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CHEMISTRY OF *TERMINALIA* SPECIES—III

CHEMICAL EXAMINATION OF *TERMINALIA PANICULATA* ROTH

ISOLATION OF 3,3′-DI-O-METHYL ELLAGIC ACID-4-GLUCOSIDE

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Abstract—A new glucoside of di-methyl ellagic acid has been isolated from the heartwood of *Terminalia paniculata* together with β-sitosterol and a new triterpene carboxylic acid. The glucoside is the 4-mono glucoside of 3,3′-di-O-methyl ellagic acid.

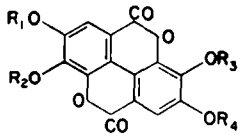
Terminalia paniculata Roth[1] is a common tree found in the forests of Western Ghats of India. The wood is greyish brown, moderately heavy and often used as a substitute for teak. The heart wood is considered ant-proof and durable under water. The wood was extracted with petroleum ether, ether and acetone successively. The petroleum ether extract furnished β-sitosterol identical with an authentic sample. A new triterpene carboxylic acid $C_{30}H_{48}O_4$ was also isolated from both the petroleum ether and ether extracts.

From the acetone extract, a compound (m.p. 214–215°) was isolated which analysed for $C_{22}H_{20}O_{13}$. The solubility in aqueous sodium carbonate and brown colour with ferric chloride, indicated a phenolic compound. Hydrolysis with 4 per cent methanolic sulphuric acid furnished a molecule of glucose* (α_D^{30}, +51·2°; osazone, m.p. 203–205°) and another of an aglucone (m.p. 338–340°) having a molecular formula of $C_{16}H_{10}O_8$. The latter analysed for a dihydroxy dilactone, containing two methoxyl groups. It formed a diacetate and a dimethyl ether, which was insoluble in cold alkali but dissolved in warm alkali and could be reprecipitated on acidification with hydrochloric acid. Its ultra-violet spectrum[2] and its chemical behaviour suggested a close relationship to ellagic acid (I). The dimethyl ether of the aglucone was identical with tetra-O-methyl ellagic acid (IV) and the aglucone could therefore be a dimethyl ether of ellagic acid. The U.V. absorption spectra of the glucoside and its aglucone were unchanged in the presence of sodium acetate strongly suggesting that the free phenolic groups may be in the 4- and 4′-positions.[3] Sodium acetate differentiates between the two pairs of hydroxyls, 3,3′ and 4,4′, the former being more strongly ionizing showing a shift of absorption peaks in the U.V. spectrum. The glucoside should, therefore, possess a 4-hydroxyl and the aglucone a 4,4′-dihydroxy system (II), clearly showing that one of these hydroxyls might have been involved in the glucoside formation. This is borne

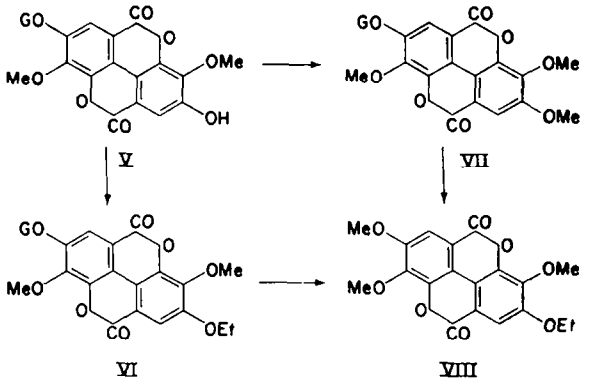
* Mannosides are extremely rare and mannose itself was not noticed in *Terminalia* species. D. E. Hathway (*Biochem. J.*, **63**, 380, 1956) identified eight different sugars by paper chromatography, but not mannose, in the fruit of *T. chebula*.

[1] R. S. Pearson and F. P. Brown, *Commercial Timbers of India* Vol. 1. pp. 525–530. Govt. of India, Central Publications, Calcutta (1932).
[2] L. Jurd, *Chem. & Ind.*, 261 (1959); *J. Amer. Chem. Soc.* **81**, 4610 (1959).
[3] D. E. Hathway, *Nature Lond.* **177**, 747 (1956).

out by the fact that the glucoside gives a monomethyl ether on methylation with diazomethane and later hydrolyses to a trimethyl ether of ellagic acid, identified as 3,3',4'-tri-O-methyl ellagic acid (III) by mixed melting point determination with an authentic sample.* The glucoside is thus a 3,3'-di-O-methyl ellagic acid-4-mono glucoside (V).



I, $R_1=R_2=R_3=R_4=H$
II, $R_1=R_4=H$; $R_2=R_3=Me$
III, $R_1=R_2=R_3=Me$; $R_4=H$
IV, $R_1=R_2=R_3=R_4=Me$



V VI VII VIII

This constitution (V) for the new glucoside is further confirmed by the following series of reactions. The glucoside was ethylated with diethyl sulphate and alkali and the resulting ethyl ether (VI) hydrolysed and methylated to give a tri-O-methyl-mono-ethyl ellagic acid identical with 3,3',4-tri-O-methyl-4'-O-ethyl ellagic acid (VIII). The latter was also obtained by hydrolysing the methyl glucoside (VII) and ethylating the resulting hydroxy tri-O-methyl ellagic acid.

It is known that ellagic acid occurs widely[4] in nature, and particularly in *Terminalia* species.[5] It also occurs as 3,3'-dimethyl ether in the roots of *Euphorbia formosanum* Hey.[6] Its occurrence as a glucoside is now noticed in the heartwood of *T. paniculata* Roth for the first time. It may be noted that 3,3',4-tri-O-methyl ellagic acid was recently isolated from the bark and wood of *Eugenia maire* A. Cunn (Myrtaceae).[7]

EXPERIMENTAL

The U.V. absorption spectra were measured on Hilger Uvispek spectrophotometer in 95% ethanol. The m.p. are not corrected. For analysis samples were dried at 100°/0·05 mm.

Extraction of T. Paniculata *heartwood.* The wood powder (2 kg) was extracted continuously with pet ether (b.p. 40–60°), ether and acetone in a soxhlet extractor.

The light yellow pet ether extract (3 l.) was concentrated to 50 ml and left. The deposited solid was separated into neutral (Compound A; m.p. 135°, yield 1·69 g) and acid (Compound B; m.p. 285–288°, yield 0·5 g) fractions by sodium hydroxide. The ether extract (4 l.), upon concentration deposited a solid identical with Compound B (m.p. 285–288°). The dark brown acetone extract (3 l.) was concentrated to a small volume (50 ml), diluted with ether (300 ml) and kept at 0° for a week, depositing a brown solid, yield 1·5 g and referred to a Compound C.

Examination of compound A: *β-sitosterol.* The compound crystallized from methanol as fine needles (1·5 g) m.p. 136–137°, $[\alpha]_D^{31}$°, −35° (*c*, 1·25 in chloroform), (Found: C, 83·76; H, 12·12 $C_{29}H_{50}O$ requires: C, 84·06 and H, 12·08%). It gives a positive Liebermann-Burchard's reaction for sterols and the m.p. with authentic β-sitosterol was not depressed. The acetate (acetic anhydride–pyridine at 100°, 30 minutes) has m.p. and mixed m.p. 124–126°.

* Kindly furnished by Professor L. H. Briggs.

[4] E. C. Bate Smith, *Chem. & Ind.* B.I.F. Review, R32, (1956).
[5] F. E. King, T. J. King and J. M. Ross, *J. Chem. Soc.* 1333 (1955).
[6] H. Shinoda and C. P. Kun, *J. Pharm. Soc. Japan* **51**, 50 (1931).
[7] L. H. Briggs, R. C. Cambie, J. B. Lowry and R. N. Seelye, *J. Chem. Soc.* 642 (1961).

Examination of compound B. The acidic fraction crystallized from methanol as prisms (420 mg) m.p. 289–290°; $(\alpha)_D^{30°}$, −72° (*c*, 0·677 in chloroform.) (Found: C, 76·35, H, 10·36; $C_{30}H_{46}O_4$ requires: C, 76·30 and H, 10·18%). The acid dissolved in conc sulphuric acid giving a yellow solution changing to red; a pink colour in the Liebermann-Burchard's reaction and light yellow with tetranitromethane. The acid (100 mg) was treated with ethereal diazomethane and the *methyl ester* crystallized from methanol in colourless needles m.p. 212–213°; $(\alpha)_D^{30°}$, −68° (*c*, 1·12 in chloroform). (Found: C, 76·23; H, 10·35, $C_{31}H_{50}O_4$ requires: C, 76·54 and H, 10·28%).

Examination of compound C. Compound C was crystallized from aqueous methanol as light brown prisms m.p. 214–215°. $(\alpha)_D^{30°}$, +79° (*c*, 0·504 in ethanol). It has U.V. absorption at λ_{max} 249 mμ (log ϵ, 4·67), and 373 mμ (log ϵ, 4·07): (Found C, 53·12, H, 4·50, OMe, 10·97, $C_{22}H_{20}O_{13}$ requires: C, 53·60, H, 4·07 and OMe 12·6%). Compound C gives a brown colour with alcoholic ferric chloride and is soluble in sodium hydroxide giving a bright yellow solution.

Compound C (100 mg) was methylated with ethereal diazomethane and the *methyl ether* crystallized from methanol as pale yellow prisms m.p. 205–207°. (Found: C, 53·80, H, 5·01, OMe, 17·5, $C_{23}H_{22}O_{13}$ requires: C, 54·54, H, 4·35 and OMe, 18·38%).

Hydrolysis of compound C: 3,3′-*di*-O-*methyl ellagic acid.* Compound C (200 mg) was refluxed with 4% methanolic sulphuric acid for 6 hr. It was cooled, concentrated *in vacuo* and after dilution with water continuously extracted with ether in a liquid–liquid extractor. Removal of ether deposited a solid which after two crystallizations from dioxan separated as colourless flakes, m.p. 338–340°, not depressed by a synthetic sample of 3,3′-di-O-methyl ellagic acid. It showed U.V. absorption at λ_{max} 249 mμ (log ϵ, 4·75) and 370 mμ (log ϵ, 4·15). (Found: C, 57·8, H, 3·45 OMe, 18·25; $C_{16}H_{10}O_8$ requires: C, 58·2, H, 3·03 and OMe, 18·78%).

The aqueous extract (150 ml) was neutralized with barium carbonate and after removal of inorganic salts was concentrated *in vacuo* to a small volume (20 ml). To one portion, phenyl hydrazine hydrochloride (100 mg) was added and heated for 10 min on a boiling water bath. The light yellow osazone was crystallized from alcohol m.p. 203–205°. Mixed m.p. with glucosazone was undepressed. Ascending paper chromatography of the aqueous solution, with butanol–acetic acid–water (4:1:5) system gave only one spot with R_f value 0·25, with aniline hydrogen phthalate. Under the same conditions, glucose has the R_f value 0·26. In a quantitative hydrolysis of the glucoside (200 mg) with 4% methanolic sulphuric acid, the sugar filtrate after the removal of the aglucone was concentrated and the optical rotation of the concentrate, made up to 50 ml, was measured. It gave a constant value $[\alpha]_D$, +51·2°. D-Glucose has a constant value $[\alpha]_D$, +52·8°.

3,3′-*Di*-O-*methyl ellagic acid diacetate.* Di-O-methyl ellagic acid (100 mg) was acetylated, with acetic anhydride and pyridine (at 100°, 1 hr) and the acetate crystallized from dioxan as prisms (70 mg) m.p. 300–302°. (Found: C, 57·82, H, 3·32, OMe 12·91; $C_{20}H_{14}O_{10}$ requires: C, 57·96, H, 3·37 and OMe, 14·5%). This diacetate was also synthetically obtained from tetraacetyl ellagic acid following the method of Jurd.[8]

Di-O-methyl ellagic acid (100 mg) was methylated with ethereal diazomethane and the *tetramethyl ellagic acid* was crystallized from dioxan as pale yellow needles (90 mg) m.p. 342–344°.

Di-O-methyl ellagic acid (100 mg) in 2N sodium hydroxide (10 ml) was shaken with dimethyl sulphate (1·5 ml) and the tetramethyl ether crystallized from dioxan as pale yellow needles (60 mg) m.p. 342–344°, unchanged by authentic tetramethyl ellagic acid, prepared by the methylation of ellagic acid. (Found: C, 59·8, H, 4·21, OMe, 31·2; $C_{18}H_{14}O_8$ requires: C, 60·3, H, 3·9 and OMe 34·6%).

Hydrolysis of the methyl ether of compound C: 3,3′,4-*tri*-O-*methyl ellagic acid.* The methyl ether of Compound C was hydrolysed with 4% methanolic sulphuric acid. The aqueous hydrolysate was extracted with ether and the ether extract on concentration, deposited a colourless solid which crystallized from dioxan as prisms of tri-O-methyl ellagic acid, m.p. 289–291°, undepressed by authentic 3,3′,4-tri-O-methyl ellagic acid (m.p. 293–294°). It has the U.V. absorption maxima at λ_{max} 249 mμ (log ϵ, 4·65) and 370 mμ (log ϵ, 4·05). (Found: C, 58·76, H, 3·60; OMe, 25·6; $C_{17}H_{12}O_8$ requires: C, 59·30, H, 3·50 and OMe, 27·5%).

4′-O-*Ethyl*-3,3′,4-*tri*-O-*methyl ellagic acid.* 3,3′,4-Tri-O-methyl ellagic acid (m.p. 290–291°, 20 mg) in 2% sodium hydroxide (5 ml) was ethylated with diethyl sulphate (0·1 ml). The ethyl ether (3,3′,4-tri-O-methyl-4′-O-ethyl ellagic acid) crystallized as pale yellow solid (15 mg) m.p. 328–330° dec (Found: C, 59·85, H, 4.85, $C_{19}H_{17}O_8$ requires: C, 61·13 and H, 4·56%).

Ethylation of the glucoside and hydrolysis. 4′-O-*ethyl*-3,3′-*di*-O-*methyl ellagic acid.* The glucoside

[8] L. Jurd, *J. Amer. Chem. Soc.* **81**, 4606 (1959).

(60 mg) in 2N sodium hydroxide was shaken with diethyl sulphate (0·2 ml) After 4 hr, it was acidified. The solid product resisted crystallization from aqueous methanol, gave no ferric chloride colour and was subjected directly to hydrolysis by refluxing with 2N methanolic sulphuric acid for 4 hr. It was extracted with ether and evaporation of the dried ether extract deposited crystalline 3,3′-O-dimethyl-4′-O-ethyl ellagic acid (30 mg) m.p. 332–334°. (Found: C, 60·16, H, 4·15, $C_{18}H_{14}O_8$ requires: C, 60·3 and H, 3·9%).

3,3′,4-*Tri*-O-*methyl*-4′-*ethyl ellagic acid.* 3,3′-di-O-methyl-4′-O-ethyl ellagic acid (25 mg) was methylated with ethereal diazomethane and the methyl ether, 3,3′,4-tri-O-methyl-4′-O-ethyl ellagic acid crystallized from dioxan as a pale yellow solid (20 mg) m.p. 326–328° (dec) undepressed by the sample obtained by ethylation of 3,3′,4-tri-O-methyl ellagic acid.

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