## 737. *Gallotannins. Part VII.* Tara Gallotannin.*

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The main constituent of Tara gallotannin gives 3,4,5-tri-*O*-galloylquinic acid on methanolysis. The acid has been converted by methylation and hydrolysis into 1-*O*-methylquinide (III) which was identified by comparison with a synthetic specimen. Tara gallotannin is probably derived by the random-wise addition of two or three galloyl groups depsidically linked to the 3,4,5-tri-*O*-galloylquinic acid core.

An important commercial tannin known as Tara powder is obtained by crushing the fruit pods of the widely distributed South American shrub *Caesalpinia spinosa*. The extract has pronounced acidic properties and this led Burton and Nursten [1] to classify the tannin present as an ellagitannin, but further investigation of this material was shown to be necessary by observations of White *et al.*[2] who subjected Tara powder to paper chromatography. This whilst demonstrating the complexity of Tara powder also showed that the principle component displayed many similarities to the known Chinese gallotannin. Some of our preliminary results on the composition of the Tara extract have been presented in an earlier communication.[3] As the main gallotannin constituent yielded, on acid or enzymic hydrolysis, a mixture of gallic and quinic acid (I; $R^1 = R^2 = R^3 = R^4 = H$) it was concluded that Tara gallotannin was a galloylated quinic acid and that it differed from other members of the hydrolysable tannin group which are based upon galloylated or ellagoylated hexose structures. Determination of the equivalent weight indicated a

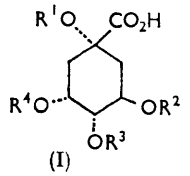
* Part VI, preceding paper.

[1] Burton and Nursten, "The Chemistry of Vegetable Tannins," Society of Leather Trades' Chemists, Croydon, 1956, p. 57.

[2] White, Kirby, and Knowles, *J. Soc. Leather Trades' Chemists*, 1952, **36**, 148.

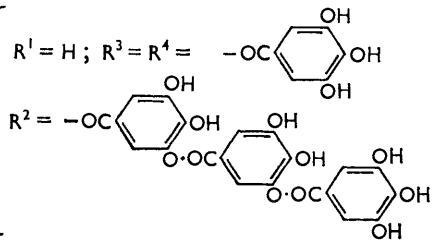
[3] Armitage, Bayliss, Gramshaw, Haslam, Haworth, Jones, Rogers, and Searle, *J.*, 1961, 1842.

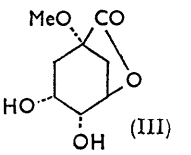
tetra- or penta-galloylated quinic acid structure, and in conjunction with other observations one of us put forward [4] structure (II) as probably representing that of the main species present in Tara gallotannin. Our initial studies have latterly received support from the work of Horler and Nursten [5] on Tara gallotannin, and in the present paper further and more detailed evidence in support of structure (II) is presented.




(I)

(II)




(III)

Paper-chromatographic examination [2,3] of the Tara extract yielded a complex pattern of substances besides the main gallotannin spot. Some observations on these minor components have been made by Horler and Nursten [5] and we have identified paper chromatographically glucose, quinic, shikimic, gallic, and *m*-digallic acid, β-glucogallin and theogallin [6] amongst them. The principle component referred to as Tara gallotannin was obtained either by counter-current distribution of the Tara extract between ethyl methyl ketone and water or by fractional precipitation as a white amorphous powder which was highly lævorotary and gave analyses for a tetra- or penta-galloylquinic acid. Paper chromatography of Tara gallotannin gave a diffuse and elongated tannin spot, and since this demonstrated the heterogeneity of the material the product was subjected further to partition chromatography on cellulose, which afforded three fractions, A, B, and C. They gave analyses for, respectively, tri-, tetra-, and penta-galloylquinic acid but since, as will be shown later, A, B, and C are all based on the same trigalloylquinic acid core, the differences between them must lie in the number and possibly the position of galloyl groups linked in the form of depsides to this core. Analysis of the gallic acid content of the gallotannin and other galloylated quinic acids was made after hydrolysis with tannase by measurement of the optical density at 280 mμ and the quinic acid by titration after acid hydrolysis and preferential elution of the quinic acid from Dowex 1.

Several observations have led to an understanding of the arrangement of galloyl groups on the quinic acid nucleus of the gallotannin. Diazomethane-methylation of Tara gallotannin gave an oily product but analysis [3] of the ratio of **3,4**-di- and **3,4,5**-tri-*O*-methylgallic acids liberated on hydrolysis of this substance indicated that approximately two galloyl groups were bound as depsides; this was confirmed by the identification of *m*-digallic acid after acid hydrolysis of the gallotannin. Parallel work [3] on Chinese, Turkish, and sumach gallotannin had revealed the useful reaction brought about by methanol at pH 6·0: methanolysis of the depside links occurred but galloyl groups directly attached to glucose were unaffected. Application of this reaction to Tara gallotannin and paper chromatography of the products showed the presence of methyl gallate, traces of gallic acid, and two galloylated quinic acids (D and E) which must represent the basic core of the gallotannin, with D clearly predominating. Methanolysis of the materials A, B, and C obtained by fractionation of Tara gallotannin revealed similarly the presence of acids D and E, thus leading to the previously stated conclusion that these substances have basically the same galloylated quinic acid core. Paper chromatography of the methanolysis products of Tara gallotannin at an intermediate stage showed the presence of methyl *m*-digallate, indicating, as with the other gallotannins, the presence in some or all of the molecules of chains of at least three galloyl groups. The mixture of galloylated

[4] Haworth, Pedler Lecture, *Proc. Chem. Soc.*, 1961, 401.
[5] Horler and Nursten, *J.*, 1961, 3786.
[6] Roberts and Myers, *J. Sci. Food Agric.*, 1958, **11**, 701.

quinic acids D and E was separated by a combination of partition chromatography and counter-current distribution, and both acids were isolated as amorphous solids whose analyses indicated that they were trigalloylquinic acids. The structure of acid E was not ascertained in detail on account of small amounts available, but it was proved that D and E are isomers of a structural or conformational type since subjecting the former to the conditions of the methanolysis slowly gave the latter; further work on this change and the relations of acids D and E is in progress.

The isolation of the trigalloylquinic acid D indicated that one of the hydroxyl groups on the quinic acid core of the gallotannin was unesterified and in order to identify its position methylation studies were carried out. Diazomethane-treatment of both Tara gallotannin and the methanolysis product D gave amorphous products whose infrared spectra still showed the presence of a free hydroxyl group. Further methylation with silver oxide and methyl iodide followed by alkaline hydrolysis gave in both cases an amorphous derivative of quinic acid whose $R_F$ values strongly supported its assignment as a monomethyl derivative. The substance reacted with periodic acid as a vicinal diol, and when warmed in dioxan saturated with hydrogen chloride it gave a crystalline lactone identical with synthetic 1-*O*-methylquinide (III) (kindly prepared by Mr. D. A. Lawton [7]). Thus the major core of the gallotannin D must be 3,4,5-tri-*O*-galloylquinic acid (I; $R^1 = H$; $R^2 = R^3 = R^4 =$ 3,4,5-trihydroxybenzoyl) and further work on the synthesis of this compound is in progress.

The evidence quoted is thus in good agreement with the structure (II) put forward earlier for Tara gallotannin but, since we have little evidence available on the heterogeneous nature of this substance, this may represent only one of several possible structures which can be written on the evidence available and which may, as Freudenberg [8] has suggested similarly for Chinese gallotannin, all contribute to the complete gallotannin structure.

## Experimental

Paper chromatography of the galloylated quinic acids was carried out in the solvent systems (*a*) and (*b*) previously described.[3] A ferric chloride–potassium ferricyanide spray revealed the trihydric phenols as blue spots on a white background. Quinic acid and its derivatives were subjected to paper chromatography in solvent system (*c*), benzyl alcohol–t-butyl alcohol–propan-2-ol–water (3 : 1 : 1 : 1) containing 2% of 90% formic acid, and the compounds were detected with a spray of sodium metaperiodate, sodium nitroprusside, and piperazine.[9] Solutions were concentrated at 25—30° under reduced pressure with a rotary evaporator and quantitative determinations were made on samples dried to constant weight at 80°/0·005 mm.

*Preparation of Tara Gallotannin.*—Finely ground pods of *Caesalpinia spinosa* (30 g.) were shaken with water (250 c.c.) for 2 days at room temperature, the slurry was filtered, and the filtrate extracted with ethyl acetate (5 × 200 c.c.). Removal of the ethyl acetate and freeze-drying of the residue from water gave a light brown amorphous solid (5·0 g.). The crude extract when subjected to paper chromatography showed the pattern tabulated.

Table 1.

| Fraction | $R_F$ (*a*) | $R_F$ (*b*) | $NH_3$/U.v. | Compound |
|---|---|---|---|---|
| (i) | 0·37 | 0·50 | Violet | Gallotannin |
| (ii) | 0·00—0·41 | 0·44—0·62 | Absorption | Gallotannin |
| (iii) | 0·49 | 0·70 | Violet | Gallic acid |
| (iv) | 0·32 | 0·77 | Violet | *m*-Digallic acid |
| (v) | 0·52 | 0·48 | Violet | Unknown |

Two such extracts (10 g.) were dissolved in ethyl acetate (100 c.c.), benzene (60 c.c.) was added, and the supernatant liquid was decanted from the precipitated gum. The gum was triturated with benzene (40 c.c.), yielding a white solid which was removed by decantation and filtration from the residual gum. This process was repeated a further 6 times, and the solids

[7] Haslam, Haworth, and Lawton, unpublished work.
[8] Freudenberg, " Tannin, Cellulose, and Lignin," Verlag Chemie, Berlin, 1933, p. 38.
[9] Cartwright and Roberts, *Chem. and Ind.*, 1955, 230.

were combined and freeze-dried from water, to give Tara gallotannin (6·4 g.) (Found: C, 52·6; H, 3·7; quinic acid, 24·3. Calc. for $C_{35}H_{28}O_{32}$: C, 52·5; H, 3·5; quinic acid 24·0%). Paper chromatography showed Tara gallotannin to contain fractions (i) and (ii) with traces of (iii) and (v) (Table 1).

*Fractionation of Tara Gallotannin.*—Tara gallotannin (3·5 g.) in N-acetic acid (50 c.c.) was applied to a column of cellulose (75 × 7 cm.) and elution carried out with the same solvent. Fractions (15 c.c.) were collected and analysed by measurement of their optical density at 320 mμ. Four main peaks were thus obtained and the solvent was removed from each at 30°. Fraction 1, on crystallisation from water, gave gallic acid (0·2 g.), m. p. and mixed m. p. 245—250°. Fraction 2, on freeze-drying from water, gave material A (0·50 g.) as an amorphous white powder (Found: C, 51·6; H, 3·9; quinic acid, 29·9. Calc. for $C_{28}H_{24}O_{18}$: C, 51·8; H, 3·7; quinic acid, 29·6%). Paper chromatographic analysis showed the presence of materials (i) $R_F$ (*a*) 0·34, (*b*) 0·54 and (ii) $R_F$ (*a*) 0·31, (*b*) 0·58 (Table 1). Fraction 3, on freeze-drying from water, gave material B as a white amorphous powder (0·82 g.) (Found: C, 52·4; H, 3·7; quinic acid, 21·0; gallic acid, 88·2. Calc. for $C_{42}H_{32}O_{26}$: C, 52·8; H, 3·4; quinic acid, 20·2; gallic acid, 89·3%), $[\alpha]_D^{21}$ −116·1° (*c* 1·2 in acetone), $R_F$ (*a*) 0·22, (*b*) 0·38. Fraction 4, on freeze-drying from water, gave an amorphous white powder (1·03 g.) (Found: C, 52·7; H, 3·6; quinic acid 20·2; gallic acid, 89·4. Calc. for $C_{42}H_{32}O_{26}$: C, 52·8; H, 3·4; quinic acid, 20·2; gallic acid, 89·3%), $[\alpha]_D^{21}$ −109·1° (*c* 2·9 in acetone), and $R_F$ (*a*) 0·11, (*b*) 0·40.

*Determination of Quinic Acid in Galloylated Quinic Acids.*—The compound (0·010 g.) was heated at 100° for 24 hr. with N-hydrochloric acid (3·0 c.c.), and the solution passed down a column of Dowex 1 (3 g.; 5 × 1 cm., acetate form). Elution was continued with 2·5N-acetic acid (100 c.c.), and fractions (10 c.c.) were collected in tubes which were then suspended in a thermostat-bath at 45° and evaporated by passage of air across the top of the tubes. The contents were twice re-dissolved in water, re-evaporated to dryness, and then titrated to phenolphthalein against N/300-barium hydroxide under a stream of nitrogen. Control experiments were simultaneously performed with and without quinic acid.

*Methanolysis of Tara Gallotannin.*—Tara gallotannin (7·0 g.) was dissolved in methanol (450 c.c.) containing 0·5N-acetate buffer (pH 6·0; 50 c.c.), and the solution was kept at 37° under nitrogen. After 7 days the solution was concentrated to 50 c.c. at 30°, the pH was then adjusted to 6·5 with saturated sodium hydrogen carbonate solution, and the mixture was extracted with ethyl acetate (10 × 50 c.c.). The aqueous layer was passed down a column of ZeoKarb 215 (5 × 15 cm.; $H^+$), and the eluate concentrated and dried to give, after freeze-drying from water, a light brown amorphous solid (5·0 g.). Paper chromatography of this material gave the following pattern:

TABLE 2.

| Fraction | | $R_F$ (*a*) | $R_F$ (*b*) | $NH_3$/U.v. | Compound |
|---|---|---|---|---|---|
| (i) | E | 0·27 | 0·43 | Violet-blue | Galloylated quinic acid |
| (ii) | D | 0·35 | 0·52 | Violet | Galloylated quinic acid |
| (iii) | | 0·63 | 0·46 | Violet | Unknown |
| (iv) | | 0·49 | 0·20 | Violet | Gallic acid |

This brown solid (5·0 g.) was applied in N-acetic acid (75 c.c.) to a column of cellulose (77 × 5·5 cm.) and elution continued with N-acetic acid. Fractions (15 c.c.) were collected and analysed by measurement of their optical density at 320 mμ. Three main peaks were thus obtained and the solvent was removed from each at 30°. Fraction 1 gave small amounts of gallic acid and material (iii). Fraction 2 gave, on freeze-drying, an amorphous solid (1·54 g.) which when analysed by paper chromatography contained acid D and gallic acid. Countercurrent distribution of this solid D in the system propan-1-ol–butan-1-ol–cyclohexane–water (3 : 1 : 1 : 7; 50 transfers), analysis of the contents of each tube by paper chromatography in solvent system (*a*), concentration of tubes 5—19, and freeze-drying from water gave acid D as an amorphous white solid (0·99 g.) (Found: C, 51·7; H, 4·0; quinic acid, 29·9; gallic acid, 78·4. Calc. for $C_{28}H_{24}O_{18}$: C, 51·8; H, 3·7; quinic acid, 29·6; gallic acid, 78·7%), $[\alpha]_D^{22}$ −129·0° (*c* 0·8 in acetone). Fraction 3 gave, on freeze-drying, an amorphous solid (2·21 g.) which paper chromatographic analysis showed to contain acids D and E. Fraction 3 (0·8 g.) was twice chromatographed on cellulose with propan-1-ol–acetic acid–water (4 : 1 : 5) as solvent. The eluate was collected in fractions (10 c.c.) which were analysed by measurement of their optical density at 320 mμ. Concentration of the appropriate fraction gave acid *E* (0·05 g.) which after freeze-drying from water was obtained as a white amorphous solid (Found: C, 51·4; H, 4·5; quinic

acid, 30·8. Calc. for $C_{28}H_{24}O_{18}$: C, 51·8; H, 3·7; quinic acid 29·6%) $[\alpha]_D^{21}$ — 89·0° (*c.* 0·5 in acetone; pH 6·0).

*Methanolysis of Products* A, B, C, *and* D.—Fraction A, B, or C (0·02 g.) was dissolved in methanol (1·0 c.c.) containing 0·5N-acetate buffer (pH 6·5; 0·2 c.c.), and the solution was kept at 37° under nitrogen, then analysed after 6 days by paper chromatography. The patterns were identical with that shown in Table 2, except that acid C did not show fraction (iii) and in all cases the pattern showed the additional presence of methyl gallate, $R_F$ (*a*) 0·54, (*b*) 0·84. Acid D, similarly treated and analysed after 3 days, showed the presence of material E, $R_F$ (*a*) 0·32, (*b*) 0·35.

*Methylation of Methanolysis Product* D.—The substance D (1·3 g.), in acetone (10 c.c.), was treated at 0° for 2 days with ethereal diazomethane (75 c.c.), and after removal of the solvents the process was twice repeated. The resultant gum was dissolved in benzene (10 c.c.) and filtered through alumina (30 g.); the eluate after removal of the solvent gave an amorphous solid (0·7 g.) (Found: C, 57·9; H, 5·7; OMe, 39·6. Calc. for $C_{38}H_{44}O_{18}$: C, 57·9; H, 5·6; OMe, 39·3%). The latter amorphous solid was refluxed with methyl iodide (15 c.c.) in the presence of silver oxide (0·40 g.) for 5 days; additional amounts of methyl iodide and silver oxide were added after 2 and 4 days. The solution was extracted with ether (10 × 20 c.c.), the extract was filtered, and the solvent removed, yielding a gum which was refluxed for 18 hr. with methanolic 50% potassium hydroxide (20 c.c.). The solution, after dilution with 50% aqueous methanol (50 c.c.), was passed down a column of ZeoKarb 215 ($H^+$; 18 × 4 cm.), and elution continued with the same solvent (200 c.c.). Concentration of the eluate and extraction with ether (6 × 30 c.c.) gave 3,4,5-tri-*O*-methylgallic acid (0·45 g.), m. p. and mixed m. p. 167°. The aqueous liquors were subjected to paper chromatography in solvent system (*c*) and showed the presence of a substance $R_F$ (*c*) 0·40, giving a positive reaction to the periodate–piperazine–sodium nitroprusside spray.[9] Removal of the water at 30° gave a gum which was refluxed in dioxan (30 c.c.) containing dry hydrogen chloride (1·5 g.) for 6 hr.; potassium carbonate (8·0 g.) was then added and the solution refluxed for 20 min. before filtration. Removal of the dioxan and crystallisation of the residue from ethyl acetate–light petroleum (b. p. 60—80°) gave 1-*O*-methylquinide as colourless needles (0·01 g.), m. p. and mixed m. p. 152—153° (Found: C, 50·9; H, 6·3; OMe, 16·6. Calc. for $C_8H_{12}O_5$: C, 51·1; H, 6·7; OMe, 16·5%). The infra-red spectra of the isolated and the synthetic sample of 1-*O*-methyl-quinide were identical.

Similar treatment of Tara gallotannin also gave 1-*O*-methylquinide.

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