360. *Gallotannins. Part III.* The Constitution of Chinese, Turkish, Sumach, and Tara Tannins.*

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The isolation and purification of the tannins of Chinese galls and the leaves of Sicilian and Stagshorn sumach are described, and analysis suggests the products are essentially the same octa- or nona-galloylated glucose. The presence of a glucose core is established by hydrolysis with the purified esterase obtained from tannase (Part I). By using the methanolysis reaction (Part II) it is shown that the gallotannin contains a β-penta-*O*-galloylglucose nucleus to which some three or four additional galloyl groups are attached by depsidic linkages, and evidence is advanced favouring the presence of chains containing at least three galloyl residues. β-Penta-*O*-galloylglucose has been synthesised from tri-*O*-benzylgalloyl chloride and β-glucose, with subsequent hydrogenolysis, and the amorphous product is indistinguishable from the major methanolysis product of gallotannin. β-Tetra-*O*-galloylglucose has also been prepared synthetically and is indistinguishable from a minor methanolysis product of Chinese gallotannin or the tannin of old sumach leaves.

Preliminary results of an investigation of Turkish gallotannin are described, and the main constituent of tara tannin is shown to be based upon a new galloylated quinic acid type of structure, probably containing four or five galloyl groups united to a quinic acid nucleus.

Fischer,[1] Freudenberg,[2] and Karrer *et al.*[3] in their classical researches on the gallotannins concluded that these were not only mixtures of isomers but also of substances

* Part II, preceding paper.

[1] Fischer, *Ber.*, 1919, **52**, 809.
[2] Freudenberg, " Naturliche Gerbstoffe," Verlag Chemie, Berlin, 1920, p. 101.
[3] Karrer, Salomon, and Payer, *Helv. Chim. Acta*, 1923, **6**, 17.

of differing empirical formulæ all having in common a galloylated glucose structure. More recently White [4] has claimed that alkaline hydrolysis of Chinese gallotannin gave rise to a substance identified on the basis of paper chromatography as a tri- or tetra-saccharide and he suggested that this was the carbohydrate core of the tannin. Grassmann, Stiefenhofer, and Endres [5] hydrolysed the methylated tannins from Sicilian and Stagshorn sumach and after removal of the 3,4,5-tri-*O*-methyl- and 3,4-di-*O*-methyl-gallic acid the carbohydrate core was isolated and separated from inorganic salts by paper electrophoresis. The carbohydrate then behaved on a paper chromatogram as a tetrasaccharide, and on acid-hydrolysis gave the component monosaccharides which were again identified by paper chromatography. Grassmann *et al.* were thus able to formulate the carbohydrate core of the gallotannin from Sicilian sumach (and Stagshorn sumach for which similar results were obtained) as a tetrasaccharide composed of one arabinose, one rhamnose, and two glucose units.

In the present investigations of the gallotannins one of the first aims, in view of these conflicting ideas, was to determine the nature of the carbohydrate core and then to establish the nature and the extent of esterification by gallic acid, but an examination of the methods of isolation and analysis of the gallotannins and the consequent application of more modern techniques was an essential preliminary to these problems. Recent work [6] has clearly shown that the tannin extracts are much more complex in composition than was previously realised; for example, paper chromatography has shown the myrobalans extract to consist of at least forty substances. Two-dimensional paper chromatograms of the crude tannin from aqueous or aqueous-acetone extracts of Chinese galls (*Rhus semialata*), Turkish or Aleppo galls (*Quercus infectoria*), the leaves of Stagshorn and Sicilian sumach (*Rhus typhina* and *Rhus coriaria*) and the pods of tara (*Caesalpinia spinosa*) similarly revealed complex mixtures of polyphenolic materials. The tannin was separated from these extracts in a chromatographically pure state by a number of methods varying with the source and nature of the extract. The gallotannins from Chinese galls and Aleppo galls were obtained free from acids and minor polyphenols by ethyl acetate extraction of an aqueous solution of the crude extract adjusted to pH 6·8. Similar treatment of crude Sicilian and Stagshorn sumach extract gave the gallotannin contaminated with materials of a flavanoid nature which, however, were removed by chromatography on Perlon powder. Tara tannin had acidic properties and was separated from other components of the extract by distribution between ethyl methyl ketone and water. In all cases the gallotannins were obtained as light amorphous solids by evaporation of solutions in acetone or by freeze-drying from t-butyl alcohol. The extremely hygroscopic nature of the gallotannins necessitated care in drying and presented many difficulties in weighing and microanalysis.

The constitution of the carbohydrate core of these tannins was investigated by the use of the carbohydrase-free galloyl esterase whose separation from the enzyme tannase has been described previously in Part I of this series.[7] Since this enzyme has no activity against oligosaccharide linkages the sugar resulting from its action on the gallotannins must represent the latter's complete carbohydrate core. Thus Chinese, Sicilian, and Stagshorn sumach gallotannins, when hydrolysed by this esterase, liberated glucose as the only carbohydrate. Similar conclusions resulted from a study of the alkaline hydrolysis of the methylated tannins (prepared by the action of diazomethane in acetone) under conditions which would not be expected to break any polysaccharide linkages. After removal of the methylated gallic acids by continuous ether-extraction and cations by an Amberlite resin, glucose was the only carbohydrate detected by paper chromatography.

[4] White, "The Chemistry of Vegetable Tannins," Soc. Leather Trades' Chemists, Croydon, 1956, p. 13.
[5] Grassmann, Stiefenhofer, and Endres, *Ber.*, 1956, **89**, 454.
[6] Kirby, Knowles, and White, *J. Soc. Leather Trades' Chemists*, 1951, **35**, 338; 1952, **36**, 148.
[7] Haslam, Haworth, Jones, and Rogers, *J.*, 1961, 1829.

For the Chinese and Sumach gallotannins the glucose was isolated after hydrolysis of the methylated material and characterised by its optical rotation and its β-penta-acetate. These results, whilst agreeing with those of Fischer,[1] Freudenberg,[2] and Karrer *et al.*,[3] are at variance with the more recent work of White [4] on Chinese gallotannin and of Grassmann and his colleagues [5] on the sumach gallotannins. During the course of our work White's conclusions have been withdrawn [8] and there is little doubt that the failure to remove inorganic salts from the alkaline hydrolysate of the tannins interfered with the paper-chromatographic analysis. We have confirmed Partridge's observations [9] that the presence of inorganic salts during paper chromatography may modify the R_F value of glucose until it simulates the behaviour of a tri- or tetra-saccharide. More difficulty was experienced in accounting for the results of Grassmann *et al.*[5] Some of the discrepancies may arise from contamination of the tannin with flavanoid material, and both rhamnose and glucose have been detected in the hydrolysis products of the flavanoids present in the sumach extract. Recently, however, at the Symposium of the Plant Phenolics Group at Egham, April 21st to 22nd, 1960, Dr. Endres kindly informed us that these results had been obtained from a tannin believed to be that of Stagshorn sumach, but that all attempts to reproduce the results with authentic samples of Stagshorn and Sicilian sumach have been unsuccessful; the tannins yielded only glucose and gallic acid on hydrolysis, thus confirming our views on the nature of the carbohydrate core of the sumach gallotannins.

TABLE 1.

Tannin	C (%)	H (%)	Gallic acid (%) *a*	Gallic acid (%) *b*	Glucose (%)	$[\alpha]_D^{20}$ (*c* 2 in acetone)
Chinese	53·2	4·0				
	53·1	4·0	102	99·4	12·1	+12·1°
	53·2	4·1	102	98·8	12·1	
Sicilian sumach	53·4	3·5		98·2	12·4	+12·6
	53·6	4·0			12·2	+12·2
Stagshorn sumach ...	53·3	4·0		97·8	12·1	
					11·7	+12·1
Turkish	53·8	4·1		97·5	16·4	+23·2
	53·2	4·0			16·5	+21·7

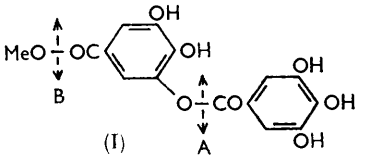
Analyses for carbon, hydrogen, gallic acid, and glucose and the $[\alpha]_D$ values of the gallotannins from Chinese galls and from Stagshorn and Sicilian sumach are shown in Table 1 and are in such close agreement as to indicate the identity of these substances with one another. The gallic acid content of the gallotannins was determined, after hydrolysis with tannase, by measurement of the optical density at 280 mμ (*a*, Table 1) or by titration of the gallic acid liberated an enzymic hydrolysis (*b*, Table 1), and the glucose was estimated after removal of the gallic acid on a column of Amberlite C.G. 400 by the method of Park and Johnson [10] in which anthrone is used. The amounts of glucose and gallic acid, which relate to the amounts of each substance liberated on hydrolysis, suggested structures containing 8—9 galloyl groups per glucose molecule, in reasonable agreement with Fischer's average formulation of Chinese gallotannin as a penta-*m*-digalloylglucose. However, the glucose and optical-rotation figures for Turkish gallotannin differ from those of the other gallotannins, and paper chromatography indicated that purification had been less successful in this case although it is not possible to decide whether the divergent figures are due to the presence of impurities or to significant structural difference. During the remainder of this paper, therefore, the term gallotannin will refer to the tannin from Chinese galls and Sicilian and Stagshorn sumach.

The arrangements of galloyl groups in the tannins was first derived from analysis of the ratio of the 3,4-di-*O*-methyl- and 3,4,5-tri-*O*-methyl-gallic acid liberated by hydrolysis

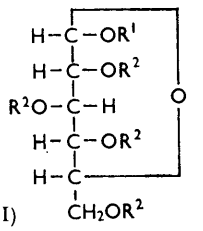
[8] White and King, *Chem. and Ind.*, 1958, 683.
[9] Partridge, *Nature*, 1949, **164**, 443.
[10] Park and Johnson, *J. Biol. Chem.*, 1949, **181**, 150.

of the methylated tannin. The total amount of acid liberated on hydrolysis was determined by titration and the proportion of 3,4-di-*O*-methylgallic acid found by measurement of the colour developed with Folin's reagent.[11] The ratio of the two methylated acids from Chinese gallotannin methylated with diazomethane was approximately 1 : 1 and indicated the presence of as many gallic acid residues linked as depsides as there were linked as normal aliphatic esters in the original tannin; this agrees approximately with figures derived by Fischer, based on the weights of the two acids isolated on hydrolysis of methylated gallotannin.

Further evidence of the extent of the depside linkages between the gallic acid residues in the tannins was derived by partial degradation of the tannin with aqueous methanol at pH 5—6; under these conditions methanolysis of the depside linkages, *e.g.*, A in formula (I), occurred to yield methyl gallate, but aliphatic ester linkages, *e.g.*, B in formula (I), were unaffected as described in Part II.[12] In order to prevent secondary reactions, probably due to oxidation, it was preferable to carry out methanolysis of the gallotannins in an atmosphere of nitrogen. After 7 days' methanolysis of Chinese and sumach gallotannins, paper chromatography revealed the presence of methyl gallate, a trace of gallic acid, and two galloylated glucoses (C) and (D) of which (C) constituted some two-thirds of the total methanolysis products. Paper-chromatographic analysis at an intermediate stage showed the presence of methyl *m*-digallate (I), thus indicating chains of at least



(I)



(II)

three galloyl groups in the tannin. The two galloylated glucoses were separated by chromatography on cellulose powder and the major component (C) was obtained as an amorphous powder, homogeneous on the basis of paper chromatography and counter-current distribution experiments, which gave analytical figures agreeing closely with those required by a penta-*O*-galloylglucose. The identification was supported by methylation, first with diazomethane and then with methyl iodide and silver oxide, to a product which on hydrolysis gave glucose and 3,4,5-tri-*O*-methylgallic acid uncontaminated with di-*O*-methylgallic acid. β-Penta-*O*-galloylglucose (II; $R^1 = R^2 =$ 3,4,5-trihydroxybenzoyl) was therefore synthesised; as Fischer and Bergmann's method[13] using tri-*O*-acetylgalloyl chloride has been shown[14] to give a mixture, tri-*O*-benzylgalloyl chloride was treated with β-glucose and pyridine in chloroform, to yield amorphous β-pentakis-*O*-(tri-*O*-benzylgalloyl)glucose which on hydrogenation afforded β-penta-*O*-galloylglucose. The amorphous powder gave analytical figures and optical-rotation and optical-density values identical with those of the major methanolysis product (C) of the gallotannin, which was indistinguishable from the latter by paper-chromatographic and counter-current analysis. Both the methanolysis product and the synthetic specimen were recovered unchanged when subjected to further methanolysis in absence of air, and at 100° both showed a change in optical rotation and of paper-chromatographic pattern probably as a result of partial isomerisation. When freeze-dried from glacial acetic acid and analysed by paper chromatography, both samples were partly transformed into a compound of unknown constitution, but the product reverted completely to β-penta-*O*-galloylglucose when subjected

[11] Swain and Hillis, *J. Sci. Food Agric.*, 1959, **11**, 63.
[12] Preceding paper.
[13] Fischer and Bergmann, *Ber.*, 1918, **51**, 1760.
[14] Asquith, *Nature*, 1951, **168**, 3738.

to methanolysis. The effects of heat and glacial acetic acid on β-penta-*O*-galloylglucose are being studied more closely.

The minor component (D) of the galloylated glucose mixture derived from the methanolysis of the tannins was less readily available, but analysis suggested a tetra-*O*-galloylglucose structure, and methylation followed by hydrolysis gave 3,4,5-tri-*O*-methylgallic acid free from di-*O*-methylgallic acids. Controlled hydrolysis of β-pentakis-*O*-(tri-*O*-benzylgalloyl)glucose gave a crystalline substance, formulated as β-2,3,4,6-tetrakis-*O*-(tri-*O*-benzylgalloyl)glucose. Hydrogenation of this crystalline substance gave an amorphous compound identical analytically and paper-chromatographically with the minor component (D) which is provisionally regarded as β-2,3,4,6-tetra-*O*-galloylglucose (II; R^1 = H, R^2 = 3,4,5-trihydroxybenzoyl) since it gave a positive reaction with aniline hydrogen phthalate [15] indicative of a potential aldehyde group.

The presence of the small amount of β-tetra-*O*-galloylglucose in the anærobic methanolysis products of the gallotannins requires further consideration. Thus Chinese and sumach gallotannins may be mixtures of closely related substances, the majority of which have as their basic core β-penta-*O*-galloylglucose, together with a small proportion which have a β-tetra-*O*-galloylglucose core. Alternatively, the small amounts of β-tetra-*O*-galloylglucose may arise initially by a partial breakdown of the natural tannin before or during isolation. To test this supposition freshly collected leaves of Stagshorn sumach were extracted at 20° with ethyl acetate to give an extract free from gallic acid, and the tannin was purified from chlorophyll and other impurities by chromatography on Perlon powder at 0°. Methanolysis of this tannin gave β-penta-*O*-galloylglucose and methyl gallate only, thus indicating that the naturally occurring tannin has a β-penta-*O*-galloylglucose core which may be modified by changes in older leaves or galls or during isolation of the tannin. However, it is not inferred that the tannins themselves are homogeneous and further work is in progress using counter-current and electrophoretic methods to test the nature and extent of any heterogeneity.

The occurrence of methanolysis may also explain some anomalous results of earlier workers; thus Fischer and Freudenberg [16] and Herzig [17] isolated methyl 3,4,5-tri-*O*-methylgallate and penta-*O*-methyl-*m*-digallate respectively on methylation of tannins with diazomethane in methanol. The alcoholysis is, in addition, of practical consequence in the extraction of tannins. Some workers, including Iljin,[18] have recommended the use of ethanol for tannin extractions, but the products differ considerably from those used by Fischer which were obtained by aqueous extraction. Thus prolonged extraction or evaporation with aqueous ethanol leads to the production of ethyl gallate which has been isolated by chloroform-extraction and identified. The residual tannin contained 13·6% of glucose and must therefore be partially degraded; criticism of Fischer's work based on the analysis of such materials is obviously invalid. Also there is no evidence to support White's [4] claim that penta-*O*-galloylglucose occurs in gallotannin extracts since the paper-chromatographic spot used for the identification of the penta-*O*-galloylglucose can be ascribed to ethyl gallate produced by ethanolysis of the tannin.

Tara tannin was different from the gallotannins described above in its pronounced acidic character,[19] and hydrolysis with the galloyl esterase from tannase gave gallic and quinic acid, thus revealing a hitherto unknown type of galloylated quinic acid tannin structure. These conclusions were confirmed by isolation of both gallic and quinic acid after acid-hydrolysis of the tannin. Analysis of the gallic and quinic acid in the tannin indicated a ratio of 4—5 : 1. The gallic acid was determined as outlined for Chinese and sumach gallotannins, and the quinic acid by titration of the free carboxyl group of the

[15] Hough, *J.*, 1950, 1702.
[16] Fischer and Freudenberg, *Ber.*, 1914, **47**, 2485.
[17] Herzig, *Ber.*, 1923, **56**, 221.
[18] Iljin, *Ber.*, 1914, **47**, 485.
[19] Burton and Nursten, "The Chemistry of Vegetable Tannins," Soc. Leather Trades' Chemists, Croydon, 1956, p. 61.

tannin, but alternative methods for the analysis of quinic acid are being developed and it is hoped more accurate analyses will soon be available. Methanolysis of tara tannin yielded methyl gallate and a complex mixture of galloylated quinic acid derivatives which have not yet been separated.

Investigations of the possibilities of separating the natural tannins by counter-current and electrophoretic methods, and further studies on Turkish and tara tannin, are in progress.

Experimental

"Light petroleum" refers to the fraction of b. p. 60—80° and alumina to Peter Spence's grade "H." Unless otherwise stated, quantitative determinations on gallotannins and galloylated glucoses were made on samples dried to constant weight at 105°/0·005 mm.

Solutions were concentrated at 25—30° under reduced pressure with a rotary evaporator.

Paper Chromatography.—Whatman No. 2 paper was used except where otherwise stated, and chromatograms were developed at 20° ± 3°.

(i) *Phenols.* Substances were chromatographed in two dimensions with solvent systems composed of (*a*) 6% acetic acid and (*b*) butan-2-ol–acetic acid–water (14 : 1 : 5). *o*-Dihydroxyphenols were revealed with a spray (*a*) composed of ferric chloride and potassium ferricyanide,[20] and materials of a flavanoid nature were detected by their absorption or fluorescence in ultraviolet light (normally also in the presence of ammonia vapour) and also by use of a citric acid–boric acid spray (*b*),[21] which revealed these substances in ultraviolet light as green fluorescent spots.

(ii) *Carbohydrates.* Carbohydrates were chromatographed with the solvent system (*b*) above, or (*c*) composed of butan-1-ol–ethanol–water (40 : 11 : 9) and detected by sprays of silver nitrate,[22] aniline hydrogen phthalate,[15] or sodium metaperiodate and benzidine.[23]

(iii) *Methylated phenolic acids.* Substances were chromatographed two dimensionally in solvent systems (*a*) and (*b*) above or in system (*d*) composed of butan-1-ol saturated with 1·5N-ammonia and 1·5N-ammonium carbonate. Phenols with a free *para*-position and those with a carboxyl group in the *para*-position were detected by spraying with a 0·1% methanolic solution of 2,6-dibromobenzoquinone 4-chloroimide (Gibbs reagent) followed by sodium hydrogen carbonate solution.

Counter-current Distribution.—Analytical counter-current distributions were carried out with solvent systems (*e*), composed of propan-1-ol–butan-1-ol–cyclohexane–water in the ratios of 33 : 11 : 7 : 49. Tubes (upper phase 15 c.c., lower phase 10 c.c.) were homogenised by the addition of propan-1-ol (9·0 c.c.) and analysed by measurement of the optical density at 320 mμ.

Extraction of Chinese Galls and Paper Chromatography of the Extract.—Crushed Chinese galls (10 g.) were shaken with water (100 c.c.) for 2 days at room temperature, and the aqueous

Table 2.

Spot	R_F (*a*)	R_F (*b*)	Spray (*a*)	U.v.–NH_3	Substance
(I)	0·00—0·30	0·32—0·54	+	Absorbs	Gallotannin
(II)	0·03	0·18	+		Unknown
(III)	0·14—0·27	0·26	+	Violet	Unknown
(IV)	0·20	0·10	+		Unknown
(V)	0·30	0·74	+		Unknown
(VI)	0·33	0·72	+	Blue	*m*-Digallic acid
(VII)	0·48	0·30	+		Unknown
(VIII)	0·49	0·70	+	Blue	Gallic acid

solution was extracted with ethyl acetate (8 × 100 c.c.). Removal of the ethyl acetate gave a gum (2 g.) which was freeze-dried from water to give a buff amorphous powder. Paper chromatography of this extract in solvent systems (*a*) and (*b*) revealed the pattern shown in Table 2.

[20] Kirby, Knowles, and White, *J. Soc. Leather Trades' Chemists*, 1953, **37**, 283.
[21] Wilson, *J. Amer. Chem. Soc.*, 1939, **61**, 2303.
[22] Trevelyan, Porter, and Harrison, *Nature*, 1950, **166**, 144.
[23] Gordon, Thornberg, and Werum, *Analyt. Chem.*, 1956, **28**, 849.

Purification of Chinese gallotannin. The aqueous extract of Chinese galls or commercial tannic acid (4·4 g.) was added to M-phosphate buffer (pH 6·8; 100 c.c.), and the solution extracted with ethyl acetate (8 × 100 c.c.) until the extract no longer reacted with ferric chloride. The ethyl acetate was removed at 30° and the residue dissolved in water (100 c.c.) and re-extracted with ethyl acetate. The resultant gum was taken up in water (10 c.c.) and freeze-dried for 12 hr. to give Chinese gallotannin (3·95 g.) as a white amorphous powder. Paper chromatography revealed in solvent systems (*a*) and (*b*) only the gallotannin, R_F (*a*) 0·00—0·30 and (*b*) 0·30—0·54.

Extraction of Sicilian or Stagshorn Sumach Leaves and Chromatography of the Extract.—Dried powdered sumach leaves (40 g.) were shaken with chloroform (100 c.c.) for 2 days, the residue was dried, and the polyphenols were removed by shaking at room temperature with 40% aqueous acetone (800 c.c.) for 24 hr. This procedure was repeated twice and the combined extracts were concentrated to 150 c.c. at 30° and extracted with ethyl acetate (8 × 100 c.c.). Removal of the ethyl acetate at 30° gave a tan-coloured gum which when freeze-dried gave a yellow powder (10 g.). Paper-chromatographic analysis in systems (*a*) and (*b*) is recorded in Table 3.

TABLE 3.

Spot	R_F (*a*)	R_F (*b*)	Spray (*a*)	Spray (*b*)	U.v.	U.v.–NH_3	Substance
(I)	0·05	0·25	—	+	—	Yellow	Unknown
(II)	0·05	0·35	—	+	—	Yellow	Unknown
(III)	0·04	0·49	—	+	—	Green	Unknown
(IV)	0·04	0·66	—	+	—	Yellow	Unknown
(V)	0·04—0·15	0·56	—	+	—	Green	Unknown
(VI)	0·13	0·68	—	+	—	Green	Unknown
(VII)	0·00—0·30	0·32—0·54	+	—	Absorbs	Absorbs	Gallotannin
(VIII)	0·10—0·23	0·44	—	+	Absorbs	Yellow	Unknown
(IX)	0·33	0·63	+	—	Absorbs	Blue	*m*-Digallic acid
(X)	0·15—0·38	0·59	+	+	Absorbs	Yellow	Unknown
(XI)	0·16—0·39	0·68	+	+	Absorbs	Yellow	Unknown
(XII)	0·21—0·38	0·75	—	+	Absorbs	Yellow	Unknown
(XIII)	0·45	0·64	+	—	—	Blue-violet	Gallic acid
(XIV)	0·50	0·60	—	—	—	Blue	Unknown
(XV)	0·53	0·27	+	—	—	Violet	Unknown
(XVI)	0·58	0·32	+	—	—	Violet	Unknown
(XVII)	0·56	0·40	+	—	—	Violet	Unknown
(XVIII)	0·54	0·56	—	—	—	Blue-green	Unknown
(XIX)	0·60	0·54	—	—	—	Blue	Unknown
(XX)	0·58	0·68	—	—	—	Blue	Unknown

Purification of Stagshorn or Sicilian sumach gallotannin. The extract (6 g.) was dissolved in methanol (20 c.c.) and chromatographed at 0° on a column of Perlon (200 g.; 60 × 3·5 cm.). At 0° methanolysis of the crude tannin did not occur and methyl gallate was not detected in the eluate. Elution at 0° with methanol (200 c.c.) removed the flavanoid material after which elution was continued with ethyl methyl ketone–water azeotrope as solvent; fractions (10 c.c.) were collected and their optical density measured at 335 mμ. After an initial peak containing the remaining flavanoids and gallic acid, the gallotannin was eluted in a peak (2 l.). Concentration of this eluate gave a gum which was freeze dried from water or t-butyl alcohol to give sumach gallotannin as a white amorphous powder (1·5 g.), R_F (*a*) 0·00—0·30 and (*b*) 0·32—0·54.

Extraction of Turkish (Aleppo) Galls and Chromatography of the Extract.—Powdered Turkish galls (10 g.) were shaken with water (100 c.c.) for 2 days at room temperature, and the aqueous solution was then extracted with ethyl acetate (8 × 100 c.c.). Removal of the ethyl acetate gave a gum (2 g.) which was freeze-dried from water to give a buff amorphous powder. Paper chromatography of this powder in solvent systems (*a*) and (*b*) revealed the pattern shown in Table 4.

Purification of Turkish gallotannin. The extract (2 g.) was purified in an identical manner to that of Chinese galls, to give Turkish gallotannin (1·4 g.) as a white amorphous powder, R_F (*a*) 0·02—0·37 and (*b*) 0·36—0·50.

Determination of Glucose in Gallotannins.—The method used was an adaption of that employed by Park and Johnson.[10] Gallotannin (10 mg.) was dissolved in 0·5N-acetate buffer

TABLE 4.

Spot	R_F (*a*)	R_F (*b*)	Spray (*a*)	U.v.–NH_3	Substance
(I)	0·02	0·27—0·41	—	Blue-green	Ellagic acid
(II)	0·02—0·37	0·36—0·50	+	Absorbs	Gallotannin
(III)	0·22	0·71	+	Blue	Unknown
(IV)	0·36	0·70	+	Blue	*m*-Digallic acid
(V)	0·39	0·33	+	Violet	Unknown
(VI)	0·47	0·65	+	Blue	Gallic acid
(VII)	0·51	0·02	+	Absorbs	Unknown
(VIII)	0·51	0·18	+	—	Unknown
(IX)	0·51	0·38	+	Absorbs	Unknown
(X)	0·55	0·30	+	Violet	Unknown
(XI)	0·60	0·32	+	Violet	Unknown
(XII)	0·68	0·45	—	Blue-violet	Unknown

pH 6·0 (2 c.c.), 0·025% tannase solution (1 c.c.) and a few drops of toluene were added, and the whole was incubated at 37° for 2 days; gallic acid was then the only phenol detectable by paper chromatography in systems (*a*) and (*b*). Samples (1 c.c.) were passed down Amberlite C.G. 400 columns (0·7 × 3 cm.), the columns were washed with distilled water, and the eluates collected in graduated flasks (10 c.c.). The volume of each was made up to 10 c.c., samples (0·2, 0·4, 0·6, 0·8, and 1·0 c.c.) were withdrawn and placed in test tubes, and distilled water was added to 1 c.c. To these solutions, cooled in ice, a mixture of ethanol and concentrated hydrochloric acid (1 : 1; 1·5 c.c.) was added followed by a 0·1% solution of anthrone in " AnalaR " 98% sulphuric acid (3 c.c.), and the solutions were mixed with a glass rod. The tubes were suspended in boiling water for 7 min., then kept at room temperature for 20 min., whereafter the optical density was measured at 620 mμ. The results were plotted and compared with the graph obtained with one or more standard glucose solutions treated similarly. Results are shown in Table 1.

Determination of Gallic Acid in Gallotannin.—(a) *Optical-density measurement.* The extinction coefficient $E^{1\,cm.}_{mole}$ at 280 mμ of gallic acid in acetate buffer pH 6·0 was $3{\cdot}65 \times 10^3$. Gallotannin (10 mg.) was dissolved in 0·5N-acetate buffer of pH 6·0 (10 c.c.), 0·025% tannase (0·5 c.c.) added, and the solution incubated for 24 hr. at 37°. Aliquot parts (2·0 c.c.) were diluted to 100 c.c. each and the optical density measured at 280 mμ. Enzyme controls were also determined. Results are shown in Table 1.

(b) *Titration.* The theoretical end-point for the titration of gallic acid with 0·5N-sodium hydroxide was determined at 37° to be pH 6·5. Gallotannin (2 mg.) was dissolved in water (1·0 c.c.) and placed in a small cell (5 c.c.) at 37°; a fine stream of oxygen-free nitrogen passed through the solution. The pH was adjusted to 5·5 with an autotitrator, and 0·05% tannase (1 c.c.) added. As the hydrolysis proceeded addition of 0·5N-sodium hydroxide by the autotitrator maintained the pH at 5·5 and after 6 hr., when the reaction was complete, the pH was adjusted to 6·5 and the alkali consumed determined. Enzyme controls were also determined. Results are shown in Table 1.

Action of Purified Tannase Esterase on Chinese and Sumach Gallotannins.—The tannin (4 mg.) was dissolved in 0·5N-acetate buffer of pH 6·0 (4·0 c.c.) and incubated for 48 hr. with the purified tannase esterase (0·1% solution; 0·4 c.c.) prepared as described in Part I of this series. At this stage paper chromatography in solvent system (*a*) showed gallic acid to be the only phenol present. Sodium ions were removed on a small column of Dowex " 50 " (H^+), and the tannin hydrolysate was examined for sugars by paper chromatography. Similarly treated solutions of glucose, maltose, and raffinose were used as standards. Glucose, R_F (*b*) 0·20, was the only sugar detected in the tannin hydrolysates.

Isolation of Glucose from Sicilian Sumach and Chinese Gallotannin.—Sicilian sumach tannin (or Chinese gallotannin) (1·16 g.), dissolved in acetone (50 c.c.), was treated with an excess of ethereal diazomethane for 24 hr. and the process repeated twice. The solvents were removed at 30° and the residue was treated with 12% methanolic potassium hydroxide (40 c.c.) for 48 hr. at 0°, potassium ions were removed by passage down a column of ZeoKarb 215, and the resultant solution was continuously extracted with ether (4 days) to remove 3,4-di-*O*-methylgallic and 3,4,5-tri-*O*-methylgallic acid. Concentration of the aqueous phase gave a brown gum (0·133 g.) which paper-chromatographic analysis in solvent systems (*b*) and (*c*) showed to contain glucose, R_F (*b*) 0·20, as the only carbohydrate. The glucose was freeze-dried, refluxed for 3 hr. with a mixture of anhydrous sodium acetate (0·1 g.) and acetic anhydride (3 c.c.), and

poured into water (100 c.c.). The precipitated oil separated, after several crystallisations, from ethanol as crystals, m. p. 130° undepressed on admixture with glucose β-penta-acetate.

Detection of Rhamnose in Sicilian Sumach Extract.—Crude Sicilian sumach tannin (10 mg.) was hydrolysed for 24 hr. at 100° with 1% sulphuric acid (2 c.c.), and the sulphate ions were removed on a small column of Dowex " 2 " (acetate form), or alternatively by addition of 0·2M-barium hydroxide (1 c.c.) with separation of the barium sulphate on a centrifuge. The hydrolysate when examined paper-chromatographically in systems (*b*) and (*c*) was seen to contain glucose, R_F (*b*) 0·20, and rhamnose, R_F (*b*) 0·30. Examination by this procedure of the fractions obtained by chromatography on Perlon powder of this crude Sicilian sumach tannin showed rhamnose, R_F (*b*) 0·30, to be present in the flavanoids eluted with methanol.

Determination of Ratio of 3,4,5-*Tri*-O-*methylgallic and* 3,4-*Di*-O-*methylgallic Acid produced on Hydrolysis of Methylated Chinese and Sumach Gallotannin.*—The gallotannin (0·20 g.) was methylated in acetone (5 c.c.) with an excess of diazomethane as described by Fischer and Freudenberg.[24] After removal of the acetone the residue was taken up in fresh acetone (5 c.c.), methyl iodide (3 c.c.) and silver oxide (1·0 g.) were added, and the solution was refluxed for 5 days with exclusion of moisture. The silver oxide and acetone were removed, and the residual methylated gallotannin (0·20 g.) was dissolved in 12% methanolic potassium hydroxide (4·0 c.c.). After 48 hr. at 0°, the solution was diluted to 100 c.c. with water. Aliquot parts (1 c.c.) were titrated against 0·1N-hydrochloric acid to pH 7. A blank determination without the methylated gallotannin gave the total weight of the methylated acids produced on hydrolysis. The percentage of free phenol in the mixture was then determined with Folin's reagent according to the method of Swain and Hillis,[11] and the ratio of the two methylated acids liberated was then calculated.

Methanolysis of Chinese Gallotannin.—Chinese gallotannin (3 g.) was dissolved in a solution of 0·5N-acetate buffer (pH 6·0; 30 c.c.) in methanol (300 c.c.) which had previously been de-oxygenated by boiling under nitrogen; the mixture was kept at 37° for 7 days with a very slow nitrogen stream passing through it. Removal of the solvents at 30°, solution of the residue in water (100 c.c.), and extraction with ethyl acetate (7 × 100 c.c.) gave a gum which when analysed by paper chromatography in solvents (*a*) and (*b*) revealed the following pattern shown in Table 5.

TABLE 5.

Spot	R_F (*a*)	R_F (*b*)	U.v.–NH_3	Substance
(I)	0·02—0·14	0·58	Absorbs	C
(II)	0·20	0·60	Absorbs	D
(III)	0·47	0·70	Blue	Gallic acid
(IV)	0·52	0·82	Blue	Methyl gallate

The gum was dissolved in 6% aqueous acetic acid (20 c.c.) and applied to a cellulose column (B.W. 200; 6·5 × 66 cm.) packed in the same solvent. Elution was carried out with 6% acetic acid and 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 solvents were removed from each at 30°.

Fraction I yielded methyl gallate which recrystallised from aqueous methanol in needles (1·2 g.), m. p. and mixed m. p. 196°, R_F (*a*) 0·52, (*b*) 0·82, and fraction 2 gave gallic acid, m. p. and mixed m. p. 240°, R_F (*a*) 0·47, (*b*) 0·70. A solution of the gummy fraction 3 in acetone was filtered and evaporated at 30°. The residue was taken up in ethyl acetate (100 c.c.), washed with water (3 × 100 c.c.), and recovered at 30°. The residue, freeze-dried from water or t-butyl alcohol, yielded *component D* as a white amorphous powder (0·08 g.) (Found: C, 51·4; H, 4·0; glucose, 22·1, 22·3. $C_{34}H_{28}O_{22}$ requires C, 51·8; H, 3·6; glucose, 22·8%) with R_F (*a*) 0·20 and (*b*) 0·60. Similar treatment of fraction 4 gave *component C* as a white amorphous powder (1·08 g.) (Found: C, 52·1, 52·4; H, 3·6, 3·9; glucose, 19·0, 19·2. $C_{41}H_{32}O_{26}$ requires C, 52·3; H, 3·4; glucose, 19·2%) with R_F (*a*) 0·02—0·14, (*b*) 0·58.

Methyl Tri-O-*benzylgallate.*—This was prepared by a modification of the method described by Clinton and Geissman.[25] Methyl gallate (75 g.) was added to a stirred mixture of potassium

[24] Fischer and Freudenberg, *Ber.*, 1914, **47**, 2485.
[25] Clinton and Geissman, *J. Amer. Chem. Soc.*, 1943, **65**, 85.

carbonate (200 g.), potassium iodide (50 g.), and acetophenone (600 c.c.) at 100°. The temperature was raised to 140—150° and stirring continued in an atmosphere of carbon dioxide during the addition, in 1 hr., of benzyl chloride (220 c.c.) and then for a further 4 hr. The mixture was steam-distilled and, after cooling, the solid non-volatile product was collected. By chromatography on alumina (45 × 5·2 cm.) with benzene as eluant and crystallisation twice from ethyl acetate–light petroleum, *methyl tri-O-benzylgallate* (174 g., 94%) was obtained as colourless needles or prisms, m. p. 100·5—101·5° (Found: C, 76·7; H, 6·0. $C_{29}H_{26}O_5$ requires C, 76·6; H, 5·8%). Crystallisation from benzene–light petroleum afforded white needles of a *solvate*, m. p. 90·5—91·5° (Found: C, 77·6, 77·5; H, 6·1, 5·9. $C_{29}H_{26}O_5,\frac{1}{2}C_6H_6$ requires C, 77·9; H, 5·9%) containing benzene (Ramsden test [26]) which was eliminated by drying at 60°/0·002 mm. Clinton and Geissman [25] describe a compound, m. p. 89—90°, crystallising from benzene–light petroleum as the unsolvated form.

Tri-O-benzylgalloyl Chloride.—The foregoing ester (50 g.) was suspended in boiling methanol (400 c.c.), and 20% aqueous potassium hydroxide (46 c.c., 1·5 mol.) added during 1 hr. After a further hour on the steam-bath the solution was cooled and acidified with dilute hydrochloric acid, and tri-*O*-benzylgallic acid was collected, washed with water, dried, and crystallised from ethyl acetate–light petroleum; white needles (44 g., 91%), m. p. 191—191·5°, were obtained (Schmidt and Schach [27] give m. p. 192°). The acid was converted quantitatively into tri-*O*-benzylgalloyl chloride which separated from benzene–light petroleum in colourless needles, m. p. 116·5—117° (Schmidt and Schach [27] give m. p. 115—116°).

Pentakis-O-(tri-O-benzylgalloyl)-β-glucose.—Powdered β-glucose [28] (2·5 g.), $[\alpha]_D^3$ +18·1 ± 0·03° (*c* 9 in H_2O), tri-*O*-benzylgalloyl chloride (38·4 g., 6 mol.), pyridine (15 c.c.), and chloroform (100 c.c.) were stirred until the glucose dissolved (24—30 hr.). The pale yellow solution was heated at 61° for 18—21 days during which pyridine hydrochloride separated and the solution darkened. The cooled mixture was diluted with chloroform (250 c.c.), shaken successively with water, dilute sulphuric acid, and aqueous sodium hydrogen carbonate, dried ($MgSO_4$), and evaporated at 20°. The viscous residue was dissolved in benzene, the solution was evaporated to small volume at 20°, and *tri-O-benzylgallic anhydride* removed; crystallisation from ethyl acetate–light petroleum gave needles (3·9 g.), m. p. 166·5—167° (Found: C, 78·1; H, 5·85. $C_{56}H_{46}O_9$ requires C, 78·0; H, 5·4%). Schmidt and Schach [27] give m. p. 165·5° but no analytical results. The filtrate and benzene washings were further concentrated, freed from a second crop (1·1 g.) of anhydride, and adsorbed on ethyl acetate-washed alumina (30 × 3·5 cm.). The column was eluted with benzene (2 l.), the eluate evaporated at 20°, and the residual glass freeze-dried from benzene. After drying at room temperature and 0·002 mm., the product (30·5 g., 97·5%) was obtained as a light buff-coloured powder $[\alpha]_D^{24}$ −2·1° ± 0·3° (*c* 3 in ethyl acetate). This material was repeatedly chromatographed on ethyl acetate-washed alumina (20 × 3·5 cm.), covered by a layer (4 cm.) of alumina, with benzene (1·5 l.) as eluant until no change in optical rotation was observed. After freeze-drying, *pentakis-O-(tri-O-benzylgalloyl)-β-glucose* was obtained as a white amorphous powder (Found: C, 76·1, 76·5; H, 5·7, 5·9. $C_{146}H_{122}O_{26}$ requires C, 76·7; H, 5·4%), $[\alpha]_D^{22·5}$ −6·4° ± 0·4° (*c* 3 in ethyl acetate), readily soluble in acetone, benzene, chloroform, methylene dichloride, ether, ethyl acetate, dioxan, and tetrahydrofuran, but sparingly soluble in methanol and ethanol.

2,3,4,6-*Tetrakis-O-(tri-O-benzylgalloyl)-β-glucose*.—A solution of the foregoing ester (10 g.) in benzene (250 c.c.) was diluted with an equal volume of light petroleum and shaken intermittently with acetic acid-washed alumina (250 g., activated at 140—150° for 24 hr.) for 16 days at room temperature. This mixture was poured on a column (40 × 3·5 cm.), the solvents were removed, and the alumina was exhaustively eluted with benzene (2 l.) and with benzene–chloroform (1 : 1; 2 l.). Hydrogenation followed by paper chromatography indicated that the fraction (1·5 g.) recovered from benzene contained pentakis(tribenzylgalloyl)glucose, and the fraction (6·0 g.) from the benzene–chloroform eluate consisted of a mixture of tetrakis- and pentakis-(tribenzylgalloyl)glucoses. This mixture was rechromatographed on ethyl acetate-washed alumina (18 × 2 cm.), and eluted with benzene (1·5 l.), and then with benzene–chloroform (1 : 1; 1 l.). Evaporation at 25° of the benzene–chloroform eluate afforded a pale yellow glass, which was dissolved in warm ether (70 c.c.), and, after 12 hr., the gelatinous precipitate was removed and the filtrate nucleated; the first crystals were obtained by careful

[26] Bradley, Robinson, and Schwarzenbach, *J.*, 1930, 812.
[27] Schmidt and Schach, *Annalen*, 1951, **571**, 29.
[28] Hudson and Dale, *J. Amer. Chem. Soc.*, 1917, **39**, 320.

removal of the benzene followed by prolonged contact and trituration with fresh quantities of ether. After several days, 2,3,4,6-*tetrakis*-O-(*tri*-O-*benzylgalloyl*)-β-*glucose* was collected and thrice recrystallised from methylene dichloride–ether, to give colourless needles (3·15 g.) (Found: C, 76·2; H, 5·7. $C_{118}H_{100}O_{22}$ requires C, 75·8; H, 5·4%), gradually shrinking on heating to a turbid gum, which cleared at 97—98°. A further quantity (0·85 g.) was recovered from the mother-liquors.

Penta-O-*galloyl*-β-*glucose*.—The pentakis(tribenzylgalloyl)glucose (6·42 g.) in tetrahydrofuran (50 c.c.) was reduced at room temperature over 10% palladised charcoal (0·65 g.) which had been washed with water until the washings had pH 7·0. Hydrogen absorption ceased after 8 hr.; the mixture was filtered through a layer of cellulose powder, and the catalyst washed rapidly with a little deoxygenated ethanol. The filtrate and washings were evaporated at 25° and the residual glass was thrice dissolved in acetone and recovered at 25°. The product, freeze-dried from t-butyl alcohol, was a white amorphous powder (Found: C, 52·7, 52·4, 52·0; H, 3·8, 3·7, 3·7; glucose, 19·1, 18·9. $C_{41}H_{32}O_{26}$ requires C, 52·3; H, 3·4; glucose, 19·2%) with R_F (*a*) 0·02—0·14, (*b*) 0·58.

Pentagalloylglucose and compound C (above) were readily soluble in methanol, ethanol, acetone, tetrahydrofuran, ether, dioxan, and 2-methoxyethanol, moderately soluble in t-butyl alcohol, and sparingly soluble in water, but insoluble in benzene, chloroform, or light petroleum. Samples dried to constant weight at 20°/0·005 mm. gave (*c* 2 in acetone) $[\alpha]_D^{23\cdot5}$ 17·7° ± 0·3° for synthetic material, and 17·6°, 17·4° ± 0·3° for material from methanolysis of gallotannin. Counter-current distribution in solvent system (*e*) showed that both samples were homogeneous (at 20°, *K* 1·04), and the distribution pattern was unaffected on admixture. The extinction coefficient measured in solvent system (*e*) [top phase (10 c.c.), lower phase (15 c.c.), homogenised with propan-1-ol (9 c.c.)] was $6\cdot5 \times 10^3$, and hydrolysis with tannase at pH 6·0 and 37° yielded glucose and gallic acid. The products were recovered after being heated with 90% aqueous methanol adjusted to pH 6·0 for 48 hr. After methylation and hydrolysis as described above, the hydrolysate was passed down a column (20 × 1·5 cm.) of ZeoKarb 215(H^+) to remove potassium ions and concentrated to 1 c.c. at 30°; paper chromatography in solvent system (*d*) and spraying with Gibbs's reagent showed only 3,4,5-tri-*O*-methylgallic acid.

2,3,4,6-*Tetra*-O-*galloyl*-β-*glucose*.—2,3,4,6-Tetrakis-*O*-(tri-*O*-benzylgalloyl)-β-glucose (0·504 g.) was reduced as described for the preparation of pentagalloylglucose (above). The product, after freeze-drying from t-butyl alcohol, was a colourless amorphous powder (Found: C, 52·1; H, 4·0; glucose, 22·4, 22·5. $C_{34}H_{28}O_{22}$ requires C, 51·8; H, 3·6; glucose, 22·8%) with R_F (*a*) 0·20, (*b*) 0·60; $[\alpha]_D^{24\cdot5}$ 55·4° ± 0·6° (*c* 2 in acetone).

2,3,4,6-*Tetragalloylglucose* and compound D (above) had solubilities as described for pentagalloylglucose and gave brown colours with aniline hydrogen phthalate in moist butan-1-ol (under similar conditions pentagalloylglucose gave no colour). Methylation and hydrolysis gave 3,4,5-tri-*O*-methylgallic acid only.

Isolation of Ethyl Gallate from Chinese Galls Extracted with Ethanol.—Crushed Chinese galls (5 g.) were extracted by shaking with 50% aqueous ethanol (250 c.c.) for 48 hr. The extract was concentrated at 30° to 100 c.c., adjusted to pH 6·8 with 0·2M-potassium dihydrogen phosphate (50 c.c.) and M-potassium hydroxide and extracted with ethyl acetate (4 × 150 c.c.). Removal of the ethyl acetate and freeze-drying of the residue gave a buff-coloured powder (1·2 g.) which paper-chromatographic analysis showed to contain the gallotannin and a substance with R_F (*a*) 0·55 and (*b*) 0·88, giving a positive reaction with the spray (*a*). The tannin extract was continuously extracted with chloroform (Soxhlet) for 96 hr.; removal of the chloroform gave a yellow gum from which ethyl gallate was obtained by sublimation (100°/0·05 mm.) and recrystallisation from methanol–water as needles (0·08 g.), m. p. and mixed m. p. 140°. Ethyl gallate subjected to paper-chromatographic analysis had R_F (*a*) 0·55 and (*b*) 0·88.

Extraction of Pods of Caesalpinia spinosa *and Chromatography of the Extract*.—Finely ground pods of *Caesalpinia spinosa* (40 g.) were shaken for 24 hr. with water (500 c.c.). Filtration, removal of water at 30°, and freeze-drying of the residue gave the tara extract (21—24 g.) as a buff solid. Its paper chromatography is recorded in Table 6. Quinic and shikimic acid were revealed with a spray of sodium metaperiodate followed by sodium nitroprusside and piperazine.[29]

The extract (10 g.) was dissolved in ethyl methyl ketone saturated with water (1 : 1), and this solution extracted 9 times with water saturated with the ketone (1·5 l. in all). The ketone

[29] Cartwright and Roberts, *Chem. and Ind.*, 1955, 231.

phase was evaporated to 50 c.c., water added, the whole evaporated, and the residue freeze-dried from water to give *tara tannin* (0·95—1·05 g.) as an off-white powder (Found: C, 52·0; H, 4·0. $C_{35}H_{28}O_{22}$ requires C, 52·5; H, 3·5%), R_F (*a*) 0·03—0·24, (*b*) 0·46—0·67.

Isolation of Gallic and Quinic Acid from Tara Tannin.—A mixture of tara tannin (1 g.) and 3N-hydrochloric acid (67 c.c.) was heated at 100° for 8½ hr., the solution becoming dark brown. After filtration, the solution was extracted with ether (10 × 70 c.c.). The extracts yielded

TABLE 6.

Spot	R_F (*a*)	R_F (*b*)	Spray (*a*)	U.v.–NH_3	Substance
(I)	0·02	0·28—0·41	—	Blue-green	Ellagic acid
(II)	0·02—0·37	0·35—0·50	+	Absorbs	Tara tannin
(III)	0·17	0·70	+	Blue-violet	Trigallic acid?
(IV)	0·29	0·69	+	Blue-violet	*m*-Digallic acid
(V)	0·20—0·39	0·30—0·40	+	Violet	Unknown
(VI)	0·45	0·66	+	Blue-violet	Gallic acid
(VII)	0·47	0·45	+	Absorbs	Unknown
(VIII)	0·49	0·35	+	Absorbs	Unknown
(IX)	0·64	0·40	+	Violet	Unknown
(X)	0·64	0·52	—	Green	Unknown
(XI)	0·64	0·66	—	Green	Unknown
(XII)	0·66—0·78	0·33	+	Violet	Unknown
(XIII)	0·70	0·75	—	Blue	Unknown
(XIV)	0·72	0·58	—	Green	Unknown
(XV)	0·75	0·70	—	Blue	Unknown
(XVI)	0·83	0·48	—	—	Shikimic acid
(XVII)	0·84	0·18	—	—	Quinic acid
(XVIII)	0·96	0·10	—	—	Carbohydrates

crude gallic acid (0·75 g.) which crystallised from water in needles, m. p. and mixed m. p. 240° (decomp.), and on treatment with excess of ethereal diazomethane in acetone afforded methyl 3,4,5-tri-*O*-methylgallate, m. p. and mixed m. p. 80°. The aqueous solution, after ether-extraction, was evaporated at 30°, diluted with water, and re-evaporated to remove all the hydrochloric acid. The residue was dissolved in water (25 c.c.) and filtered through charcoal (3 × 3 cm.) to remove residual polyphenols. After washing of the charcoal with water, the filtrate and washings were combined, concentrated at 30° to 20 c.c., and applied to a column of Dowex " 2 " (acetate form; 40 × 1 cm.). The column was washed with water (75 c.c.) and successive portions (100 c.c.) of 0·2N-, 0·5N-, N-, 2N-, and 4N-acetic acid, and each eluted fraction (100 c.c.) was concentrated to 10 c.c. and tested for quinic acid.[29] Evaporation at 30° of the 2N-eluate gave quinic acid (0·11 g.), which crystallised from ethanol in prisms, m. p. and mixed m. p. 174—175° (Found: C, 44·0; H, 6·4. Calc. for $C_7H_{12}O_6$: C, 43·8; H, 6·3%). This acid had an infrared spectrum identical with authentic quinic acid and on treatment with acetic anhydride [30] gave triacetylquinide, m. p. and mixed m. p. 133—134°.

Determination of Gallic Acid in Tara Tannin.—Gallic acid was determined as in Chinese or sumach gallotannin by measurement of (*a*) the optical density at 280 mμ after hydrolysis with tannase or (*b*) the alkali consumed during hydrolysis by tannase [Found: (*a*) 86·9, 87·8; (*b*) 83·0, 83·7%).

Determination of the Equivalent Weight of Tara Tannin.—Titration of mixtures of quinic acid and β-glucogallin (1 : 4) indicated that titration of the carboxyl group of quinic acid in presence of the gallate ion was complete at pH 5·4 at 37° or at pH 5·8 at 20°. Tara tannin (0·016 g.) was dissolved in water (10 c.c.), and aliquot parts (2 c.c.) were titrated with 0·05N-sodium hydroxide (Found: equiv., 873).

We thank the Department of Scientific and Industrial Research for a postgraduate fellowship to J. W. G. and for a maintenance grant to K. J.; the University for a Henry Ellison fellowship and E. H. Linley scholarship to H. J. R. and G. S. B. respectively; the Leverhulme Trust and Thos. Hedley & Co. for fellowships to T. S. and R. A. respectively; and Dr. R. O. B. Wijisekera who made some preliminary experiments on the synthesis of pentagalloylglucose. We also thank Miss M. A. McKinnon, B.A., and Miss V. Dufty of the microanalytical laboratory of this Department for extremely valuable work in the microanalysis of the hygroscopic materials.

THE UNIVERSITY, SHEFFIELD. [*Received, November 9th*, 1960.]

[30] Erwig and Koenigs, *Ber.*, 1889, **22**, 1459.