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TWO ELLAGITANNINS FROM THE STEM BARK OF *CAESALPINIA PULCHERRIMA**

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Abstract—Two ellagitannins have been isolated from the stem bark of *C. pulcherrima*. These tannins have been assigned structures with glucose as the carbohydrate core, esterified with two galloyl and one hexahydroxydiphenoyl group and with a galloyl, a hexahydroxydiphenoyl and a *m*-digalloyl group, respectively.

INTRODUCTION

The presence of hydrolysable tannins has been reported previously in *Caesalpinia brevifolia*, *C. coriaria* and *C. spinosa* [1]. The stem bark of *C. pulcherrima* is highly astringent, the astringency diminishing with the age of the plant, and it is widely used as an abortifacient and as an emmenagogue. The aim of the present work was to determine the nature of the chemical constituents responsible for the special properties of the stem bark.

RESULTS AND DISCUSSION

The stem bark of plants *ca* 1-year-old was extracted and the presence of two ellagitannins, (**1**) and (**2**), was established along with other minor constituents, viz. sitosterol, sebacic acid, quercimeritrin, prodelphinidin, gallic acid and ellagic acid.

Constitution of tannin **1**

Tannin **1** was obtained as a crystalline, hygroscopic, chromatographically (PC and TLC) homogeneous entity. A dark blue colour with ferric chloride indicated its polyphenolic nature. A positive Molisch's test suggested the presence of a carbohydrate moiety and a positive test with aniline hydrogen phthalate indicated a free reducing group in the sugar. Therefore, **1** could be a sugar ester. Both acid and alkaline hydrolyses of **1** gave gallic and ellagic acids and glucose. Ellagic acid was confirmed by mmp and CO-PC. UV λ_{max}255 nm: and superimposable IR with a synthetic sample [2]. Gallic acid was confirmed by mmp, CO-PC and superimposable IR with an authentic marker. Quantitative acid hydrolysis of the tannin confirmed the presence of 2 mol of gallic acid (38.5%) and 1 mol of ellagic acid (38.6%) per mol of glucose (22.6%) ($C_{34}H_{26}O_{22}$ requires: 38.9, 38.7 and 22.3%, respectively).

Acetyl estimation [3, 4] of the acetate (Found: Ac, 43.2. $C_{34}H_{13}O_{22}$ $(Ac)_{13}$ requires: Ac, 41.96%) showed the presence of thirteen hydroxyl groups in the tannin. The tannin after methylation with diazomethane and subsequent hydrolysis with 5% alcoholic NaOH gave trimethylgallic acid, hexamethoxydiphenic acid and glucose. The proportion of the two acids was 2:1, as determined by chromatographic comparison with artificial mixtures of the two acids. Thus tannin **1** has a glucose core, which is esterified in a random fashion with two galloyl groups and one hexahydroxydiphenoyl group.

Constitution of tannin **2**

The presence of ethyl gallate in the ethanolic extract of the bark could be explained as an artefact formed by alcoholysis of the depside links in the genuine tannin [5]. Therefore, the method of extraction was modified. The bark was extracted with de-ionized water at room temperature (*ca* 20°), mineral matter was removed by de-ionization over a mixed bed of ion-exchange resins and the extract concentrated. No ethyl gallate could be detected after this treatment. The dark brown syrup was extracted successively with petrol, ether and ethyl acetate. The petrol extract did not contain any phenolics, the ether extract showed traces of free gallic acid and the ethyl acetate extract gave prodelphinidin (confirmed by co-PC with an authentic sample extracted from *Solanum melongena* fruit coat hydrolysate [6]) along with traces of gallic acid and tannin **2**. Extraction of the residue with acetone and separation of the acetone extract on a deactivated silica gel column yielded micro-crystalline chromatographically homogeneous tannin **2**. Tannin **2** was found to be a sugar ester by a positive Molisch's test and aniline hydrogen phthalate [7]. Both acid and alkaline hydrolysis gave glucose, gallic acid and ellagic acid. The quantitative acid hydrolysis calculated to 3 mol of gallic acid (49.0%) and 1 mol of ellagic acid (32.0%) per mol of glucose (20.1%) ($C_{41}H_{30}O_{26}$ requires: 48.8; 32.4 and 18.7% respectively). Acetyl estimation [3, 4] of the acetate (Found: Ac, 40.5, $C_{41}H_{15}O_{26}$ $(Ac)_{15}$ requires: Ac, 41.13%) showed the presence of 15 hydroxyl groups in the tannin. Methylation of the tannin with diazomethane and subsequent hydrolysis gave glucose along with trimethylgallic acid (two parts), dimethylgallic acid (one part) and hexamethoxydiphenic acid (one part). The presence of dimethylgallic

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acid suggested depside links in the tannin, i.e. a polygalloylated chain may be present.

On controlled methanolysis **2** gave traces of gallic acid, methyl gallate and another tannin which was found to be identical with **1** in all respects. Therefore, the latter appears to be an artefact. As no *m*- tri-, or digallic acid could be detected during methanolysis the possibility of a polygalloylated chain was excluded. Tannin **2** thus has a glucose core, which is esterified randomly with galloyl, *m*-digalloyl and hexahydroxydiphenoyl units. This compound is unusual in containing a *m*-digalloyl unit in addition to a hexahydroxydiphenoyl group. A digalloylated chain has not been found in any of the naturally occurring ellagitannins reported in the literature.

EXPERIMENTAL

Plant material. Plant material was supplied by United Chemical and Allied Products, Calcutta, India.

Solvents used for chromatography. PC: solvent a_1: *n*-BuOH–HOAc–H_2O (4:1:5); solvent b_1: *n*-BuOH saturated with NH_3. TLC: solvent a_2: $CHCl_3$–MeOH (9:1).

Isolation of tannin **1**. Stem bark (1 kg) was extracted with boiling EtOH (4 × 11.). The combined and conc extract was fractionated into petrol, Et_2O and EtOAc soluble fractions, respectively, by continuous liquid–liquid extraction. The petrol extract gave sitosterol, the Et_2O extract gave sebacic acid along with gallic acid, ethyl gallate and traces of ellagic acid and the EtOAc extract yielded mainly quercimeritrin and prodelphinidin along with small amounts of ellagitannin **1**. These three compounds were separated by PLC (solvent a_2). The remaining EtOH extract on concn and extraction with Me_2CO and subsequent crystallization from an Me_2CO–Et_2O mixture gave a colourless microcrystalline substance, tannin **1**; yield (0.1%), mp 249° (d). It gave single spot on PC, R_f 0.28 (solvent a_1; spray: EtOH–$FeCl_3$). (Found: C, 51.4; H, 3.6. $C_{34}H_{26}O_{22}$ requires: C, 51.9; H, 3.3%). UV λ_{max}nm: 262; IR γ_{max}^{KBr} cm^{-1}: 3550 (OH), 3150, 1710 (ester), 1610, 1550, 1450, 1390, 1310, 1250, 1110, 1020, 990, 820, 800.

Quantitative acid hydrolysis of **1**. The tannin (0.0605 g) dissolved in EtOH–H_2SO_4 (15 ml, 7%) was refluxed for 2 hr, the soln cooled to room temp., the solid centrifuged down and washed with H_2O, dried at 100° and weighed. After removing ellagic acid the remaining soln was continuously extracted with Et_2O. The Et_2O extract was concd and the residue dissolved in H_2O made up to 100 ml. The acids were estimated by potentiometric titration against 0.1 N soln of KOH. The remaining soln was neutralized with $BaCO_3$, $BaSO_4$ ppt. filtered and washed with H_2O. The filtrate and washings were combined and made up to 50 ml. The sugar present was estimated by Folin's and Wu's colorimetric method [Found: ellagic acid, 0.0232 g (38.6%); gallic acid, 0.0233 g (38.5%); glucose, 0.0137 g (22.6%). $C_{34}H_{26}O_{22}$ requires: ellagic acid, 38.7; gallic acid, 38.9; glucose, 22.3%].

Methylation and hydrolysis of **1**. The tannin (0.20 g) on methylation with CH_2N_2 gave a white solid which crystallized from Me_2CO–Et_2O as colourless crystals, mp 240° (d). The methylated tannin (0.15 g) was taken in EtOH (5 ml), the soln added to NaOH (5 ml, 5%) and the mixture kept for 48 hr at room temp. out of contact with air. The mixture was acidified and extracted with Et_2O. The Et_2O extract on PC gave two compds R_f 0.14 and 0.38 (solvent b_1; spray: bromophenol blue) corresponding to hexamethoxydiphenic acid and trimethylgallic acid. The hydrolysate and artificial mixtures prepared from authentic trimethylgallic and hexamethoxydiphenic acids in different proportions, viz. 1:1, 2:1, 3:1, 4:1, and 3:2, were put on a descending strip chromatogram (solvent b_1; spray: bromophenol blue). The intensity of the spots of the acids in the hydrolysate was the same as the intensity of the artificial mixture in the proportion 2:1. The trimethylgallic acid was prepared by methylation of gallic acid using Me_2SO_4 and NaOH [8], the dimethylgallic acid by controlled methylation using a calculated amount of Me_2SO_4 and refluxing in Me_2CO soln in the presence of K_2CO_3 for 4 hr. Hexamethoxydiphenic acid was prepared by Ullmann condensation of 2-iodo-3,4,5-tri-*O*-methylgallic acid [9].

Acetylation of **1**. The tannin on acetylation with Ac_2O–NaOAc gave a colourless solid mp 238°(Found: C, 53.8; H, 3.8; Ac, 43.2. $C_{60}H_{52}O_{35}$ requires: C, 54.05; H, 3.9; Ac, 41.96%).

Isolation of tannin **2**. The fresh stem bark (1 kg) was extracted with dist. H_2O (3 × 2 l.) at room temp. (*ca* 25°) for 4 days. The dark brown extract (conductance 2800 mho) was passed repeatedly through a column of mixed cation (IR-120) and anion exchange (IR-45) resins until the conductance was constant (800 mho). This soln was concd below 40° and the residue extracted with boiling petrol, Et_2O and finally with hot EtOAc. From the EtOAc extract prodelphinidin was obtained with traces of gallic acid and some tannin **2**. The former crystallized as colourless prisms on adding petrol and by repeated crystallization was freed from gallic acid and tannin **2**. The EtOAc residue was extracted with small amounts of boiling Me_2CO, the extract concd and Et_2O added slowly. Dark-coloured impurities were removed by decantation, more Et_2O added and on keeping a cream-coloured microcrystalline substance was obtained, which was separated on a Si gel column. The fraction eluted with C_6H_6–Me_2CO (1:1) was chromatographically homogeneous tannin **2**. On PC it gave a single blue spot with R_f 0.30 (solvent a_1; spray EtOH–$FeCl_3$). It gave purple–brown spot when sprayed with aniline hydrogen phthalate. It crystallized from Me_2CO–Et_2O as microcrystalline prisms, mp 240° (d), yield 0.6 g (0.12%). (Found: C, 52.8; H, 3.6. $C_{41}H_{30}O_{26}$ requires: C, 52.4; H, 3.2%). UV λ_{max}nm: 265. IR γ_{max}^{KBr} cm^{-1}: 3550 (OH), 3040, 1725 (depside), 1610 (aromatic) 1590, 1510, 1440, 1400, 1320, 1200, 1110, 1060, 920, 890 and 760.

Acetylation of **2**. The acetate was a colourless crystaline solid, mp 258° (d) (Found: C, 54.20; H, 3.9; Ac, 40.5. $C_{71}H_{60}O_{41}$ requires: C, 54.33; H, 3.82; Ac, 41.13%).

Quantitative acid hydrolysis of **2**. Tannin **2** (0.0555 g) was hydrolysed (Found: ellagic acid, 0.0178 g (32.0%); gallic acid, 0.0272 g (49.0%); glucose, 0.0112 g (20.1%). $C_{14}H_{30}O_{26}$ requires: ellagic acid, 32.4; gallic acid, 48.8; glucose, 18.7%).

Methylation and hydrolysis of **2**. The CH_2N_2 methylated tannin **2** (0.2 g), mp 235° (d), was taken in Et_2O, NaOH soln (5 ml, 10%) added and the mixture kept for 48 hr at room temp. (*ca* 25°) out of contact with air. The mixture was acidified with HCl and continuously extracted with Et_2O. The Et_2O extract on PC gave three compds, R_fs 0.59, 0.38 and 0.14, respectively (solvent b_1; spray: bromophenol blue), which corresponded to synthetic samples of 3,4-dimethylgallic acid, trimethylgallic acid and hexamethoxydiphenic acids, R_fs 0.58, 0.36, and 0.15, respectively. The ratio of 3,4-dimethylgallic acid, 3,4,5-trimethylgallic acid and hexamethoxydiphenic acid was ascertained as 1:2:1 by comparative PC using artificial mixtures of these acids in different proportions, viz. 1:1:1, 1:2:1, 1:3:1 and 2:3:1.

Methanolysis. The tannin (0.1 g) was dissolved in 0.5 N acetate buffer (10 ml, pH 6.0) and to it MeOH (40 ml) was added. The soln was kept at 35° for 7 days. The MeOH was removed by distillation under red. pres. and the residue continuously extracted with EtOAc. The EtOAc extract on PC gave gallic acid and methyl gallate (co-PC R_fs 0.74, and 0.82, respectively in solvent a_1; spray: EtOH–$FeCl_3$. The residue after EtOAc extraction was dissolved in EtOH. On PC it (co-chromatographed (R_f 0.28, solvent a_1; spray: EtOH–$FeCl_3$) with tannin **1** and the two compds were found to be identical in all respects.

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REFERENCES

1. Haslam, E. (1966) *Chemistry of Vegetable Tannins*. Academic Press, London.
2. Perkin, A. G. and Nierenstein, M. (1905) *J. Chem. Soc. Trans. Sec.* **87**, 1412.
3. Weisenberger, E. (1947) *Mikrochemie* **33**, 51.
4. Belcher, R. and Godbert, A. I. (1954) *Semi-micro Quantitative Organic Analysis*, p. 164. Longman Green, New York.
5. Haslam, E., Haworth, R. D. and Rogers, H. J. (1961) *J. Chem. Soc.* 1836.
6. Hayashi, K. (1962) *The Chemistry of Flavonoid Compounds* (Geissman, T. A., ed.) p. 270. Pergamon Press, Oxford.
7. Hough, L., Jones, J. K. and Wadman, W. H. (1950) *J. Chem. Soc.* 1702.
8. Gilman, H. and Blatt, A. H. (1961) *Organic Synthesis Collective*, Vol. I, p. 537. John Wiley, New York.
9. Cremlyn, R. J. W. and Still, R. H. (1967) *Named and Miscellaneous Reactions in Practical Organic Chemistry*, p. 137. Heinemann Educational, London.