Tannins and Related Compounds. Part 5.[1] Isolation and Characterization of Polygalloylglucoses from Chinese Gallotannin

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

Along with 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose, the homologous series of hexa- and hepta-galloylglucoses have been isolated from Chinese gallotannin, and their structures established on the basis of ^{13}C n.m.r. spectroscopic analysis, methanolysis, and partial hydrolysis. Octa–undeca-galloylglucoses have also been partially purified, and their structures characterized mainly by using ^{13}C n.m.r. spectroscopy. From the isolation and structural studies of these galloylglucoses, Chinese gallotannin has been shown to be a mixture consisting mainly of penta–undeca-galloylglucoses which have depside galloyl group(s) randomly distributed at the C-2, C-3, and C-4 position(s) on a penta-*O*-galloyl-β-D-glucose core. The evidence for the occurrence of *m*- and *p*-depside linkages in a galloyl chain is also described.

In earlier studies by Fischer[2] and Freudenberg,[3] Chinese gallotannin derived from twig galls of *Rhus semialata* L. was shown to be a mixture of polygalloylglucoses having depsidically linked galloyl groups. The average molecular weight corresponded to that of penta-*O*-*m*-digalloyl-β-D-glucose, and this structural assignment was supported by 3,4-di-*O*-methylgallic acid and tri-*O*-methylgallic acid being obtained on hydrolysis of methylated Chinese gallotannin; further, the tannin liberated on hydrolysis approximately ten gallic acids per glucose molecule. However, the structure and composition of this tannin were not clearly defined.

In the 1960's, more detailed studies on Chinese gallotannin were reported by Haslam and his co-workers.[4] They revealed that Chinese gallotannin was composed of only D-glucose and gallic acid on the basis of the results obtained by hydrolysis with tannase (galloyl esterase).[5] They also showed that depsidically linked galloyl groups are preferentially cleaved by methanolysis with aqueous methanol[6] to give 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (1). Thus, they concluded that Chinese gallotannin has the basic structure (1). The depsidically linked galloyl groups in Chinese gallotannin have been presumed to be attached to the 2-hydroxy-group of the glucose residue by analysis of the ^1H n.m.r. spectra.[7,8] However, the proposed structure (9) has many uncertainties, since due to difficulties in the isolation of the individual components a mixture of gallotannins has been used for studies.

Our recent studies of gallotannins in *Paeoniae radix* have revealed the fact that each component can be separated according to the degree of galloylation by a combination of Sephadex LH-20 chromatography and normal-phase high performance liquid chromatography (h.p.l.c.). In the present study the gallotannins with the same galloyl contents were separated from Chinese gallotannin by using these methods, and the structural isomers having the same molecular weights were further separated by means of reverse-phase h.p.l.c.

Results and Discussion

The ethyl acetate-soluble portion of the acetone extract showed eight peaks due to penta—dodeca-galloylglucoses (G5—G12) by normal-phase h.p.l.c. [Figure 1, (A)]. Amongst them penta-, hexa-, hepta-, and octa-galloylglucoses (G5, G6, G7, and G8) were separated by repeated chromatography on Sephadex LH-20 using a solvent system of ethanol–water–acetone (Table 1).

Pentagalloylglucose (G5) showed a single peak in the reverse phase h.p.l.c. [Figure 1, (B)], and was identified as 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (1), by comparison of the ^{13}C and ^1H n.m.r. spectra with those of the sample obtained from *Paeoniae radix*.[9]

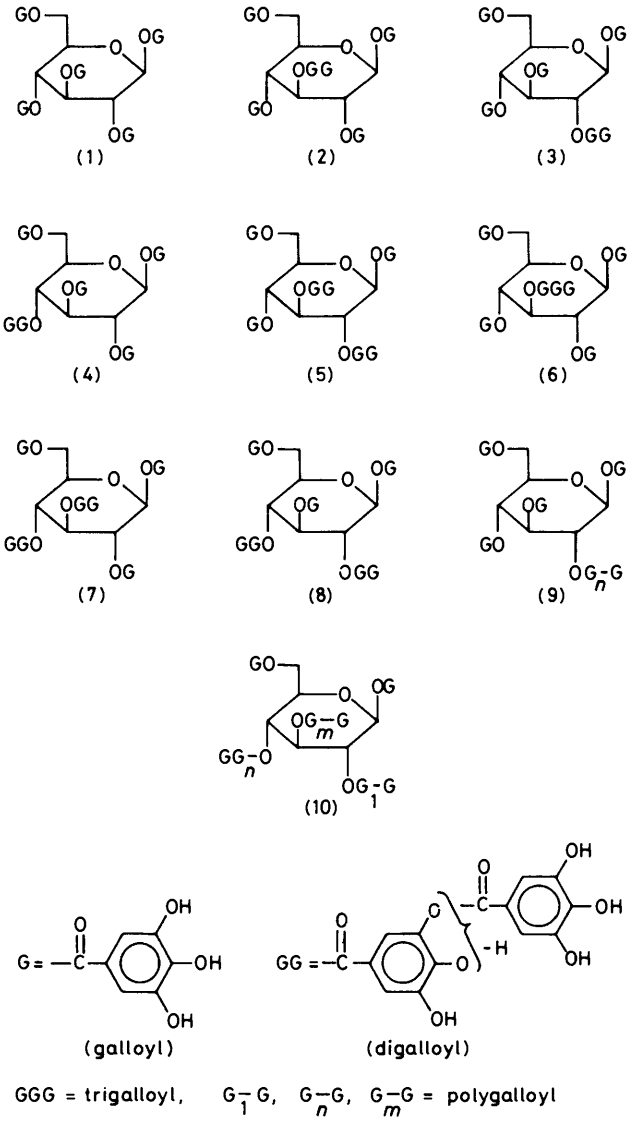


Table 1. Fractionation of gallotannins in Chinese galls on Sephadex LH-20 [a]

Fraction	Eluant (ml) EtOH–H_2O–acetone	Components	Yields (g)
1	100 : 0 : 0 (500)	Gallic acid, digallic acid	1.45
2	90 : 10 : 0 (200)	Digallic acid, trigallic acid	0.09
3	80 : 20 : 0 (200)		0.05
4	70 : 30 : 0 (200)	G5 + unknown	0.034
5	60 : 40 : 0 (100)	$\underline{G5}$ [b] + unknown	0.051
6	60 : 40 : 0 (100)	$\underline{G5}$ + G6	0.065
7	54 : 36 : 10 (100)	$\underline{G5}$ + G6	0.061
8	54 : 36 : 10 (100)	G5 + $\underline{G6}$	0.121
9	48 : 32 : 20 (100)	$\underline{G6}$ + G7	0.372
10	48 : 32 : 20 (100)	$\underline{G6}$ + G7 + G8	0.540
11	42 : 28 : 30 (100)	$\underline{G7}$ + G8 + G9	1.423
12	42 : 28 : 30 (100)	G7 + $\underline{G8}$ + G9 + G10	1.316
13	36 : 24 : 40 (100)	$\underline{G8}$ + G9 + G10 + G11	1.802
14	36 : 24 : 40 (100)	$\underline{G9}$ + G10 + G11 + G12	0.889
15	30 : 20 : 50 (100)	$\underline{G10}$ + G11 + G12	0.520
16	30 : 20 : 50 (200)	$\underline{G11}$ + $\underline{G12}$	0.184

[a] A mixture of gallotannins (9.2 g) was applied to a 3.5 cm i.d. × 17 cm column. [b] Single underlining shows a major component in the fraction.

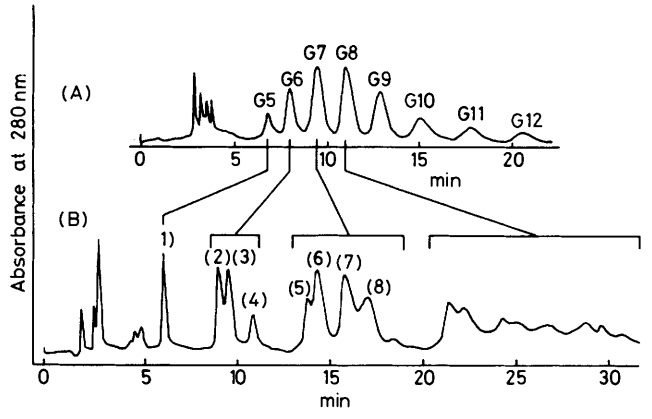


Figure 1. H.p.l.c. of gallotannins in Chinese galls: (A) normal-phase, column Nucleosil 50—10 (3 mm i.d. × 300 mm); solvent n-hexane–methanol–tetrahydrofuran–formic acid (55 : 33 : 11 : 1) (oxalicacid 450 mg/l); (B) reverse-phase, column Nucleosil 5 C_{18} (4 mm i.d. × 250 mm); solvent acetonitrile–water (21 : 79) (oxalic acid 2 g/l)

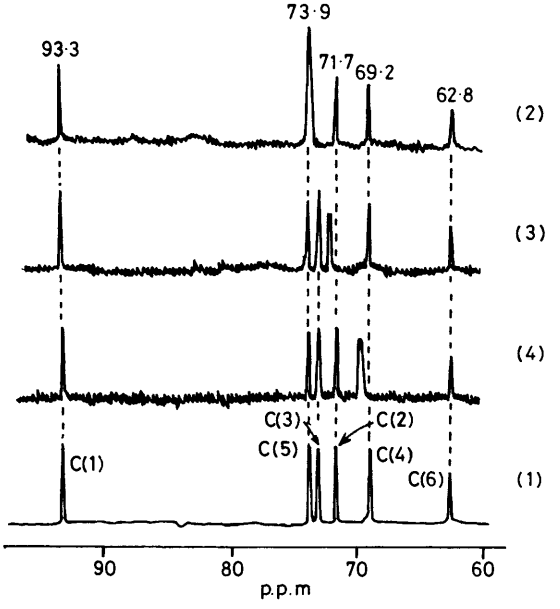


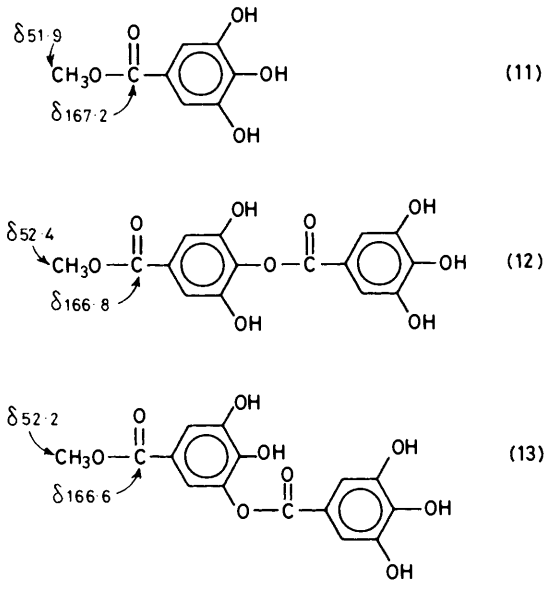
Figure 2. ^{13}C N.m.r. spectra of pentagalloylglucose (1) and three isomers of hexagalloylglucoses (2), (3), and (4)

The hexagalloylglucose (G6) fraction showed three peaks by reverse-phase h.p.l.c. [Figure 1, (B)]. The components of this fraction corresponding to these three peaks [G6-A (2), G6-B (3), and G6-C (4)] were isolated by preparative reverse-phase h.p.l.c. on Nucleosil 10 C_{18} or Wakogel LC-ODS 30K using an acetonitrile–water–acetic acid system as eluant. All these isomers gave equimolar amounts of 1,2,3,4,6-penta-*O*-galloylglucose and methyl gallate on treatment with aqueous methanol (acetate buffer, pH 5.5).[6] Thus, G6-A, G6-B, and G6-C were shown to have a 1,2,3,4,6-penta-*O*-galloylglucose core to which one galloyl group was depsidically linked.

The position of the additional galloyl residue on the penta-*O*-galloylglucose core was determined by comparison of the ^{13}C n.m.r. spectra of the compounds with that of (1).* In the spectrum of G6-A, the signal due to C-3 in the glucose residue was shifted downfield by 0.6 p.p.m., and overlapped with the C-5 signal. This downfield shift was similar to that of the ester methyl carbon observed when methyl digallate was formed from methyl gallate by galloylation. Similarly, signals due to C-2 in G6-B, and C-4 in G6-C were shifted downfield by 0.5 and 0.4 p.p.m., respectively (Figure 2). Therefore, the structures of G6-A, G6-B, and G6-C have been concluded to be 3-*O*-digalloyl-1,2,4,6-tetra-*O*-galloyl-β-D-glucose (2), 2-*O*-digalloyl-1,3,4,6-tetra-*O*-galloyl-β-D-glucose (3), and 4-*O*-digalloyl-1,2,3,6-tetra-*O*-galloyl-β-D-glucose (4), respectively.

The heptagalloylglucose (G7) fraction was composed of five homologues as revealed by h.p.l.c. analysis [Figure 1, (B)], of which four isomers corresponding to the peaks of G7-A (5), G7-B (6), G7-C (7), and G7-D (8) were isolated. All these isomers furnished on methanolysis 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose and methyl gallate in the molar ratio 1 : 2, indicating that these isomers have a 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose core with two depsidically linked galloyl

* Glucose carbon signals were assigned by comparing the chemical shifts with those of penta-*O*-acetyl-β-D-glucose.[10]



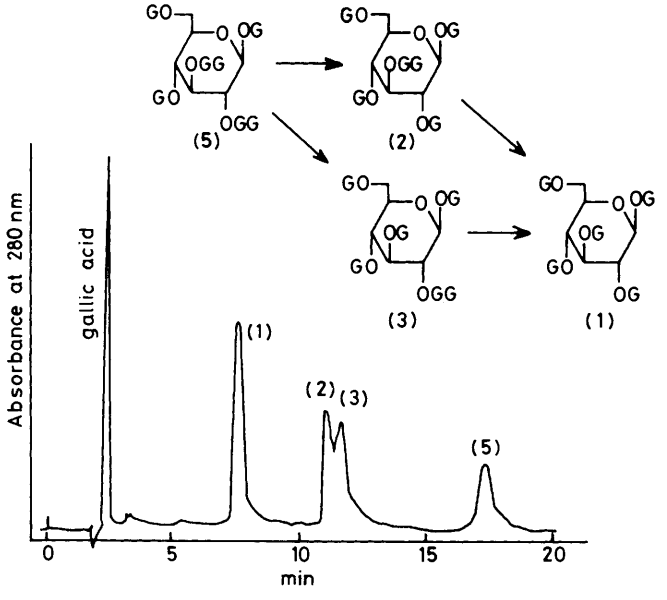


Figure 3. Reverse-phase h.p.l.c. of partial hydrolysis products of compound (5)

groups. The positions of the depside galloyl groups were determined by using ^{13}C n.m.r. spectroscopy and by partial hydrolysis. In the spectra, signals due to C-2 and C-3 in G7-A, C-3 in G7-B, C-3 and C-4 in G7-C, and C-2 and C-4 in G7-D were shifted downfield by 0.4—0.6 p.p.m. as compared with those of (1) (Table 2). Furthermore, hydrolysis of G7-A in aqueous solution afforded a mixture of compounds (1), (2), (3), and gallic acid as examined by reverse-phase h.p.l.c. (Figure 3). Similarly, G7-B, G7-C, and G7-D were partially hydrolyzed in aqueous solution to give a mixture of compounds (1) and (2), gallic acid, and digallic acid; a mixture of compounds (1), (2), and (4) and gallic acid; and a mixture of compounds (1), (3), and (4) and gallic acid, respectively.

Thus, the structures of G7-A, G7-B, G7-C, and G7-D were elucidated as 2,3-bis-*O*-digalloyl-1,4,6-tri-*O*-galloyl-β-D-glucose (5), 3-*O*-trigalloyl-1,2,4,6-tetra-*O*-galloyl-β-D-glucose (6), 3,4-bis-*O*-digalloyl-1,2,6-tri-*O*-galloyl-β-D-glucose (7), and 2,4-

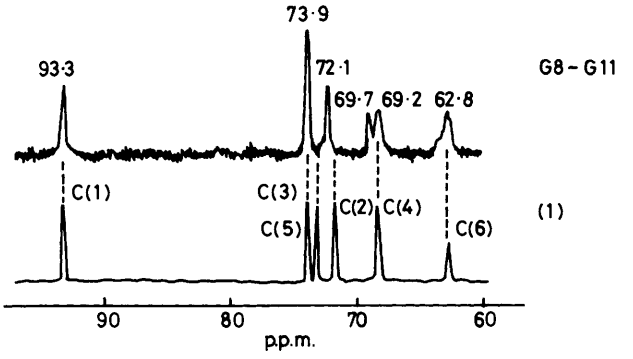


Figure 4. ^{13}C N.m.r. spectra of pentagalloylglucose (1) and a mixture of octa—undeca-galloylglucoses (G8—G11)

bis-*O*-digalloyl-1,3,6-tri-*O*-galloyl-β-D-glucose (8), respectively.

The octagalloylglucose (G8) fraction and a mixture of octa—undeca-galloylglucoses (G8—G11) gave compound (1) and methyl gallate as the methanolysis products, indicating that these gallotannins are composed of a 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose core. Reverse-phase h.p.l.c. analysis [Figure 1, (B)] of these fractions revealed many peaks, suggesting the occurrence of complicated structural isomers. Owing to difficulties in isolating the individual components in these fractions, the distribution of the depsidically linked galloyl groups was examined by means of ^{13}C n.m.r. spectroscopy without further separation. In the spectrum of the G8—G11 fraction (Figure 4), signals due to C-2 (δ 72.1) and C-3 (δ 73.9) carbons in the glucose residue were observed at lower field than those of compound (1). This indicated that in all the isomers both C-2 and C-3 have more than one depsidically linked galloyl group. On the other hand, signals corresponding to C-4 and C-6 were split into two peaks, one being shifted downfield due to the presence of the depside galloyl group(s), and the other remaining unchanged. From the intensities of these split signals at δ 69.2 and 69.7, the proportion of the gallotannins having depsidically linked galloyl group(s) at C-4 was estimated to be one half. Similarly, this proportion at C-6 was estimated to be less than one tenth.

^{13}C N.m.r. chemical shifts of glucose and carbonyl carbons of gallotannins isolated in this study are summarized in Table 2. The carbonyl carbon signals of the proximal galloyl moieties in di- and tri-galloyl chains were observed at *ca.* 0.6 p.p.m. upfield compared with those of the galloyl groups which have no depside linkage; this is in agreement with the upfield shift of the carbonyl carbon in methyl gallate caused by galloylation. On the basis of these facts, the locations of the depside galloyl groups in the glucose moiety were also predictable from the chemical shifts of the carbonyl carbons. In the spectra of compounds (3) and (8), signals due to C-2 in the glucose residue appeared as two signals at δ 72.1 and 72.2, and this splitting was presumed to be due to the occurrence of *m*- and *p*-digalloyl groups at this position.

The depsidically linked galloyl groups in gallotannins have been reported to be attached exclusively through *m*-depside linkages to a carbohydrate core. This conclusion was derived from the fact that 3,4-di-*O*-methylgallic acid and tri-*O*-methylgallic acid were obtained by hydrolysis of methylated gallotannins.[11,12] However, in the ^{13}C n.m.r. spectra of hexa- and hepta-galloylglucoses, signals which do not correspond to a *m*-digalloyl group were observed in the aromatic carbon region. In order to assign these signals, a careful examination was made of the spectrum of methyl digallate (obtained by partial methanolysis of commercial tannic acid with methan-

Table 2. ^{13}C N.m.r. chemical shifts of gallotannins (δ values)

	Glucose						Carbonyl				
	C-1	C-2	C-3	C-4	C-5	C-6	C-1	C-2	C-3	C-4	C-6
(1)	93.3	71.7	73.3	69.2	73.9	62.8	164.9	165.6	165.8	165.5	166.3
(2)	93.3	71.7	*73.9*	69.2	73.9	62.8	164.9	165.6	*165.3* [a]	165.6	166.3
(3)	93.3	*72.1*	73.3	69.2	73.9	62.8	164.8	*165.0* [a]	165.8	165.5	166.3
		72.2									
(4)	93.3	71.7	73.3	*69.7*	73.9	62.8	164.8	165.6	165.8	*165.1* [a]	166.3
(5)	93.3	*72.0*	*73.9*	69.2	73.9	62.8	164.8	*165.0* [a]	*165.3* [a]	165.6	166.3
(6)	93.3	71.7	*73.9*	69.2	73.9	62.8	164.8	165.6	*165.3* [a]	165.6	166.3
(7)	93.3	71.7	*73.9*	69.6	73.9	62.8	164.9	165.5	*165.3* [a]	*165.0* [a]	166.3
(8)	93.3	*72.1*	73.3	*69.7*	73.9	62.8	164.9	*165.0* [a]	165.8	*165.0* [a]	166.3
		72.2									

[a] Carbonyl carbon signal of the proximal galloyl moiety in a galloyl chain.

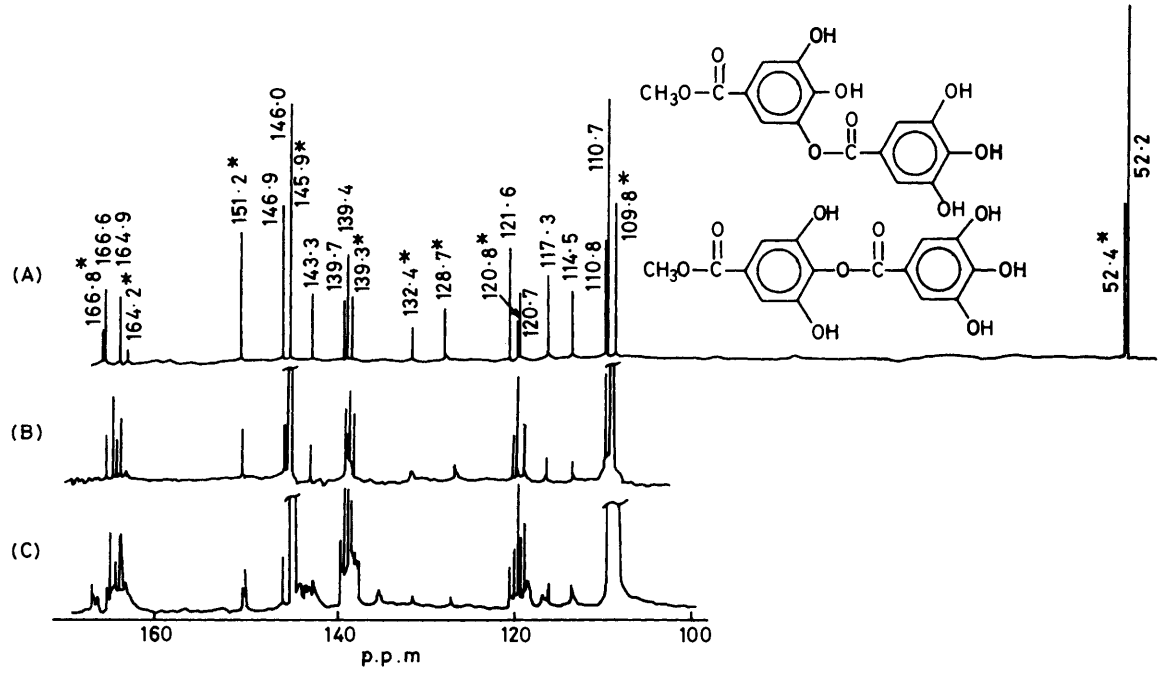
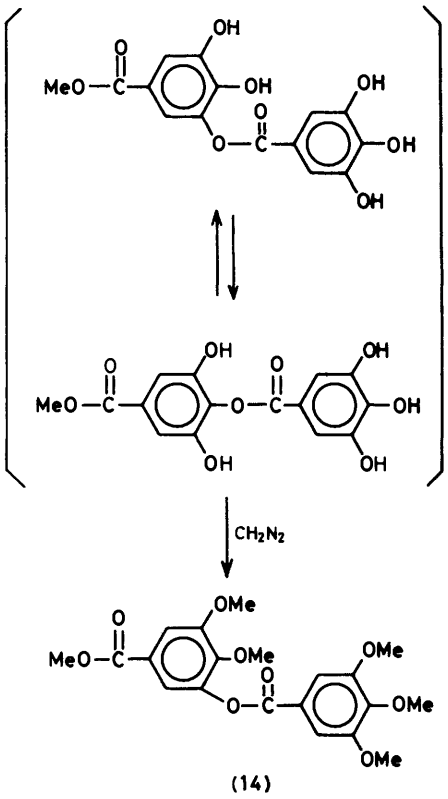


Figure 5. ^{13}C N.m.r. spectra of (A) methyl digallate, (B) G6-A (3), and (C) a mixture of gallotannins. * Signal arising from methyl *p*-digallate

olic hydrochloric acid followed by column chromatography on Sephadex LH-20). Although methyl digallate thus obtained showed a single spot on t.l.c. and a single peak on h.p.l.c., the ^{13}C n.m.r. spectrum exhibited 24 peaks (Figure 5), of which thirteen were assignable to those of methyl *m*-digallate. Next, we synthesized methyl *m*-digallate according to the method described in the literature [6] (condensation of methyl 3,4-diphenylmethylenedioxy-5-hydroxybenzoate with tri-*O*-benzylgallic acid followed by reductive deprotection), and this compound, contrary to our expectations, showed in its ^{13}C n.m.r. spectrum 24 signals analogous to those of the sample obtained from tannic acid. Moreover, a mixture of *p*- and *m*-digallates gave only methyl penta-*O*-methyl-*m*-digallate on methylation with diazomethane. On the basis of these findings, it is presumed that the depsidically linked galloyl groups in Chinese gallatannin migrate between *m*- and *p*-hydroxy-groups of proximal galloyl groups, and also that methyl digallate exists as an equilibrium mixture of *m*- and *p*-isomers. The formation of penta-*O*-methyl-*m*-digallate as the sole product on methylation of the mixture could be explained on the basis that the *p*-hydroxy-group is more reactive to diazomethane due to its stronger acidity, and thus is preferentially methylated.

In conclusion, the structure of Chinese gallotannin has been shown to be that illustrated by formula (10). The relative compositions of penta—dodeca-galloylglucoses in Chinese gallotannin analyzed by normal-phase h.p.l.c. are summarized in Table 3. From this result, Chinese gallotannin has been shown to contain 8.3 galloyl groups per glucose molecule, and the average molecular weight is, therefore, calculated to be 1 434 in close agreement with the values reported by Fischer [2] and Haslam.[4]

* Signals were assigned by the substituent chemical shifts using methyl gallate as a model compound.[13]



(14)

Experimental

^1H and ^{13}C N.m.r. spectra were determined on a JEOL FX-100 spectrometer at 100 MHz and 25.05 MHz, respectively, in CD_3COCD_3 unless otherwise stated, with $SiMe_4$ as internal standard; chemical shifts are given as δ (p.p.m.) values. I.r. spectra were recorded on a Hitachi model 260-10 spectrometer, u.v. spectra on a Hitachi model 200-10 spectrometer, and optical rotations on a Perkin-Elmer model 243 digital polarimeter. M.p.s were determined on a Yanako MP-S3 micro-melting point apparatus. Mass spectra were taken on a Shimadzu LKB-9000 mass-spectrometer. H.p.l.c. was performed on a Hitachi model 638 liquid chromatograph, equipped with a Hitachi variable wavelength spectrophotometric detector. A Nucleosil 50-10 (Macherey-Nagel) column (3 mm i.d. × 300 mm, glass) was used for normal-phase h.p.l.c. The mobile phase was prepared by dissolving 1.0 g of oxalic acid in 1 000 ml of n-hexane–methanol–tetrahydrofuran–formic acid (55 : 33 : 11 : 1). The flow rate was 1.8 ml/min. A Nucleosil $5C_{18}$ column (4 mm i.d. × 250 mm) was used for reverse-phase h.p.l.c. The mobile phase was prepared by dissolving 2.0 g of oxalic acid in 1 000 ml of acetonitrile–water (21 : 79). The flow rate was 1.3 ml/min. The preparative reverse-phase h.p.l.c. system consisted of a Milton-Rhoy pump, a Nucleosil $10C_{18}$ column (8 mm i.d. × 250 mm), and a Pharmacia UV-2 dual-path monitor. A column of Wakogel LC-ODS 30K (Wako Pure Chemicals, 15 mm i.d. × 300 mm) with a Fluid Metering low-pressure pump was also used for the preparative-scale separation.

Isolation of Gallotannins.—Powdered Chinese galls (160 g) were extracted twice with acetone (500 ml) at room temperature for 12 h. The combined extracts were evaporated to dryness under reduced pressure, and the residue was dissolved in water (500 ml). The aqueous solution was partitioned with ethyl acetate–benzene (1 : 1, 300 ml × 2), and then extracted with ethyl acetate (300 ml × 2). The ethyl acetate layer was washed with water, dried over Na_2SO_4, and evaporated to give a mixture of gallotannins (67.4 g, 42.1%). A part of this mixture (9.1 g) in ethanol was applied on a Sephadex LH-20 column (35 mm i.d. × 250 mm), and fractionated using a solvent system as shown in Table 1. Each fraction was analyzed by normal-phase h.p.l.c. On repeated chromatography on Sephadex LH-20, penta(G5)-, hexa(G6)-, hepta(G7)-, and octa(G8)- galloylglucose fractions were obtained; G5, 65 mg; G6, 250 mg; G7, 225 mg; G8, 425 mg. Hexagalloylglucoses (150 mg) and heptagalloylglucoses (180 mg) were subjected to preparative reverse-phase h.p.l.c., and three isomers (G6-A, G6-B, and G6-C) and four isomers (G7-A, G7-B, G7-C, and G7-D) were obtained, respectively, as light brown amorphous powders, 3-O-*digalloyl*-1,2,4,6-*tetra*-O-*galloyl*-β-D-*glucose* (2) (G6-A), $[\alpha]_D^{20}$ +45.0° (*c* 0.32 in acetone); 2-O-*digalloyl*-1,3,4,6-*tetra*-O-*galloyl*-β-D-*glucose* (3) (G6-B), $[\alpha]_D^{20}$ +11.2° (*c* 0.25, in acetone); 4-O-*digalloyl*-1,2,3,6-*tetra*-O-*galloyl*-β-D-*glucose* (4) (G6-C), $[\alpha]_D^{20}$ +14.1° (*c* 0.17, in acetone); 2,3-*bis*-O-*digalloyl*-1,4,6-*tri*-O-*galloyl*-β-D-*glucose* (5) (G7-A), $[\alpha]_D^{20}$ +24.4° (*c* 1.35, in acetone); 3-O-*trigalloyl*-1,2,4,6-*tetra*-O-*galloyl*-β-D-*glucose* (6) (G7-B), $[\alpha]_D^{20}$ +13.4° (*c* 1.35, in acetone); 3,4-*bis*-O-*digalloyl*-1,4,6-*tri*-O-*galloyl*-β-D-*glucose* (7) (G7-C), $[\alpha]_D^{20}$ +36.4° (*c* 1.62, in acetone); 2,4-*bis*-O-*digalloyl*-1,3,6-*tri*-O-*galloyl*-β-D-*glucose*, $[\alpha]_D^{20}$ +12.0° (*c* 1.27, in acetone).

1,2,3,4,6-*Penta*-O-*galloyl*-β-D-*glucose* (1), obtained as a light brown amorphous powder, had $[\alpha]_D^{20}$ +18.3° (*c* 0.38, in acetone); δ_H 4.50 (2 H, m, 6-H), 4.61 (1 H, m, 5-H), 5.61 (1 H, t, *J* 8 Hz, 2-H), 5.66 (1 H, t, *J* 8 Hz, 4-H), 6.03 (1 H, t, *J* 8 Hz, 3-H), 6.35 (1 H, d, *J* 8 Hz, 1-H), and 6.98, 7.02, 7.06, 7.12, and 7.16 (each 2 H, s, galloyl H).

Pentadeca-O-methyl Ether of (1).—A solution of (1) (340 mg) in methanol was treated four times (3 h each) with ethereal diazomethane. After removal of solvent the product was purified by silica-gel column chromatography followed by crystallization from ethanol to yield the pentadeca-*O*-methyl ether as colourless needles, m.p. 96.5—97 °C, $[\alpha]_D^{20}$ +15.2° (*c* 3.2, in acetone); $\nu_{max.}$ (Nujol) 1 725 ($ArCO_2$) and 1585 cm^{-1} (Ar); *m/z* 1 150 $(M)^+$, 406, and 195 (base peak); δ_H ($CDCl_3$) 6.30 (1 H, d, *J* 8 Hz, 1-H). This methylate was shown to be identical with pentakis-*O*-(tri-*O*-methylgalloyl)-β-D-glucose synthesized according to the reported procedure.[14]

Methanolysis of Compounds (2)—(8).—A solution of each gallotannin (1 mg each) in 0.05M acetate buffer (1 ml) and methanol (2 ml) was kept for 24 h at room temperature. The reaction products were analyzed by reverse-phase h.p.l.c., and the ratio of methyl gallate and (1) was determined by

Table 3. Relative compositions of galloylglucoses in Chinese gallotannin

	Ratio (%) [a]	Components
Penta-GG [b]	4	(1)
Hexa-GG	12	(2), (3), (4)
Hepta-GG	19	(5), (6), (7), (8)
Octa-GG	25	More than 8 components
Nona-GG	20	More than 9 components
Deca-GG	13	More than 7 components
Undeca-GG	6	
Dodeca-GG	2	

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

measuring their peak heights. The ratios of methyl gallate to (1) estimated for (2), (3), (4), (5), (6), (7), and (8) were 0.99, 1.01, 0.94, 1.84, 1.84, 1.90, and 2.00, respectively.

Partial Hydrolyses of Compounds (5)—(8).—A solution of each gallotannin in water (1 mg/1 ml) was heated for 0.5 h in a boiling water-bath. After cooling, the products were examined by reverse-phase h.p.l.c.; the results are described in the text.

Methyl Digallate from Tannic Acid.—Commercial tannic acid (10 g) was treated for 36 h with 5% methanolic hydrochloric acid (30 ml) at room temperature. The reaction mixture was diluted with water (100 ml), and extracted with ethyl acetate (200 ml × 2). The ethyl acetate layer was washed with water, dried over Na_2SO_4, and evaporated. The residue in ethanol (20 ml) was subjected to Sephadex LH-20 chromatography (35 mm i.d. × 170 mm). Elution with ethanol yielded methyl digallate (450 mg) which showed a single spot on t.l.c. [silica gel, chloroform–ethyl acetate–formic acid (5 : 4 : 1)], and a single peak on normal-phase h.p.l.c. The ^{13}C n.m.r. spectrum of the compound showed the presence of *m*- and *p*-isomers, δ_C (*m*-isomer) 52.2 (Me), 110.7 (C-2′ and C-6′),* 114.5 (C-6), 117.3 (C-2), 120.8 (C-1), 121.6 (C-1′),* 139.4 (C-4), 139.7 (C-4),* 146.0 (C-3′ and C-5′),* 146.9 (C-5), 164.9 (CO_2),* and 166.6 (CO_2); (*p*-isomer) 52.4 (Me), 109.8 (C-2 and C-6), 110.8 (C-2′ and C-6′),* 120.7 (C-1′),* 128.7 (C-1), 132.4 (C-4), 138.7 (C-4′),* 145.9 (C-3′ and C-5′),* 151.2 (C-3 and C-5), 164.2 (CO_2),* and 166.8 (CO_2). This compound gave methyl gallate on methanolysis, and gallic acid on hydrolysis with tannase.

Methylation of Methyl Digallate.—A solution of methyl digallate (60 mg) isolated from tannic acid in acetone was treated twice with etheral diazomethane at room temperature for 2 h. The reaction mixture showed three spots on t.l.c. [silica gel, chloroform–methanol (200 : 1)]. Subsequent separation by preparative t.l.c. afforded penta-*O*-methyl-*m*-digallate (30 mg) as colourless needles (methanol–water), m.p. 128.5—129.5 °C (Found: C, 58.9; H, 5.5. Calc. for $C_{20}H_{22}O_9$: C, 59.1; H, 5.5%); $\lambda_{max.}$ (EtOH) 262 nm (ε 181 100); *m/z* 406 $(M)^+$, 375, and 195 (base peak); δ_C 52.7, 59.7, 60.7, 61.0 (OMe), 108.4 (C-2′ and C-6′),* 112.6 (C-6), 117.9 (C-2), 124.8 (C-1),* 126.1 (C-1), 144.2 (C-4′),* 145.0 (C-4), 146.3 (C-3), 154.3 (C-5), 154.3 (C-3′ and C-5′),* 164.7 (CO_2),* and 166.2 (CO_2), methyl tetra-*O*-methyldigallate as a colourless amorphous powder (24 mg), *m/z* 392 $(M)^+$ and 181 (base peak); $\nu_{max.}$ (Nujol) 3 350 cm^{-1} (OH), and methyl tri-*O*-methyldigallate as a colourless amorphous powder (15 mg), *m/z* 378 $(M)^+$ and 167 (base peak); $\nu_{max.}$ (Nujol) 3 350 cm^{-1} (OH). Further treatment of the last two compounds with diazomethane afforded penta-*O*-methyl-*m*-digallate, and finally 65 mg of penta-*O*-methyl ether was obtained (96% yield in total). The spectral data of this penta-*O*-methyl ether were identical with those of the sample prepared from methyl 3,4-di-*O*-methylgallate and tri-*O*-methylgalloyl chloride.[6]

* Signals arising from depsidically linked galloyl groups.

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