be observed that the UV spectra of 4,4'-di-O-methyl ellagic acid¹⁰ and 3,3'-di-O-methyl ellagic acid¹⁰ are quite different from the spectra of 3,4-di-O-methyl ellagic acid isolated from Sapium sibiricum. As expected, sodium acetate caused a bathochromic shift (12 m μ) of the

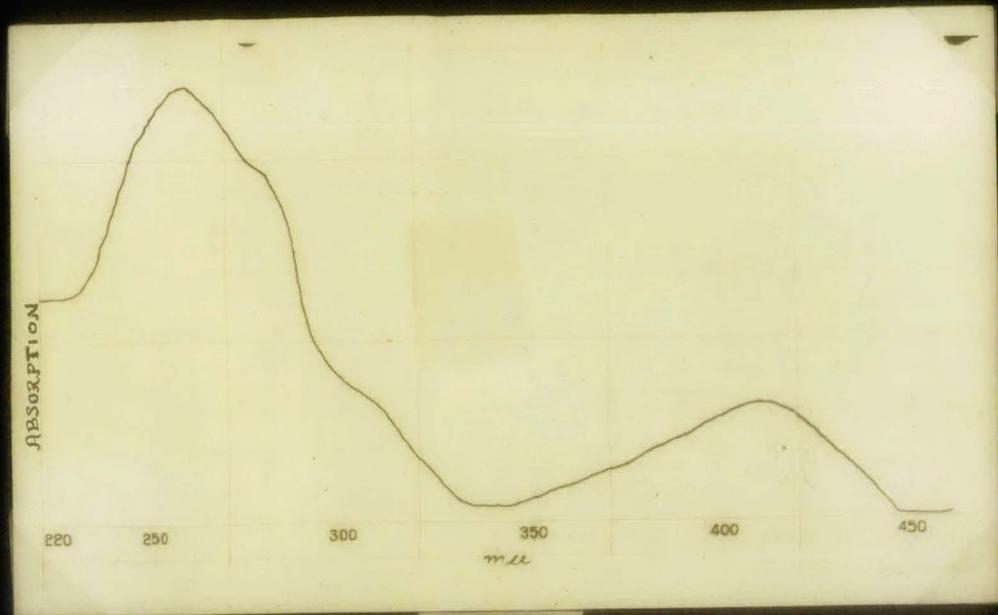


Fig. 13

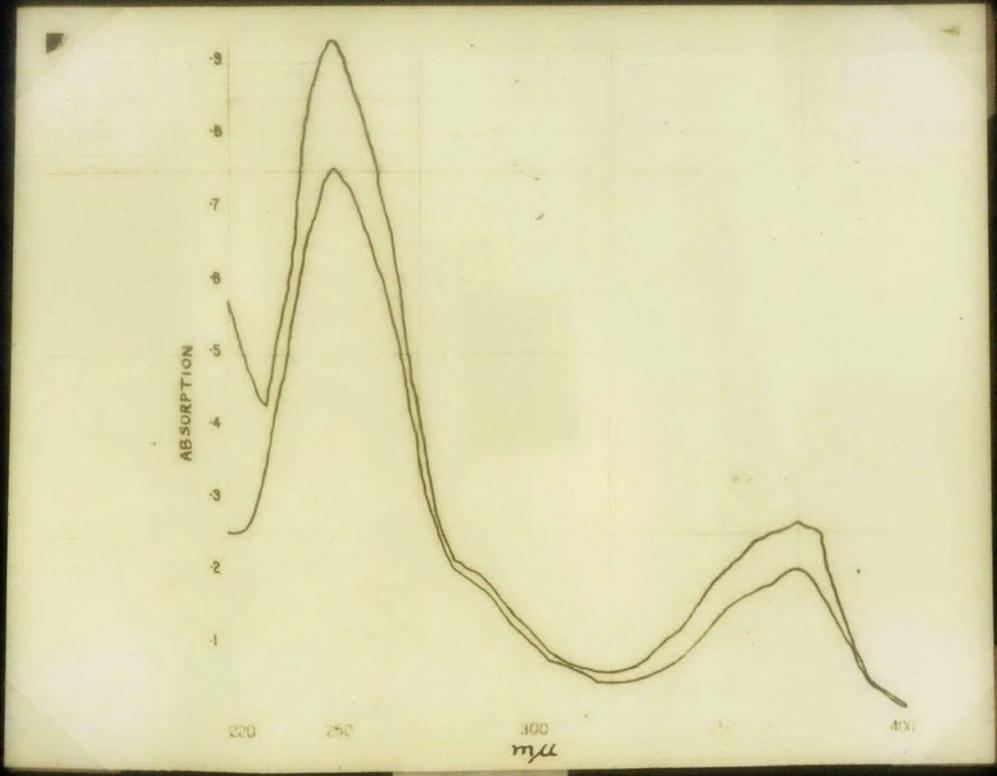
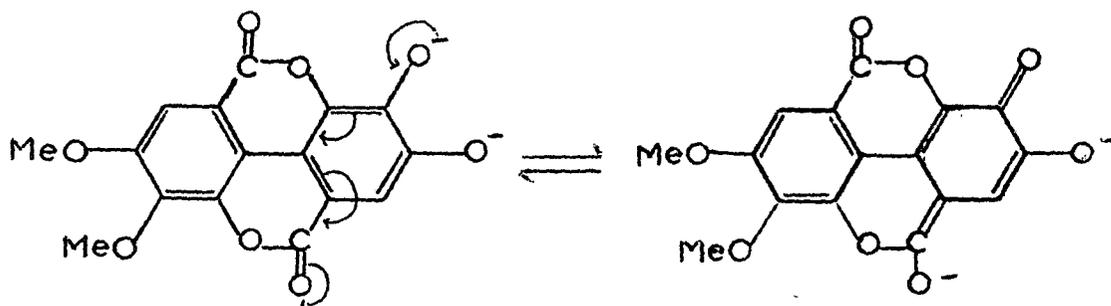
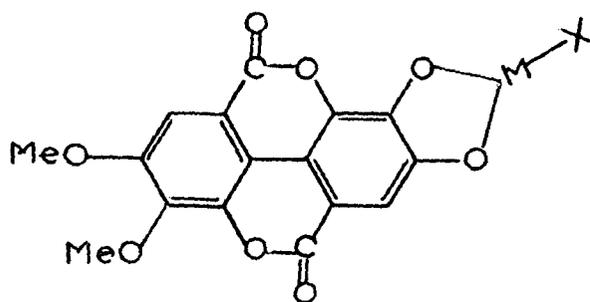


Fig. 14

short wavelength band and 5 m μ of the long wavelength band. Sodium ethylate also showed a similar effect in the UV spectra, the bathochromic shift in the long wavelength band, however, being about 25 m μ . Most probably, sodium ethylate which produces a deep yellow solution with this compound ionises both the 3',4'-hydroxyls giving rise to quinonoidal resonance forms as shown below:



The effect of boric acid and aluminium chloride was studied and their spectra showed a similar pattern (Fig.14). Both the reagents caused a hypsochromic shift of 11-12 m μ in the short wavelength band and 38 m μ in the long wavelength band. The yellow colour of the compound in ethanol solution became colourless when these reagents were added. These effects may be explained by assuming that chelation occurs with these reagents and 1,2-dihydroxy system to form a complex of the type :-



The diacetate of the compound 1 also showed a hypsochromic shift (4-5 m μ) of the short wavelength band and the long wavelength band.

Thus support for the structure 1 for the new compound as 3,4-di-O-methyl ellagic acid is furnished from the above UV absorption spectral data and clearly showed that it was different from the previously known di-O-methyl ellagic acid derivatives. The only other possible alternative structure having di-O-methyl group must be 3,4-di-O-methyl ellagic acid. This is further supported by the negative Griessmayer reaction¹¹ which shows the absence of 4,4'-dihydroxy system in the new compound.