736. *Gallotannins. Part VI.* Turkish Gallotannin.*

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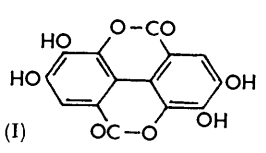
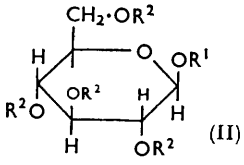
The main constituent of Turkish galls is shown to be a hexa- or hepta-galloylated glucose based upon a mixture of tetra-*O*-galloylglucose cores in which the 2- or 4-hydroxyl group of the glucose is unesterified. The tannin is derived by the addition (on average) of two or three galloyl groups which are depsidically linked to these cores and are probably randomly distributed.

Although extracts of Chinese galls (*Rhus semialata*) and Turkish or Aleppo galls (*Quercus infectoria*) had been used in the tanning industry for many years, Feist [1] in 1912 was the first to point out that remarkable differences existed in the nature of the two extracts, notably in the presence of ellagic acid (I) in the free and the combined state in that from Turkish galls. Feist also claimed the presence of a glucogallic acid in Turkish galls, but subsequent workers [2] were unable to confirm this observation. Fischer and Freudenberg [2] obtained Turkish gallotannin as an amorphous powder and confirmed the view that it was more complex than the related Chinese gallotannin, since it contained ellagic acid (2·7—3·8%) in the form of a water-soluble glucoside. Hydrolysis of the tannin gave glucose and gallic acid in the ratio 1 : 5—6, and methylation with diazomethane and subsequent hydrolysis of the methylated tannin gave 3,4,5-tri-*O*- and small amounts of 3,4-di-*O*-methylgallic acid. Thus, although these workers were able to discuss the similarities of the tannin to the synthetic penta-*O*-galloyl-β-glucose (II; $R^1 = R^2 =$ 3,4,5-trihydroxybenzoyl), they also indicated that some of the gallic acid was bound in the



(I)



(II)

form of depsides. Karrer, Widmer, and Staub [3] precipitated Turkish gallotannin with aluminium hydroxide and obtained fractions differing significantly in optical rotation and in gallic acid and ellagic acid (1·65—8·8%) content; since, however, the glucose content of these fractions was approximately the same it was suggested that the ellagic acid was not present as an individual glucoside, but that it replaced some of the gallic acid

* Part V, *J.*, 1962, 2944.

[1] Feist, *Ber.*, 1912, **45**, 1493.
[2] Fischer and Freudenberg, *Ber.*, 1914, **47**, 2485.
[3] Karrer, Widmer, and Staub, *Annalen*, 1923, **433**, 288.

in the gallotannin structure. Treatment of the precipitated fractions with hydrobromic acid in acetic acid and subsequent acetylation did not give the amorphous 1-*O*-acetyltetrakistri-*O*-acetylgalloylglucose, as occurred with Chinese gallotannin,[4] but, instead, amorphous products having a greater acetyl content and thus it was suggested that in Turkish gallotannin not all the glucose hydroxyl groups were esterified. Freudenberg[5] later summarised this early work and concluded that, on average, in Turkish gallotannin, one of the five glucose hydroxyl groups is free, one is esterified with *m*-digallic acid, and the remainder with gallic acid. More recent work has not altered this view appreciably, although White,[6] from a paper-chromatographic examination of the extract, whilst confirming the presence of ellagic acid, has stated that penta-*O*-galloylglucose is " of little if any significance " in Turkish gallotannin; recently this claim was criticised,[7] as the spot used for the identification of penta-*O*-galloylglucose may be ascribed to ethyl gallate produced by ethanolysis of the gallotannin during extraction.

At the commencement of these studies the structure of Turkish gallotannin was therefore far from clear, and in particular the number and position of unesterified glucose hydroxyl groups and the part played by ellagic acid in the gallotannin structure were subjects which required further investigation. Turkish gallotannin was obtained[7] as a white amorphous powder by extraction with ethyl acetate of an aqueous extract of crushed galls buffered to pH 6·8 with sodium phosphate. The substance gave analyses (carbon, hydrogen, glucose, and gallic acid) as for a hexa- to hepta-galloylglucose, although the diffuse and elongated nature of the gallotannin spot on paper chromatograms indicated its heterogeneity to be greater than that of Chinese gallotannin. The optical rotation also differed appreciably from that of Chinese gallotannin, and with aniline hydrogen phthalate[8] the substance gave a positive reaction indicative of free reducing groups. Chromatography on cellulose separated Turkish gallotannin into three fractions, whose glucose content varied from 14·4 to 18·6%, with corresponding differences in optical rotation, but, as will be shown later, these differences between the fractions represent differences in the number of depsidically linked galloyl groups on the same galloylglucose cores. Although ellagic acid (I) was readily isolated from the aqueous extract of crushed Aleppo galls, it was not detected either in the purified Turkish gallotannin or in the products following its hydrolysis with acid, and thus it is concluded, contrary to previous suggestions,[3] that this substance has no part to play in the structure of the gallotannin. Hydrolysis of Turkish gallotannin with the pure galloyl esterase isolated from the enzyme tannase as described in Part I[9] gave glucose and gallic acid only; since no other carbohydrate was detected the gallotannin must be a galloylated glucose derivative, and this view was substantiated by the isolation of glucose, characterised as its β-penta-acetate, after alkaline hydrolysis of diazomethane-methylated gallotannin.

Methanolysis of Turkish gallotannin or the three fractions obtained by partition chromatography of the gallotannin gave methyl gallate, a mixture of non-reducing tetra-*O*-galloylglucoses and two reducing tri-*O*-galloylglucoses. The separation and study of these products are discussed later, but the absence of penta-*O*-galloyl-β-glucose (II; $R^1 = R^2 =$ 3,4,5-trihydroxybenzoyl) is noteworthy and shows that the core of Turkish gallotannin, unlike that of Chinese gallotannin, is based on a mixture of tetra- and trigalloylglucose derivatives. This observation indicated, as previous workers had suggested,[3,5] the presence of free hydroxyl groups on the glucose nucleus, and in order to determine their position methylation studies were made. Turkish gallotannin methylated with diazomethane gave an amorphous compound whose infrared spectrum showed the

[4] Karrer, Salomon, and Peyer, *Helv. Chim. Acta*, 1923, **6**, 1.
[5] Freudenberg, " Tannin, Cellulose, and Lignin," Verlag Chemie, Berlin, 1933, p. 38.
[6] White, " The Chemistry of the Vegetable Tannins," Society of Leather Trades' Chemists, Croydon, 1956, p. 13.
[7] Armitage, Bayliss, Gramshaw, Haslam, Haworth, Jones, Rogers, and Searle, *J.*, 1961, 1842.
[8] Partridge, *Nature*, 1949, **164**, 443.
[9] Haslam, Haworth, Jones, and Rogers, *J.*, 1961, 1829.

presence of a free hydroxyl group and which still gave a positive reaction with aniline hydrogen phthalate. Hydrolysis gave glucose and a mixture of 3,4,5-tri- and 3,4-di-*O*-methylgallic acids in the ratio 1·8 : 1, indicative, for a hexagalloylglucose structure, of approximately two galloyl groups linked as depsides. Further, but incomplete, methylation of the methylated gallotannin by the silver oxide procedure of Purdie and Irvine,[10] followed by transesterification of the product with sodium methoxide in methanol, gave a carbohydrate fraction which on paper chromatographic analysis was shown to consist of glucose, a major product whose R_F values in various solvent systems was identical with that of a mono-*O*-methylglucose and traces of substances tentatively designated as di-*O*-methylglucoses. More prolonged methylation by the same procedure succeeded in producing a carbohydrate fraction which contained no glucose, but in which the presence of other artefacts, arising probably by degradation during methylation, was now evident, and for this reason we have preferred throughout this study to work with products which were incompletely methylated. Similar results were obtained by the barium oxide–dimethylformamide technique recently developed by Kuhn, Baer, and Seeliger,[11] although the time of reaction was considerably reduced. Carefully controlled conditions standardised so that pentakistri-*O*-methylgalloyl-β-glucose gave glucose only after being subjected to the Kuhn procedure and subsequent transesterification were employed. Thus it was ensured that under the basic conditions of the reaction secondary products, simulating paper-chromatographically di- and tri-*O*-methylglucoses and probably arising by hydrolysis of the acyl groups, did not result. The carbohydrate fraction obtained by further methylation of Turkish gallotannin (by either of the above methods) was resolved by paper electrophoresis, in both borate [12] and alkaline germanate [13] buffer, into glucose, 2-*O*-methylglucose, and 4-*O*-methylglucose. These results, therefore, indicate that the structure of Turkish gallotannin is based predominantly on a mixture of glucose cores having unesterified hydroxyl groups at positions 2 and 4, severally.

Confirmation of these ideas came from a study of the previously mentioned methanolysis products of Turkish gallotannin. In a previous communication [7] it was shown that the action of methanol at neutral pH on Chinese and sumach gallotannins removed galloyl groups linked as depsides but left those directly attached to glucose unaffected. In this way the main constituent of the core of Chinese gallotannin was isolated and identified as penta-*O*-galloyl-β-glucose; small amounts of 2,3,4,6-tetra-*O*-galloylglucose were also isolated. Similar treatment of Turkish gallotannin and subsequent separation of the products gave methyl gallate, a non-reducing tetra-*O*-galloylglucose (A) and, in very small amount, two reducing tri-*O*-galloylglucoses (B and C), one of which (B) was isolated as a crystalline hydrate. The detection by paper chromatography of methyl *m*-digallate during the methanolysis was indicative, as with Chinese gallotannin, of the presence of a trigalloyl chain in some or all of the gallotannin molecules. Methylation of the tetragallate A with diazomethane gave a product which still exhibited in the infrared spectrum a free-hydroxyl band, and further methylation by the silver oxide method, followed by alkaline hydrolysis, gave 3,4,5-tri-*O*-methylgallic acid and a carbohydrate fraction which, when analysed by paper electrophoresis, showed the presence of 2- and 4-*O*-methylglucose, glucose, and traces of a substance tentatively formulated as a di-*O*-methylglucose. Thus material A, which represents the major core of the gallotannin, must be a mixture of tetra-*O*-galloylglucoses, some having the 2-hydroxyl and some the 4-hydroxyl group unesterified. Attempts to separate this material A into its components by cellulose chromatography or counter-current distribution have however failed.

The investigations on Chinese and sumach gallotannins had indicated that the 1-*O*-galloyl group attached to glucose was more easily removed under a variety of conditions

[10] Purdie and Irvine, *J.*, 1903, 1621.
[11] Kuhn, Baer, and Seeliger, *Annalen*, 1958, **611**, 236.
[12] Foster, *Adv. Carbohydrate Chem.*, 1957, **12**, 80.
[13] Lindberg and Swan, *Acta Chem. Scand.*, 1960, **14**, 1043.

than galloyl groups attached to glucose at other positions, and this was thought to account for the presence of small amounts of 2,3,4,6-tetra-*O*-galloylglucose on methanolysis of the gallotannins derived from Chinese galls or old sumach leaves. It was expected, therefore, that in the products of methanolysis of Turkish gallotannin the minor products B and C would probably be derived from A by loss of the 1-*O*-galloyl group, and this was confirmed by complete methylation (diazomethane followed by the silver oxide procedure), transesterification of the methylated product, and analysis of the carbohydrate fractions by the methods outlined. Under these conditions material B gave 2-*O*-methylglucose, and C gave 4-*O*-methylglucose plus a trace of glucose and consequently these substances must be 3,4,6- and 2,3,6-tri-*O*-galloylglucose, respectively. Attempts to confirm these structures by synthesis are in progress. Several unsuccessful attempts were made to increase the availability of both materials B and C; for instance, acid hydrolysis or aerobic hydrolysis of material A at neutral pH [14] gave B and C, but in both cases the products were contaminated with other artefacts and isolation was difficult. As expected, β-glucosidase had no action on material A since this enzyme requires both the 2- and the 3-hydroxyl group to be unsubstituted before reaction will occur at the glucosidic carbon atom.[15]

The major part of the evidence for the structure of Turkish gallotannin is dependent on the interpretation of the results of methylation of partially galloylated glucoses, and earlier work [16] on the methylation of acyl sugars has clearly demonstrated the possibilities of acyl migration under these conditions. Schmidt and Schmadel [17] recently showed the ready migration, under basic conditions, of the galloyl group in 1-*O*-galloyl-α-glucose to 2-*O*-galloylglucose, and similarly we have demonstrated the isomerisation, under a variety of conditions, of 2-*O*-galloylglycerol [18] to 1-*O*-galloylglycerol. Schmidt and Schmadel [17] have, however, demonstrated that both 2-*O*-galloylglucose and 1-*O*-galloyl-β-glucose are not isomerised under basic conditions and thus that there was no migration of a galloyl group attached to glucose in an equatorial position to an adjacent hydroxyl group. Hence it is concluded that the results of methylation of Turkish gallotannin and its methanolysis products reveal the true core of the gallotannin and are not complicated by migration of galloyl groups during methylation.

The disposition of the two further galloyl groups on the mixed tetra-*O*-galloylglucose core of Turkish gallotannin is still under investigation. The methanolysis experiments indicate the presence of chains of at least 3 galloyl groups in some or all of the gallotannin molecules, but the evidence available does not allow identification of the positions of attachment of these chains, nor does it indicate the extent to which the two additional galloyl groups are involved in such chains in the gallotannin as a whole.

Experimental

Determinations of the glucose and gallic acid contents, paper chromatography, and countercurrent distribution of the gallotannins and related products were carried out as described previously.[7] Paper electrophoresis of methylated sugars was carried out in an apparatus similar to that described by Foster,[19] with both 0·2M-borate buffer (pH 10·0) [12] at 900 v and 20° for 2 hr., and 0·05M-germanate buffer (pH 10·7) [13] at 1500 v for 1½ hr. at 37°.

Solutions were concentrated at 25—30° under reduced pressure with a rotary evaporator, and quantitative determinations on gallotannins and galloylated glucoses were made on samples dried to constant weight at 105°/0·005 mm.

Turkish Gallotannin.—Turkish gallotannin, extracted and isolated as described previously,[7] was obtained as a white amorphous powder (Found: C, 53·2, 52·8; H, 3·4, 3·7; gallic acid, 97·5; glucose, 16·4, 16·5. Calc. for $C_{48}H_{36}O_{30}$; C, 52·7; H, 3·3; gallic acid, 93·4; glucose,

[14] Hathway, *J.*, 1957, 519.
[15] Veibel, "The Enzymes," Academic Press, New York, 1950, Vol. I, p. 583.
[16] Sugihara, *Adv. Carbohydrate Chem.*, 1953, **8**, 1; Bonner, *J. Org. Chem.*, 1959, **24**, 1388.
[17] Schmidt and Schmadel, *Annalen*, 1961, **649**, 157.
[18] Schmidt and Blank, *Chem. Ber.*, 1956, **89**, 283.
[19] Foster, *Chem. and Ind.*, 1952, 1050.

16·5%). [A sample of Turkish gallotannin provided by Professor O. Th. Schmidt gave glucose, 14·9% ($C_{55}H_{39}O_{34}$ requires glucose, 14·5%).] Turkish gallotannin had $[\alpha]_D^{20}$ +23·2°, + 21·7° (*c* 2·0 in acetone) and on paper chromatograms had R_F (*a*) 0·02—0·37, (*b*) 0·36—0·50 [for the systems (*a*) and (*b*) see ref. 7].

Fractionation of Turkish Gallotannin.—Turkish gallotannin (2·0 g.) in N-acetic acid was applied to a cellulose column (85 × 6 cm.) and eluted with the same solvent. Fractions (15 c.c.) were collected and analysed by measurement of their optical density at 320 mμ. Appropriate fractions were concentrated at 30°; the residue was taken up in acetone, filtered, and the solvent removed; the residual gum was freeze-dried from water. Fraction I (0·46 g.) (Found: C, 52·9; H, 3·7; glucose, 18·5, 18·6. $C_{48}H_{32}O_{26}$ requires C, 52·3; H, 3·4; glucose, 19·2%) had $[\alpha]_D^{20}$ +32·9° (*c* 3·5 in acetone) and R_F (*a*) 0·22—0·37, (*b*) 0·30—0·42. Fraction 2 (0·26 g.) (Found: C, 52·7; H, 3·6; glucose, 16·4. $C_{48}H_{36}O_{30}$ requires C, 53·1; H, 3·1; glucose, 16·5%) had $[\alpha]_D^{20}$ +23·8° (*c* 2·8 in acetone) and R_F (*a*) 0·10—0·33, (*b*) 0·27—0·39. Fraction 3 (0·25 g.) (Found: C, 53·4; H, 3·7; glucose, 14·4. $C_{55}H_{40}O_{34}$ requires C, 53·2; H, 3·2; glucose, 14·5%) had $[\alpha]_D^{20}$ +16·2° (*c* 2·8 in acetone) and R_F (*a*) 0·08—0·22, (*b*) 0·29—0·42.

Methylation of Turkish Gallotannin.—Turkish gallotannin (2·0 g.) in acetone (10 c.c.) was treated with ethereal diazomethane (150 c.c.) at room temperature. After 24 hr. removal of the solvents gave a gum which was dissolved in chloroform (100 c.c.) and washed with 0·1N-hydrochloric acid (2 × 100 c.c.) and water (3 × 100 c.c.). Evaporation of the chloroform gave a gum which was treated a further three times with ethereal diazomethane as above. Removal of the solvents finally gave a pale yellow amorphous powder (2·2 g.) (Found: C, 58·6; H, 5·5; OMe, 38·0. $C_{64}H_{68}O_{30}$ requires C, 58·3; H, 5·2; OMe, 37·8%). The compound had $[\alpha]_D^{20}$ +23·5° (*c* 3·0 in acetone) and its infrared spectrum showed the presence of a free hydroxyl group. Determination of the ratio of 3,4-di- and 3,4,5-tri-*O*-methylgallic acid liberated on hydrolysis was carried out as previously described; [7] the results are discussed on p. 3810.

Isolation of Glucose from Diazomethane-methylated Turkish Gallotannin.—Diazomethane-methylated Turkish gallotannin (2·0 g.) was treated with 12% methanolic potassium hydroxide (150 c.c.) at 0° for 48 hr.; the methanol was then removed at 30°, and the residue dissolved in water (50 c.c.) and passed down a column of ZeoKarb 215 (H^+; 18 × 2·5 cm.). The eluate was concentrated, extracted with ether continuously for 24 hr., evaporated to dryness at 30°, and dried over phosphorus pentoxide at 50°/0·003 mm. for 24 hr. The resultant gum was heated at 100° for 2½ hr. with acetic anhydride (15 c.c.) containing anhydrous sodium acetate (2 g.). The solution was poured into ice–water, and the crude product was collected and crystallised several times from methanol, to give penta-*O*-acetyl-β-D-glucose (0·12 g.), m. p. and mixed m. p. 129—130° (Found: C, 48·9; H, 5·7. Calc. for $C_{16}H_{22}O_{11}$: C, 48·9; H, 5·6%).

Further Methylation of Diazomethane-methylated Turkish Gallotannin.—(*a*) *Silver oxide method.* A solution of diazomethane-methylated Turkish gallotannin (0·2 g.) in methyl iodide (20 c.c.) to which silver oxide (1·0 g.) was added was refluxed for 2 days. The silver salts were removed and evaporation of the methyl iodide gave a gum which was dissolved in dry methanol (25 c.c.); sodium (0·01 g.) was added. After 36 hr. at room temperature the methanol was removed and the residue was dissolved in water (10 c.c.) and set aside at 0° for 24 hr. before the methylated gallic acids were removed by filtration. The filtrate was passed down a column of ZeoKarb 215 (6 × 1 cm.), and the eluate concentrated to small bulk and subjected to paper chromatography in solvent system (*b*) and to paper-electrophoretic examination. The results are discussed on p. 3810. Fractions 1, 2, and 3 from fractionation of Turkish gallotannin, when similarly treated, gave a monomethylglucose, traces of glucose, and other unidentified materials on subsequent analysis.

(*b*) *Barium oxide–dimethylformamide method.* A dimethylformamide suspension (10 c.c.) containing methyl iodide (2 c.c.), barium oxide (2·0 g.), barium hydroxide (0·04 g.), and diazomethane-methylated Turkish gallotannin (0·5 g.) was heated at 90° for 20 min. and then poured into water (200 c.c.). The aqueous solution was shaken with chloroform (150 c.c.), and the separated chloroform layer washed with water (10 × 100 c.c.) and dried (Na_2SO_4). Evaporation of the chloroform gave a gum which was dissolved in methanol (50 c.c.) containing sodium (0·02 g.), and the carbohydrate fraction was isolated and analysed as in (*a*) above. The results are discussed on p. 3810. Pentakistri-*O*-methylgalloyl-β-glucose (0·5 g.), when similarly treated, showed glucose as the only carbohydrate on subsequent analysis.

Methanolysis of Turkish Gallotannin.—Turkish gallotannin (3·0 g.) was dissolved in 0·5N-acetate buffer (pH 6·0; 30 c.c.) and methanol (300 c.c.) (previously de-oxygenated by boiling

under nitrogen) and kept at 37° for 7 days with a slow stream of nitrogen passing through the solution. Removal of the solvent at 30°, dissolution 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 showed the pattern indicated in the Table. The gum was dissolved in N-acetic acid (20 c.c.),

	$R_F(a)$	R_F (*b*)	U.v./NH_3	Substance
(i)	0·09	0·57	Violet	A
(ii)	0·25	0·56	Violet	C
(iii)	0·33	0·38	Blue	B
(iv)	0·47	0·70	Blue	Gallic acid
(v)	0·52	0·82	Blue	Me gallate

applied to a cellulose column (B.W. 200; 60 × 6·5 cm.), and eluted with the same solvent. Fractions (15 c.c.) were collected and analysed by measurement of optical density at 320 mμ. Concentration of the appropriate fractions separated the eluate into five fractions. Fraction 1 crystallised from water in needles (0·58 g.), m. p. and mixed m. p. 196°, of methyl gallate. Fraction 2 crystallised from water in needles (0·05 g.), m. p. and mixed m. p. 245—250°, of gallic acid. An acetone solution of fraction 3 was filtered and evaporated at 30°, and the residue was dissolved in ethyl acetate (100 c.c.), washed with water (3 × 100 c.c.), and recovered at 30°; this residue, freeze-dried from water, yielded a white amorphous powder (0·13 g.), which paper chromatography showed to contain material B contaminated with C. Similar treatment of fraction 4 gave material C contaminated with B, as a white amorphous powder (0·13 g.). Fraction 5, similarly treated, gave material A as a white amorphous powder (0·9 g.) (Found: C, 51·4; H, 4·0; glucose, 22·8, 22·5. Calc. for $C_{34}H_{28}O_{22}$: C, 51·8; H, 3·6; glucose, 22·8%) which had R_F (*a*) 0·09, (*b*) 0·57 and $[\alpha]_D^{21}$ +41·3° (*c* 2·0 in acetone).

Fraction 3 (0·12 g.) was subjected to counter-current distribution in propan-1-ol–butan-1-ol–cyclohexane–water (33 : 11 : 7 : 49) (upper phase 10 c.c., lower phase 15 c.c.; 100 transfers). Analysis of the distribution by paper chromatography [solvent systems (*a*) and (*b*)] and concentration of the appropriate tubes gave product B as a gum; this was dissolved in acetone (5 c.c.), water (5 c.c.) was added, and the acetone slowly removed under reduced pressure. After addition of acetic acid (0·1 c.c.), the solution was kept at 0° for 2 days and product B (0·1 g.) collected as fine colourless needles, m. p. 188—190° (Found, on sample dried at 100°: C, 49·1; H, 4·1; glucose, 27·7. Calc. for $C_{27}H_{26}O_{19}$: C, 49·5; H, 4·1; glucose, 27·5. Found, on sample dried at 120°: C, 51·4; H, 4·0. Calc. for $C_{27}H_{24}O_{18}$: C, 50·9; H, 3·8%), $[\alpha]_D^{23}$ +124° (*c* 1·4 in acetone), R_F (*a*) 0·33, (*b*) 0·38.

Fraction 4 (0·12 g.), subjected similarly to counter-current distribution, gave product C (0·10 g.) as a white amorphous powder (after freeze-drying from water) (Found: C, 51·3; H, 3·8; glucose, 28·3, 27·5. Calc. for $C_{27}H_{24}O_{18}$: C, 50·9; H, 3·8; glucose, 28·3%), $[\alpha]_D^{23}$ +23° (*c* 1·6 in acetone), R_F (*a*) 0·25, (*b*) 0·56.

Fractions 1, 2, and 3 (0·1 g. each), obtained by partition chromatography of Turkish gallotannin (p. 3812), were separately treated at 37° for 5 days in methanol (10 c.c.) containing 0·5N-acetate buffer (pH 6·0; 1·0 c.c.), and the products were analysed by paper chromatography in solvent systems (*a*) and (*b*). The results are discussed on p. 3810.

Diazomethane-methylation of Material A.—Substance A (2·31 g.) was dissolved in acetone (75 c.c.) and an excess of ethereal diazomethane was added. After 24 hr. at room temperature the solvents were removed at 30° and the process was twice repeated, to yield a gum which was freeze-dried from benzene and obtained as a white amorphous powder (2·78 g.) (Found: C, 57·0; H, 5·8; OMe, 37·7. Calc. for $C_{46}H_{52}O_{22}$: C, 57·7; H, 5·4; OMe, 38·9%), $[\alpha]_D^{20}$ +31·1° (*c* 5·8 in acetone).

Further Methylation of Diazomethane-methylated Material A.—Silver oxide (10 g.) was added to a solution of diazomethane-methylated material A (2·73 g.) in methyl iodide (15 c.c.). After 2 days' refluxing, the solution was filtered, the solvents were removed, and the residual gum was applied in benzene (50 c.c.) to neutral alumina (14 × 2·5 cm.). Elution with benzene (500 c.c.) and evaporation of the solvent gave, after freeze-drying from benzene, a white amorphous solid (1·5 g.) (Found: OMe, 41·9. Calc. for $C_{47}H_{54}O_{22}$: OMe, 42·2%), $[\alpha]_D^{20}$ +45·1° (*c* 2·9 in acetone).

This product (1·5 g.), dissolved in methanol (10 c.c.), was treated at 0° with methanolic 12% potassium hydroxide (10 c.c.), and after 48 hr. the solution was passed down a column of ZeoKarb 215 (H^+; 20 × 2 cm.), and the eluate (250 c.c.) was continuously extracted with ether for 20 hr. Evaporation of the ether and crystallisation from water gave 3,4,5-tri-*O*-methylgallic acid, m. p.

and mixed m. p. 169°; concentration of the aqueous solution gave a pale yellow gum (0·22 g.), which when subjected to paper electrophoresis showed the presence of 2- and 4-*O*-methylglucose and traces of substances tentatively designated as di-*O*-methylglucoses.

Analytical Methylation of Products B and C.—Product B or C (0·1 g.) was treated as above, first with diazomethane (3 × 10 c.c.) and then with silver oxide (0·5 g.) and methyl iodide (5 c.c.) under reflux for 3 days. The carbohydrate fraction was isolated as above after treatment with methanolic 12% potassium hydroxide (1 c.c.) for 48 hr. at 0°. Paper electrophoresis showed the carbohydrate fractions from materials B and C to give 2- and 4-*O*-methylglucose, respectively, contaminated in the latter case with traces of glucose.

Conversion of 2-O-*Galloylglycerol into* 1-O-*Galloylglycerol.*—(i) 2-*O*-Galloylglycerol (0·01 g.) (prepared by the method of Schmidt and Blank [18]), R_F (*a*) 0·56, (*b*) 0·46, was dissolved in water (10 c.c.) and kept at 90° for 20 hr. Paper chromatography then revealed 2-*O*-galloylglycerol, 1-*O*-galloylglycerol,[18] R_F (*a*) 0·64, (*b*) 0·51, and gallic acid.

(ii) 2-*O*-Galloylglycerol (0·01 g.) in 0·5N-acetate buffer (pH 7·0; 1·0 c.c.) was kept at 37° for 24 hr. Paper chromatography then indicated the presence of 2- and 1-*O*-galloylglycerol.

(iii) 2-*O*-Galloylglycerol (0·01 g.) in methanol (0·9 c.c.) containing 0·5N-acetate buffer (pH 7·0; 0·1 c.c.) was kept at 37° for 24 hr. Paper chromatography then indicated the presence of 2- and 1-*O*-galloylglycerol.

(iv) A solution of 2-*O*-galloylglycerol (0·01 g.) in methanol (1·0 c.c.) was refluxed for 2 days. Paper chromatography then indicated the presence of 1- and 2-*O*-galloylglycerol and methyl gallate, R_F (*a*) 0·52, (*b*) 0·82.

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