571. *Gallotannins. Part V.* The Structure of Penta- and Tetra-O-galloylglucoses, and Some Observations on the Molecular Weights of the Gallotannins.*

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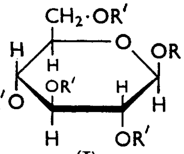
β-Pyranose structures have been established for the tetra- and penta-*O*-galloylglucoses obtained previously [1] by methanolysis of Chinese and sumach gallotannin. Molecular-weight measurements by the thermoelectric osmometer or the ultracentrifuge method indicate that the tannins are monomeric in acetone but strongly associated in aqueous solution.

In an earlier communication [1] the gallotannins from Chinese galls (*Rhus semialata*) and the leaves of stagshorn or Sicilian sumach (*Rhus typhina* and *R. coriaria*, respectively) were shown to be essentially the same octa- or nona-galloylated glucose. Methanolysis of gallotannin gave methyl gallate and two galloylated glucoses which were formulated as penta- (I; R = R′ = 3,4,5-trihydroxybenzoyl) and 2,3,4,6-tetra-*O*-galloyl-β-glucose (I; R = H, R′ = 3,4,5-trihydroxybenzoyl) after comparison with synthetic products. In this paper additional proof of the structures of these esters is given, as well as further properties of them and of the gallotannins.

The formulation of the penta- and tetra-*O*-galloylglucose as β-D-glucopyranose derivatives followed from their synthesis from β-D-glucose but further proof has been obtained as follows. As mentioned in the earlier communication,[1] treatment of pentakistri-*O*-benzylgalloyl-β-glucose (I; R = R′ = tri-*O*-benzylgalloyl) with alumina gave crystalline 2,3,4,6-tetrakistri-*O*-benzylgalloyl-β-D-glucose (I; R = H, R′ = tri-*O*-benzylgalloyl), methylation of which gave amorphous, non-reducing methyl 2,3,4,6-tetrakistri-*O*-benzylgalloyl-β-glucoside (I; R = Me, R′ = tri-*O*-benzylgalloyl), identical polarimetrically with the product of condensation of tri-*O*-benzylgalloyl chloride and methyl β-D-glucoside. The identity of these two glucosides was further demonstrated by their conversion, in good yield by removal of the tri-*O*-benzylgaloyl groups with alkali and acetylation of the products, into methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucoside (I; R = Me, R′ = Ac). This conversion indicates that 2,3,4,6-tetrakistri-*O*-benzylgalloyl-β-D-glucose, and hence the related penta- and tetra-*O*-galloylglucose obtained by degradation of Chinese and sumach gallotannins, exist in the glucopyranose configuration. A point of note in the chemistry of these substances and one previously observed by Schmidt and Schach [2] is the lack of mutarotation of tri-*O*-benzylgalloyl esters of glucose that have their glucosidic hydroxyl group unesterified.



(I)

Hydrogenation of methyl 2,3,4,6-tetrakistri-*O*-benzylgalloyl-β-D-glucoside (I; R = Me, R′ = tri-*O*-benzylgalloyl) derived by either of the methods described above gave the same methyl 2,3,4,6-tetra-*O*-galloyl-β-D-glucoside (I; R = Me, R′ = 3,4,5-trihydroxybenzoyl); the identity of the products was demonstrated by paper chromatography and polarimetry. Attempts, however, to remove the glucosidic methyl group with dilute acid from both methyl 2,3,4,6-tetrakistri-*O*-benzylgalloyl- and 2,3,4,6-tetra-*O*-galloyl-β-D-glucoside were unsuccessful and under more vigorous acidic conditions rupture of the ester linkages also occurred.

Confirmation of the structure and the β-configuration of the glucosidic galloyl group in the major methanolysis product, penta-*O*-galloyl-β-D-glucose (I; R = R′ = 3,4,5-trihydroxybenzoyl), was obtained by its methylation to the crystalline pentakistri-*O*-methylgalloyl-β-glucose (I; R = R′ = tri-*O*-methylgalloyl). The methylation products derived from synthetic penta-*O*-galloyl-β-D-glucose and the methanolysis product of the major

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* Part IV, *J.*, 1961, 1854.

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

[2] Schmidt and Schach, *Annalen*, 1959, **571**, 29.

gallotannin were identical with the product of condensation of tri-*O*-methylgalloyl chloride and β-D-glucose prepared by Fischer and Freudenberg's method.[3] The β-configuration in this crystalline compound is established because Fischer and Freudenberg [3] also prepared the 1-anomer from tri-*O*-methylgalloyl chloride and α-D-glucose and showed it to have a considerably higher positive optical rotation. Similar correlation followed from comparisons of pentagalloyl-α- and -β-glucose and their methylation products prepared by Fischer and Bergmann.[4] Condensation of methyl β-glucoside and tri-*O*-methylgalloyl chloride gave an amorphous product but further comparison with the methyl 2,3,4,6-tetra-*O*-galloyl-β-D-glucoside was not attempted.

Initially, in these studies of the constitution of the gallotannins, it had been hoped that application of countercurrent distribution [5] would solve the problem of the homogeneity of the gallotannins which has been outstanding since the work of Fischer [6] and Freudenberg.[7] The behaviour of penta- and 2,3,4,6-tetra-*O*-galloyl-β-D-glucose on countercurrent analysis in butan-1-ol–propan-1-ol–cyclohexane–water (90 transfers) now makes this appear improbable: both gave symmetrical distribution curves with distribution coefficients (K) 1·04 and 1·34, respectively; an equimolar mixture when similarly analysed showed no separation, but a Maxwellian distribution was obtained ($K = 1{\cdot}19$) which probably indicates strong association of the two species in solution. Thus any conclusions regarding the number of molecular species present in Chinese and sumach gallotannins following from analysis of the respective distribution curves must be invalid in view of this demonstration of strong association in aqueous solution of related complex polyphenols.

Similar indications of association of the polygalloyl derivatives in aqueous solution were observed during measurements of the molecular weight of gallotannins. We thank Dr. H. L. Riley of the United Coke and Chemical Co., Handsworth, Sheffield, for facilities for molecular-weight determination of the gallotannins in both acetone and aqueous solutions by the thermoelectric osmometer,[8] and Dr. Baronetsky for advice and help during the measurements. In acetone solution Chinese gallotannin gave a number average value of 1253 (±60), indicative of a hepta- or octa-galloylglucose; penta-*O*-galloyl-β-D-glucose gave by this method a value of 944 (±48) in close agreement with the theoretical value of 940. We are also deeply indebted to Dr. W. E. F. Naismith of Imperial Chemical Industries Limited, Fibres Division, Harrogate, for molecular-weight measurements of the gallotannin in acetone solution made with a Spinco ultracentrifuge and employing the sedimentation velocity method. These results also indicated that the polygalloyl derivatives exist predominantly in the monomeric state in acetone solution, and further that the gallotannin has a molecular weight 1·5 times that of penta-*O*-galloyl-β-D-glucose (calculated for gallotannin as an octagalloylglucose, 1·48), but the absolute values of the molecular weights could not be measured because of lack of diffusion data. In aqueous solution Chinese gallotannin gave results varying with the solute concentration: by the thermoelectric osmometer at 10% and 20% concentration values of 4016 and 5450 were obtained; values were reproducible after 2 days' storage of the solution, but measurements were not possible in more dilute solution as the thermoelectric-osmometer method became insensitive in these regions. We are much indebted to Professor E. F. Smith and Mr. D. M. Brown of the Biochemistry Department, University of Utah, Salt Lake City, U.S.A., for careful studies of the molecular weights of gallotannin in more dilute aqueous solution by means of sedimentation equilibrium measurements made in a Spinco model D ultracentrifuge, usually running at 29,500 r.p.m. At 1% concentration and in 0·1N-acetate buffer (pH 4·0) the value of 2500 (±125), approximating to a dimeric form for the

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[3] Fischer and Freudenberg, *Ber.*, 1914, **47**, 2485.

[4] Fischer and Bergmann, *Ber.*, 1918, **51**, 1760.

[5] L. C. Craig and D. Craig, in Weissberger's " Technique of Organic Chemistry," Vol. III, Interscience Publ. Inc., New York, 1950.

[6] Fischer, *Ber.*, 1919, **52**, 809.

[7] Freudenberg, " Naturliche Gerstoffe," Verlag Chemie, Berlin, 1920, p. 101.

[8] Hill, *Proc. Roy. Soc. A.*, 1930, **127**, 9; Baldes, *J. Sci. Instr.*, 1934, **11**, 223.

gallotannin particle, was obtained, but the expected variation in this value on change to a 0·1N-veronal buffer (pH 8·5) (when the gallotannins would be negatively charged) was not observed. Similar values were obtained for the sumach gallotannins, but Turkish gallotannin gave a lower value of 2020 (±100) (at pH 4·0 and 35,600 r.p.m.). In calculating the molecular weights from the ultracentrifuge the partial specific volume was taken as 0·43 for aqueous solution in the 1% concentration range; this value was determined by Mr. R. Armitage by the pyknometer method[9] and agrees with the value of 0·46 calculated from density data.

Adequate explanations of some of these observations, *e.g.*, absence of effect of change of pH on the molecular weight of gallotannins, cannot be advanced at the moment. The results, however, in general support the conclusion that the gallotannin is largely monomeric in acetone solution, that association occurs in aqueous solution, and that the extent of association increases with concentration.

EXPERIMENTAL

Alumina refers to Spence's grade " H," washed with ethyl acetate and reactivated at 140°; alkaline alumina refers to material prepared by treatment with an equal weight of 3% aqueous potassium hydroxide, filtration, and reactivation at 140°. Paper-chromatographic and counter-current-distribution methods and procedures used in the preparation of samples for quantitative analysis are as described in Part III.[1]

Methyl 2,3,4,6-Tetrakistri-O-benzylgalloyl-β-D-glucoside.—(*A*). A suspension of methyl β-D-glucoside (2·6 g.) and tri-*O*-benzylgalloyl chloride (33 g.) in pyridine (20 c.c.) and chloroform (70 c.c.) was stirred with exclusion of moisture until dissolution was complete (1½ hr.), and the temperature was then raised to 60°. After 9 days the mixture was cooled, diluted with chloroform (70 c.c.), repeatedly washed with water, dried, and evaporated at 25°. A solution of the residue in benzene (200 c.c.) was evaporated to small bulk and the crystalline tri-*O*-benzylgallic anhydride, m. p. 166—167° (Schmidt and Schach[2] give m. p. 165·5°), which separated was collected and washed with a little benzene. The filtrate and washings were twice chromatographed on alumina (100 g.) covered with a layer of alkaline alumina (20 g.), and the benzene eluate (1·5 l.) was evaporated to 200 c.c. and slowly added to stirred cyclohexane (2 l.). After several hours at 0° the gelatinous precipitate (23·9 g.) was collected and freeze-dried from benzene.

(*B*). 2,3,4,6-Tetrakistri-*O*-benzylgalloyl-β-D-glucose {3·05 g.; $[\alpha]_D^{21}$ +18·9° ± 0·6° (*c* 2 in acetone)}, washed silver oxide (1·55 g.), acetone (40 c.c.), and methyl iodide (20 c.c.) were refluxed for 48 hr., then the solid was removed and washed with acetone. The filtrate and washings were evaporated and the residual gum was chromatographed in benzene (20 c.c.) twice on alumina (10 g.) and alkaline alumina (2 g.). Precipitation as in method *A* from benzene–cyclohexane and freeze-drying from benzene gave *methyl 2,3,4,6-tetrakistri-O-benzylgalloyl-β-D-glucoside* (2·87 g.) as a white amorphous powder [Found (method *A*): C, 75·8; H, 5·6; OMe, 2·2; (method *B*) C, 75·6; H, 5·3; OMe, 2·1. $C_{119}H_{102}O_{22}$ requires C, 75·8; H, 5·6; OMe, 1·7%], $[\alpha]_D^{21\cdot5}$ (*A*) +16·4° ± 0·6°, (*B*) +16·3° ± 0·6° (*c* 2·5 in acetone). No mutarotation was observed during 3 days after addition of hydrochloric acid to the acetone solutions. The compound was soluble in chloroform, methylene dichloride, dioxan, and tetrahydrofuran, sparingly soluble in acetone, benzene, and ethyl acetate, and very sparingly soluble in methanol and ethanol.

Conversion of Methyl 2,3,4,6-Tetrakistri-O-benzylgalloyl-β-D-glucoside into Methyl 2,3,4,6 Tetra-O-acetyl-β-D-glucoside.—Methyl 2,3,4,6-tetrakistri-*O*-benzylgalloyl-β-D-glucoside (2·04 g.; prepared by method *A* or *B*) was refluxed for 2½ hr. with 12% methanolic potassium hydroxide (20 c.c.) and then left at 0° for 16 hr. Water (50 c.c.) was added and the solution saturated with carbon dioxide at 40°; the precipitated potassium tri-*O*-benzylgallate was collected and washed with water (10 c.c.), and the combined filtrate and washings were evaporated. The residual gum was repeatedly triturated with hot ethanol (5-c.c. portions) and the combined extracts were filtered whilst hot and then evaporated to a gum which was treated with acetic

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[9] Svedberg and Petersen, " The Ultracentrifuge," Oxford Univ. Press, 1940, p. 58.

anhydride (5 c.c.) and pyridine (5 c.c.) at room temperature for 18 hr. The mixture was poured into ice-water (100 c.c.) and after 2 hr. extracted with chloroform several times (total volume 200 c.c.). Removal of the chloroform gave a solid which was chromatographed on alumina (4 g.) in benzene; evaporation of the eluate and crystallisation from ethanol gave prisms (0·28 g.), m. p. 103—104°, undepressed on admixture with authentic methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucoside (Found: C, 50·0, 49·9; H, 6·3, 6·4. Calc. for $C_{15}H_{22}O_{10}$: C, 49·7; H, 6·1%). The infrared spectrum of the material was identical with that of an authentic sample.

Methyl 2,3,4,6-*Tetra*-O-*galloyl*-β-D-*glucoside*.—A solution of methyl 2,3,4,6-tetrakistri-*O*-benzylgalloyl-β-D-glucoside (2·15 g.; prepared by method *A* or *B*) in tetrahydrofuran (50 c.c.) was hydrogenated at room temperature over 10% palladium–charcoal (0·34 g.). When uptake of hydrogen had ceased (6 hr.) the solution was filtered through a layer of cellulose powder, and the catalyst rapidly washed with a little de-oxygenated ethanol. The filtrate was evaporated at 25° and the product thrice dissolved in acetone and recovered at 25°. The residual gum was freeze-dried from t-butyl alcohol, to give *methyl* 2,3,4,6-*tetra*-O-*galloyl*-β-D-*glucoside* as a white amorphous powder (1·04 g.) (Found: C, 51·8, 52·1; H, 4·1, 3·9. $C_{35}H_{30}O_{22}$ requires C, 52·4; H, 3·8%), $[\alpha]_D^{22}$ +45·8°, +45·5° ± 0·7° (*c* 2·5 in acetone). No mutarotation was observed during 3 days after addition of 0·1N-acetic acid to the acetone solutions. The compound gave no reaction with aniline hydrogen phthalate and had similar solubility to penta-*O*-galloyl-β-D-glucose.[1]

Pentakistri-O-*methylgalloyl*-β-D-*glucose*.—A modified form of the method described by Fischer and Freudenberg[3] was used. A mixture of β-D-glucose (2·5 g.), tri-*O*-methylgalloyl chloride (17·3 g.), pyridine (7 c.c.), and chloroform (60 c.c.) was stirred, with the exclusion of air, until dissolution was complete (2 hr.) and left at 20° in the absence of light for 6 days. The mixture was washed successively with dilute sulphuric acid, water, and sodium hydrogen carbonate solution, dried, and evaporated. The residue was chromatographed in benzene on alumina (120 g.) covered with alkaline alumina (10 g.). The benzene eluate (1 l.) was reduced to *ca.* 50 c.c. and filtered and the filtrate diluted with cyclohexane. After several days pentakis-tri-*O*-methylgalloyl-β-D-glucose (14·9 g.) was collected as needles, m. p. 135—136° (sintering at 134°) (Found: C, 58·2; H, 5·6. Calc. for $C_{56}H_{62}O_{26}$: C, 58·4; H, 5·4%) (Fischer and Freudenberg[3] give m. p. 133—134°).

Methylation of Penta-O-*galloyl*-β-D-*glucose*.—The ester (1·07 g.; prepared by synthesis or by methanolysis of Chinese gallotannin[1]) was dissolved in acetone (10 c.c.) and cooled to 0°, and ethereal diazomethane (50 c.c.; 2·5 g. of diazomethane) slowly added. The solution was left for 48 hr. at 0° and then evaporated to a gum which was treated twice again at 0° with ethereal diazomethane (50 c.c.) as above. Removal of the solvents gave a glass which was chromatographed in benzene on alumina (10 g.) covered with a layer of alkaline alumina (1 g.). Evaporation of the benzene eluate (200 c.c.), dissolution of the residue in benzene (5 c.c.), and dilution with cyclohexane gave, originally during 3—4 weeks but subsequently during 12 hr., a partially crystalline product which was recrystallised from benzene–light petroleum (b. p. 60—80°) and then from benzene–cyclohexane, to give pentakistri-*O*-methylgalloyl-β-D-glucose (0·6 g.). The product from each source was obtained as needles, m. p. 135—136°, sintering from 134°, undepressed on admixture with each other or with material prepared by Fischer and Freudenberg's method[3] (Found: C, 58·7, 58·1; H, 5·6, 5·2; OMe, 40·1. Calc. for $C_{56}H_{62}O_{26}$: C, 58·4; H, 5·4; OMe, 40·4%), $[\alpha]_D^{22}$ +17·8° ± 0·4° (*c* 2·5 in acetone). The infrared spectrum of the product was identical with that of the synthetic material prepared by Fischer and Freudenberg's method.[3]

We thank the Leverhulme Trust and the Department of Scientific and Industrial Research for fellowships to T. S. and J. W. G., respectively, and the Imperial Chemical Industries Limited for a grant.

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[*Received, January 24th,* 1962.]