Tannins and Related Compounds. Part 9.[1] Isolation and Characterization of Polygalloylglucoses from Turkish Galls (*Quercus infectoria*)

Makoto Nishizawa and Takashi Yamagishi
Hokkaido Institute of Public Health, N-12, W-19, Kita-ku, Sapporo 060, Japan
Gen-ichiro Nonaka and Itsuo Nishioka *
Faculty of Pharmaceutical Sciences, Kyushu University, Maidashi, Higashi-ku, Fukuoka 812, Japan

Two structural isomers of pentagalloylglucose and four isomers of hexagalloylglucose have been isolated from Turkish galls (*Quercus infectoria*), in addition to 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (2), and their structures were established on the basis of ^{13}C n.m.r. analysis, methanolysis, and partial hydrolysis. Tri-hepta-, and octa-galloylglucoses were partially purified, and their structures were also characterized mainly by ^{13}C n.m.r. spectroscopy. From the isolation and structural studies of these galloylglucoses Turkish gallotannin has been shown to be a mixture of two types of polygalloylglucoses; one type has a 1,2,3,6-tetra-*O*-galloyl-β-D-glucose core with depside galloyl group(s) at the C-2 and/or C-6 position of the glucose moiety, and the other has a 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose core with depside galloyl group(s) randomly distributed at the C-2, -3, -4, and -6 positions. H.p.l.c. analysis showed that Turkish gallotannin has an average molecular weight of 1 032 and the composition ranges from tri- to nona-galloylglucoses.

Extract of Turkish galls (*Quercus infectoria*) is an important commercial tannin, and is commonly used in the leather and medicinal industries. Fischer and Freudenberg,[2] in their classic work on the structure of Turkish gallotannin, had shown it to be a mixture of galloylglucoses having an average molecular weight corresponding to that of a pentagalloylglucose molecule since, on hydrolysis, the tannin liberated *ca.* five moles of gallic acid *per* mole of D-glucose. Later, Karre *et al.*[3] inferred, from observing the occurrence of a free glucose hydroxy group in Turkish gallotannin, that it had a more complicated structure and composition than the related Chinese gallotannin which consisted exclusively of a 1,2,3,4,6-pentagalloylglucose core. More recently, Haslam and his co-workers [4-6] have shown, on the basis of hydrolytic studies, that Turkish gallotannin is composed of more than two types of polygalloylglucoses, and that the main components had a 1,3,4,6-tetra-*O*-galloyl-β-D-glucose core. They also demonstrated, by means of ^{1}H n.m.r. analysis, the presence of depside galloyl groups on the C-6 position of the glucose moiety. However, their proposed structure (13) is highly speculative because a mixture of galloylglucoses was used for their studies.

In a previous paper [7] we reported the isolation of penta- to undeca-galloylglucoses from Chinese galls by a combination of Sephadex LH-20 and preparative reverse-phase high-performance liquid chromatography (h.p.l.c.), and the structures of these galloylglucoses were elucidated on the basis of ^{13}C n.m.r. analysis, methanolysis, and partial hydrolysis. In this paper we have applied these methods to the characterization of Turkish gallotannin, and the results are described herein.

Results and Discussion

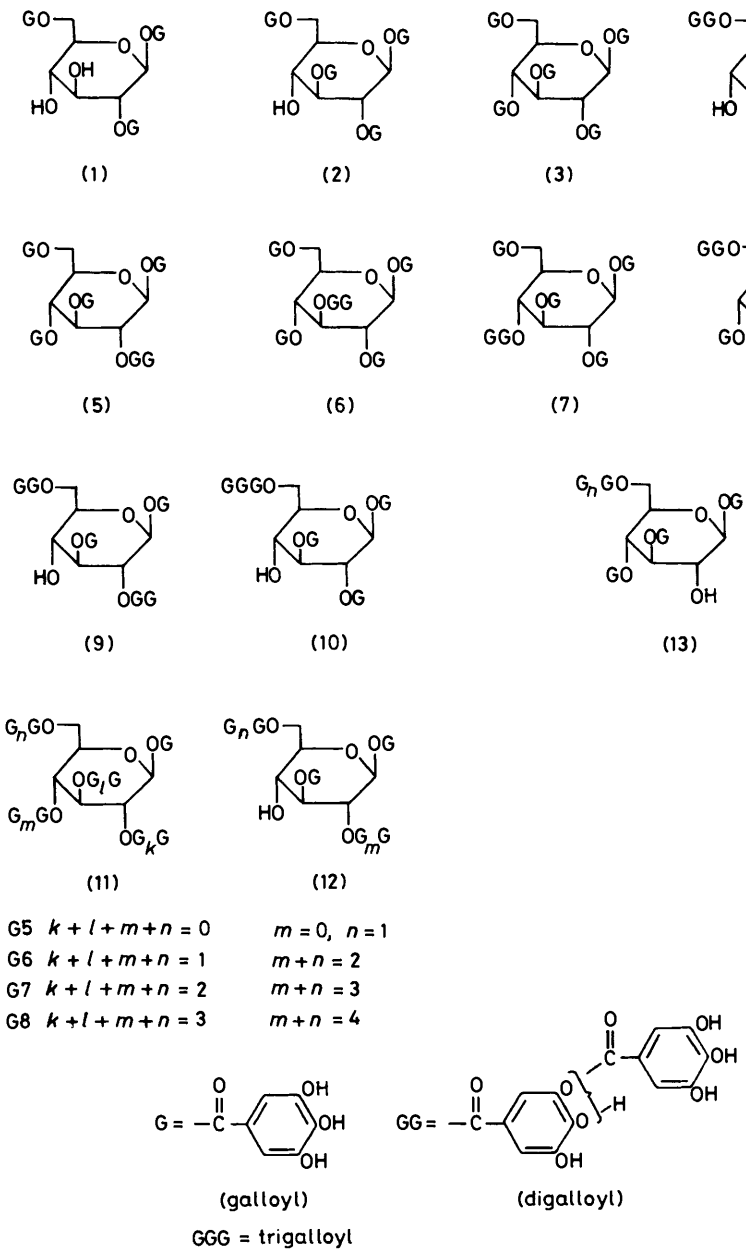
The acetone extract of Turkish galls showed seven peaks, corresponding to tri- to nona-galloylglucoses (G3)—(G9), in the normal-phase h.p.l.c. (Figure 1A). Amongst them, tri-, tetra-, penta-, hexa-, hepta-, and octa-galloylglucoses were separated by repeated chromatography on Sephadex LH-20 using a solvent system of ethanol–water–acetone.[8] The trigalloylglucose (G3) fraction displayed three peaks on reverse-phase h.p.l.c. The ^{13}C n.m.r. spectrum revealed, in the sugar-carbon region, eighteen signals including minor peaks, of which six intense signals were assigned to 1,2,6-tri-*O*-galloyl-β-D-glucose (1) by comparison of their chemical shifts with those of a sample obtained from the seed shells of *Trapa japonica*.[9] However, other minor components could not be characterized owing to our lack of model compounds.

Table 1. ^{13}C N.m.r. chemical shifts of gallotannins (δ_C values in p.p.m.) [a]

Compound	Glucose atom C-1	C-2	C-3	C-4	C-5	C-6
(1)	93.6	73.9	75.9	71.2	75.2	63.9
(2)	93.4	71.7	75.9	69.3	76.0	63.6
(3)	93.3	71.7	73.3	69.2	73.9	62.8
(4)	93.5	71.7	75.9	69.3	75.9	*64.0*
(7)	93.3	71.7	73.3	*69.7*	73.9	62.8
(8)	93.3	71.7	73.3	69.2	73.3	*63.2*
(9)	93.5	*72.0*	75.9	69.3	75.9	*63.9*
(10)	93.4	71.7	75.9	69.2	75.9	*64.0*

[a] Measured in $(CD_3)_2CO$. Chemical shift values in italics were used in the assignments of the structures of various oligogalloylglucoses (discussed in the text).

The tetragalloylglucose (G4) fraction showed a single peak on reverse-phase h.p.l.c. (Figure 1B), and the homogeneity was also confirmed by the ^{13}C n.m.r. spectrum which exhibited six signals due to glucose carbons (Table 1). The ^{1}H n.m.r. spectrum showed, in addition to four galloyl signals (δ_H 6.99, 7.07, 7.08, and 7.17), an anomeric proton doublet (δ_H 6.18, *J* 8 Hz) and glucose signals assignable to the 2-H (δ_H 5.47, t, *J* 8 Hz), 3-H (δ_H 5.68, t, *J* 8 Hz), and 6-H atoms (δ_H 4.59, m), which were all shifted downfield from the corresponding signals for D-glucose. Methylation of component (G4) with dimethyl sulphate and potassium carbonate in dry acetone yielded a dodecamethyl ether which was subsequently methylated by the Kuhn method [10] to give the corresponding permethyl ether. On alkaline hydrolysis, followed by treatment with methanolic hydrochloric acid, the permethylated derivative afforded methyl 4-*O*-methylglucopyranoside which was characterized as its acetate by gas chromatographic comparison with an authentic specimen. Accordingly, the structure of component (G4) was determined as 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (2).



The pentagalloylglucose (G5) fraction consisted of two compounds, (G5-A) and (G5-B), as revealed by reverse-phase h.p.l.c. (Figure 1B), and both components were separated by preparative reverse-phase h.p.l.c. Compound (G5-A) exhibited a ^{1}H n.m.r. spectrum clearly showing five singlets due to galloyl groups, and the compound was identified as 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (3) by direct comparisons of its ^{1}H and ^{13}C n.m.r. spectra with those of an authentic sample obtained from Chinese galls.[7] The other pentagalloylglucose, (G5-B), gave, on mild methanolysis in aqueous methanol at pH 5.5 (acetate buffer),[11] equimolar amounts of methyl gallate and 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (2), thus indicating that component (G5-B) has a core structure (2) to which was attached one galloyl group as a depside. In the ^{13}C n.m.r. spectrum of (G5-B), a signal due to the C-6 carbon in the glucose moiety was shifted downfield by 0.4 p.p.m. compared with that of C-6 in compound (2), while the other signals remained unchanged (Figure 2; Table 1). This downfield shift, rationalized by the occurrence of a depside galloyl group of C-6, was in good agreement with our previous observations on Chinese gallotannin.[7] Thus, the structure of component (G6-B) was elucidated as 6-*O*-digalloyl-1,2,3-tri-*O*-galloyl-β-D-glucose (4).

The hexagalloylglucose (G6) fraction contained more than six components (Figure 1B), of which four isomers, corresponding to major peaks [components (G6-C), (G6-D), (G6-E), and (G6-F)], were isolated by preparative reverse-phase h.p.l.c. The minor components (G6-A) and (G6-B) were tentatively identified, by comparison of their h.p.l.c. retention times with those of samples obtained from Chinese gallotannin,[7] as 3-*O*-digalloyl-1,2,4,6-tetra-*O*-galloyl-β-D-glucose (6) and 2-*O*-digalloyl-1,3,4,6-tetra-*O*-galloyl-β-D-glucose (5), respectively. Components (G6-C) and (G6-D) furnished 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (3) and methyl gallate in an equimolar ratio when they were methanolysed in the same way as for compound (4), thus suggesting that both components had a core structure (3). The assignment of structure (7) to component (G6-C) was made by examination of

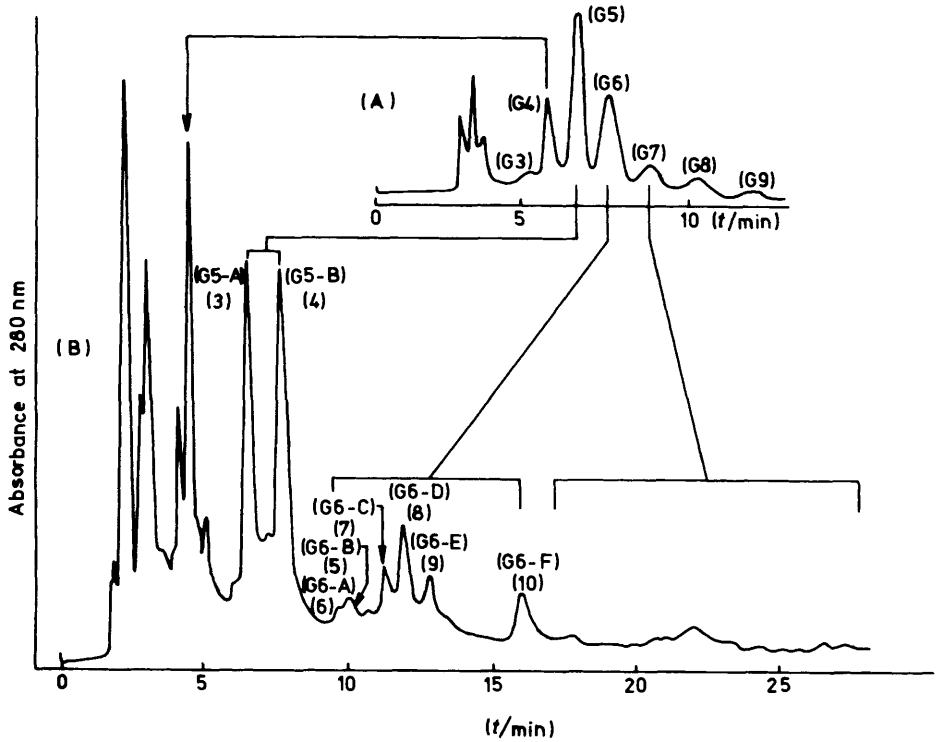


Figure 1. H.p.l.c. of gallotannins in Turkish galls. (A) Normal phase (column: Nucleosil 50—10, 3 mm i.d. × 300 mm); solvent: hexane–methanol–tetrahydrofuran–formic acid (55 : 33 : 11 : 1) together with oxalic acid (450 mg l^{-1}); (B) reverse phase (column: Nucleosil $5C_{18}$, 4 mm i.d. × 250 mm); solvent: acetonitrile–water (21 : 79) together with oxalic acid (2 g l^{-1}). The use of oxalic acid in the solvent systems gave sharper peaks on the chromatograms

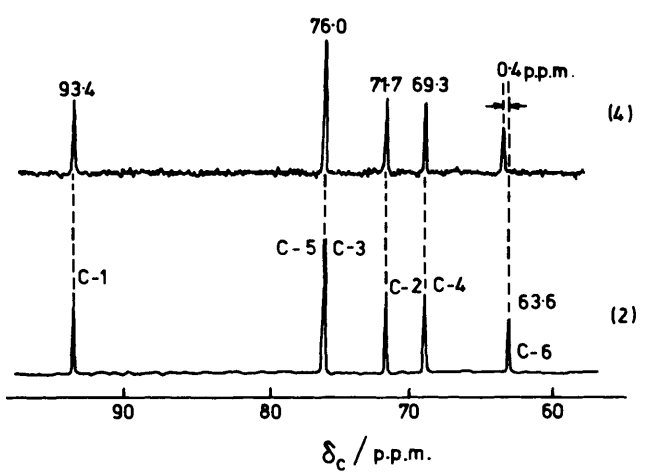


Figure 2. ^{13}C N.m.r. spectra of the tetragalloylglucose (2) and the pentagalloylglucose (4)

its ^{13}C n.m.r. spectrum in which the C-4 resonance in the glucose moiety was shifted to lower field than that of C-4 in compound (3) (Table 1). The identity of compound (G6-C) as 4-*O*-digalloyl-1,2,3,6-tetra-*O*-galloyl-β-D-glucose (7) [7] was finally established by ^{13}C n.m.r. comparison and h.p.l.c. analysis. Components (G6-E) and (G6-F), unlike (G6-C) and (G6-D), afforded 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (2) and methyl gallate in a 1 : 2 molar ratio on similar methanolysis. The assignment of the depside galloyl groups to a 1,2,3,6-tetra-*O*-galloylglucose core was made as before from ^{13}C n.m.r. results. In the spectrum of compound (G6-E), two signals corresponding to C-2 and C-6 in the glucose moiety showed characteristic downfield shifts when compared with those of C-2 and C-6 of compound (2) (Table 1). On the other hand only one signal, attributed to C-6, was shifted downfield in the spectrum of compound (G6-F). Thus, the structures of compounds (G6-E) and (G6-F) were assigned as 2,6-bis-*O*-digalloyl-1,3-di-*O*-galloyl-β-D-glucose (9) and 6-*O*-trigalloyl-1,2,3-tri-*O*-galloyl-β-D-glucose (10), respectively.

The heptagalloyl- (G7) and octagalloyl-glucose (G8) fractions contained a more complicated mixture than did fraction (G6) (Figure 1B). Owing to difficulties in isolating individual members, structural work on these components was performed without further separation. On mild methanolysis, both (G7) and (G8) gave 1,2,3,6-tetra-*O*-galloyl- and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose, together with methyl gallate; this indicated that both (G7) and (G8), as for the hexagalloylglucoses, consisted exclusively of compounds having the core structure (2) or (3). In the ^{13}C n.m.r. spectra of components (G7) and (G8) (Figure 3), signals due to C-2, C-4, and C-6 each appeared as a doublet. Comparison of the chemical shifts with those of compounds (2) and (3) (Figure 3) led to the assumptions that (i) all of the components in the fractions (G7) and (G8) have depsidically linked galloyl group(s) at the C-6 position since the two C-6 signals in (G7) and (G8) were distinctly shifted downfield, and (ii) some of the depside galloyl group(s) exist at C-2 and C-4. Furthermore, the fact that the chemical shift value (δ_C 75.9 p.p.m.) for C-3 and C-5 was closely similar to those found for compounds (2), (4), (9), and (10) suggested that the depside galloyl group(s) is (are) absent at C-3 position in components possessing a 1,2,3,6-tetragalloylglucose (2) core. Previous work on Chinese gallotannin which is based upon a 1,2,3,4,6-pentagalloylglucose core (3) has shown that in tannins having depsidically linked galloyl group(s) at C-3, the corresponding C-3 signal was not seen as a result of overlap with the C-5 signal. Taking this observation and also the above-mentioned

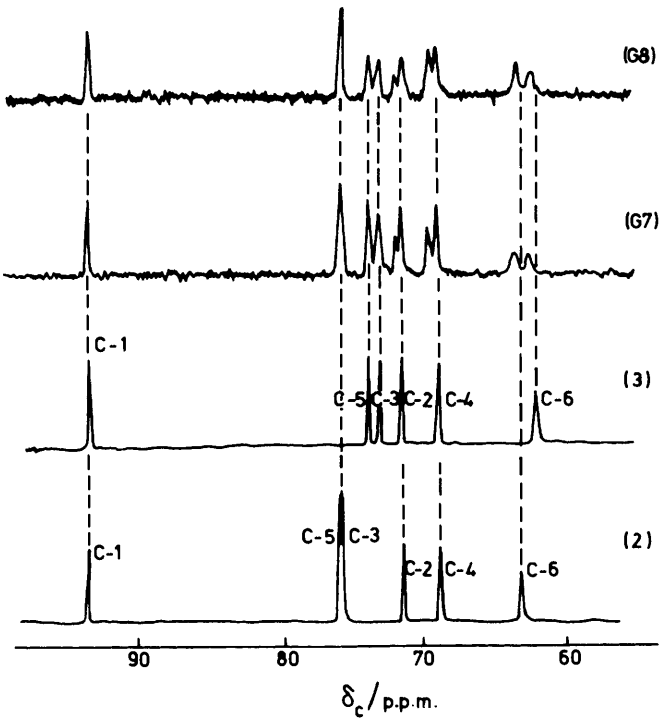


Figure 3. ^{13}C N.m.r. spectra of the tetragalloylglucose (2), the pentagalloylglucose (3), a mixture of heptagalloylglucoses (G7), and a mixture of octagalloylglucoses (G8)

Table 2. Relative composition of galloylglucoses in Turkish gallotannin

	Composition (%) [a]	Components
Tri GG [b]	1	(1) and two isomers
Tetra GG	15	(2)
Penta GG	34	(3), (4)
Hexa GG	29	(5)—(10)
Hepta GG	15	More than 8 isomers
Octa GG	5	
Nona GG	1	

[a] Determined by normal-phase h.p.l.c. analysis. [b] GG represents galloylglucose.

structures of hexagalloylglucoses into consideration, the depside galloyl group(s) may occur at C-3 in a portion of the components with a 1,2,3,4,6-pentagalloylglucose core.

In conclusion, the structure of Turkish gallotannin can be summarized by the general formulae (11) and (12). Based on the normal-phase h.p.l.c. analysis (Table 2), the main components are penta- and hexa-galloylglucoses, and the average molecular weight for Turkish gallotannin is calculated to be 1 032; the value is smaller than that of Chinese gallotannin. The complexity of Turkish gallotannin is derived from the presence of two types of galloylglucose core, (2) and (3). We could not isolate a gallotannin having a 1,3,4,6-tetra-*O*-galloyl-β-D-glucose (13) core which was previously reported to exist in Turkish gallotannin.[6] By ^{13}C n.m.r. analysis, performed as for Chinese gallotannin,[7] the occurrence of *meta*- and *para*-depside linkages in a galloyl chain has also been confirmed.

Experimental

For details of instruments and chromatographic procedures used in this work see Part 5.[7]

Isolation of Gallotannins.—Powdered Turkish galls (40 g) were extracted twice with acetone (200 ml each) at room temperature for 12 h. After evaporation of the solvent (*ca.* 40 °C), the residue was dissolved in water (200 ml) and then partitioned with ethyl acetate (200 ml × 3). The combined organic phases were dried over Na_2SO_4 and evaporated to give a mixture of gallotannins (25.7 g, 64.2%). A part (17.8 g) of this mixture, in ethanol, was applied to a Sephadex LH-20 column (3.5 cm i.d. × 22 cm). Elution with a solvent system of ethanol–water–acetone [7,8] and monitoring with normal-phase h.p.l.c., afforded tri- (G3), tetra- (G4), penta- (G5), hexa- (G6), hepta- (G7), and octa- (G8) galloylglucose fractions; (G3) (30 mg); (G4) (1.05 g); (G5) (1.36 g); (G6) (630 mg); (G7) (250 mg); and (G8) (105 mg). The (G5) (120 mg) and (G6) (170 mg) fractions were separately subjected to preparative reverse-phase h.p.l.c. and two isomers (G5-A) and (G5-B) of pentagalloylglucose and four isomers (G6-C), (G6-D), (G6-E), and (G6-F) of hexagalloylglucose were obtained as light-brown amorphous powders; (G5-B), 6-O-*digalloyl*-1,2,3-*tri*-O-*galloyl*-β-D-*glucose* (4), $[\alpha]_D^{20}$ +44.1° (*c* 0.54 in acetone); (G6-D), 6-O-*digalloyl*-1,2,3,4-*tetra*-O-*galloyl*-β-D-*glucose* (8), $[\alpha]_D^{20}$ +43.4° (*c* 0.53 in acetone); (G6-E), 2,6-*bis*-O-*digalloyl*-1,3-*di*-O-*galloyl*-β-D-*glucose* (9), $[\alpha]_D^{20}$ +39.4° (*c* 0.33 in acetone); (G6-F), 6-O-*trigalloyl*-1,2,3-*tri*-O-*galloyl*-β-D-*glucose* (10), $[\alpha]_D^{20}$ +45.2° (*c* 0.35 in acetone). Components (G5-A) and (G6-C) were identified as 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (3) and 4-*O*-digalloyl-1,2,3,6-tetra-*O*-galloyl-β-D-glucose (7), respectively, by comparison of their spectral data with those of samples obtained from Chinese galls.[7]

1,2,3,6-*Tetra*-O-*galloyl*-β-D-*glucose* (2), obtained from fraction (G4) as a light-brown amorphous powder, had $[\alpha]_D^{20}$ +31.8° (*c* 0.38 in acetone); δ_H 4.13 (2 H, m, 4- and 5-H), 4.59 (2 H, m, 6-H_2), 5.47 (1 H, t, *J* 8 Hz, 2-H), 5.68 (1 H, t, *J* 8 Hz, 3-H), 6.18 (1 H, d, *J* 8 Hz, 1-H), and 6.99, 7.07, 7.08, and 7.17 (each 2 H, s, galloyl H); δ_C 165.0 (ester carbonyl of the galloyl group attached to glucose C-1), 165.7 (ester carbonyl at C-2), 166.1 (ester carbonyl at C-3), and 166.6 p.p.m. (ester carbonyl at C-6).

Methylation of compound (2) (42 mg) with dimethyl sulphate (0.3 ml) and anhydrous potassium carbonate (0.5 g) in dry acetone afforded a *dodecamethyl ether* as a white amorphous powder (32 mg), $[\alpha]_D^{18}$ +31.5° (*c* 2.0 in acetone); δ_H 3.7—3.9 (OCH_3), 5.56 (1 H, dd, *J* 8 and 9 Hz, 2-H), 5.85 (1 H, t, *J* 9 Hz, 3-H), 6.24 (1 H, d, *J* 8 Hz, 1-H), and 7.20, 7.27, 7.30, and 7.36 (each 2 H, s, galloyl H).

The dodecamethyl ether (32 mg) was further methylated with silver oxide (0.2 g) and methyl iodide (0.2 ml) in dimethylformamide [10] (1.0 ml) to yield a tridecamethyl ether (14 mg) which was hydrolysed with 0.5M-sodium hydroxide (3 ml) under reflux for 1 h. After neutralization with Dowex 50W X8 (H^+ form), the solution was shaken with diethyl ether to remove tri-*O*-methylgallic acid. The aqueous layer was concentrated and the residue was methanolysed with 1M-hydrochloric acid in methanol. The methanolysates thus obtained were acetylated and subjected to g.l.c. analysis [column: 5% butane-1,4-diol succinate/Chromosorb W AW DMCS (1 m); column temperature 170 °C; N_2 flow rate 80 ml min^{-1}]. The retention times of the methyl glucosides were 52.8 and 60.0 min, identical with those of methyl 4-*O*-methyl-2,3,6-tri-*O*-acetyl-α-(and β-)-D-glucoside.

References

1 Part 8. G. Nonaka, O. Kawahara, and I. Nishioka, *Chem. Pharm. Bull*, 1982, **30**, 4277.
2 E. Fischer and K. Freudenberg, *Chem. Ber.*, 1914, **47**, 2485.
3 P. Karre, R. Widmer, and M. Staub, *Justus Liebigs Ann. Chem.*, 1923, **433**, 288.

4 R. Armitage, G. S. Bayliss, J. W. Gramshaw, E. Haslam, R. D. Haworth, K. Jones, H. J. Rogers, and T. Searle, *J. Chem. Soc.*, 1961, 1842.
5 R. Armitage, E. Haslam, R. D. Haworth, and T. Searle, *J. Chem. Soc.*, 1962, 3803.
6 E. Haslam, *J. Chem. Soc. C*, 1967, 1734.
7 M. Nishizawa, T. Yamagishi, G. Nonaka, and I. Nishioka, *J. Chem. Soc., Perkin Trans. 1*, 1982, 2963.
8 M. Nishizawa, T. Yamagishi, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, 1980, **28**, 2850.
9 G. Nonaka, Y. Matsumoto, I. Nishioka, M. Nishizawa, and T. Yamagishi, *Chem. Pharm. Bull.*, 1981, **29**, 1184.
10 R. Kuhn, I. Löw, and H. Trischmann, *Chem. Ber.*, 1955, **88**, 1492.
11 E. Haslam, R. D. Haworth, S. D. Mills, H. J. Rogers, R. Armitage, and T. Searle, *J. Chem. Soc.*, 1961, 1836.

Received 24*th August* 1982; *Paper* 2/1473