Phytochemistry, Vol. 25, No. 11, pp. 2599–2604, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

SCYLLO-QUERCITOL GALLATES AND HEXAHYDROXYDIPHENOATES FROM *QUERCUS STENOPHYLLA**

HIROAKI NISHIMURA, GEN-ICHIRO NONAKA and ITSUO NISHIOKA†

Faculty of Pharmaceutical Sciences, Kyushu University 62, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan

(*Received* 22 *January* 1986)

Key Word Index—*Quercus stenophylla*; Fagaceae; *scyllo*-quercitol; gallotannin; ellagitannin; gallic acid; hexahydroxydiphenic acid.

Abstract—A series of gallotannins and ellagitannins based on a *scyllo*-quercitol core have been isolated from the bark of *Quercus stenophylla*. On the basis of chemical and spectroscopic evidence, the structures of the gallotannins have been established as 2-*O*-, 1,2-di-*O*-, 1,2,3-tri-*O*-, 1,2,3,4-tetra-*O*- and 1,2,3,4,5-penta-*O*-galloyl-*scyllo*-quercitols, and the ellagitannins as 1,5-di-*O*-galloyl-2,3-(*S*)-hexahydroxydiphenoyl-*scyllo*-quercitol and 1,4-(or 4,5)-di-*O*-galloyl-2,3-(*S*)-hexahydroxydiphenoyl-*scyllo*-quercitol.

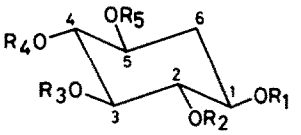
INTRODUCTION

The bark of *Quercus stenophylla* Makino (Fagaceae) is shown to produce an exceptionally complicated mixture of polyphenols (more than sixty compounds), including hydrolysable [1–5], condensed [1] and unclassifiable [6] tannins. Among these compounds, the gallo- and ellagitannins are particularly complex, containing a variety of polyalcohol cores, i.e. D-glucose (in both pyranose and open-chain forms) [6], phenol glucosides (salidroside, etc.) [1, 2], quinic acid [5], *proto*-quercitol [3, 4] and glycerol [Nishimura, H., unpublished results]. In continuation of our systematic chemical analysis of *Q. stenophylla* we have found a series of new gallotannins (**1**–**5**) and ellagitannins (**6** and **7**) based on a pentahydroxycyclohexane (*scyllo*-quercitol) core. We now report their isolation and structure determination.

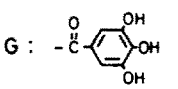
RESULTS AND DISCUSSION

Repeated Sephadex LH-20 and reverse-phase chromatography of the ethyl acetate-soluble portion of an 80% aqueous acetone extract of *Q. stenophylla* afforded compounds **3**–**7**, while compounds **1** and **2** were isolated by a similar method from the aqueous solution after extraction with ethyl acetate. All except for **1**, **6** and **7** were obtained as off-white amorphous powders, and all gave dark blue colours with ferric chloride. The ^{1}H NMR spectra of **1**–**5** showed the presence of galloyl groups in the range δ6.9–7.1, together with one methylene and five methine signals in the aliphatic regions, and the ^{13}C NMR spectra clearly indicated five methines bearing an oxygen function (δ67–80) and one methylene (δ35–39) in each molecule. On the other hand, **6** and **7** were shown by ^{1}H and ^{13}C NMR spectroscopy to possess one 4,4′,5,5′,6,6′-hexahydroxydiphenoyl ester and two galloyl groups in each molecule.

Enzymatic hydrolysis of **2** with tannase afforded gallic acid and a polyalcohol (**8**). The ^{13}C NMR spectrum of **8** shows six aliphatic resonances consisting of one methylene (δ37.5) and five hydroxy-bearing methines [δ69.3 (2 × C), 74.8, and 77.6 (2 × C)], and from this signal pattern, it is evident that **8** is a symmetrical pentahydroxycyclohexane (quercitol) derivative. Among ten possible configurational isomers of pentahydroxycyclohexane derivatives, four (*scyllo*-, *muco*-, *cis*- and *neo*-quercitols) are symmetrical *meso*-types [7]. On the other hand, the ^{1}H NMR spectrum of **2** displays an unsymmetrical signal pattern, showing, together with two galloyl peaks (δ7.04 and 7.10, each 2H, *s*), methine resonances, two of which are shifted considerably downfield (δ5.10 and 5.36) by galloylation. The observation of large coupling constants of all of these signals suggests that these methine protons have axial orientations. The identity of **8** with *scyllo*-quercitol was confirmed by comparison of the ^{1}H NMR

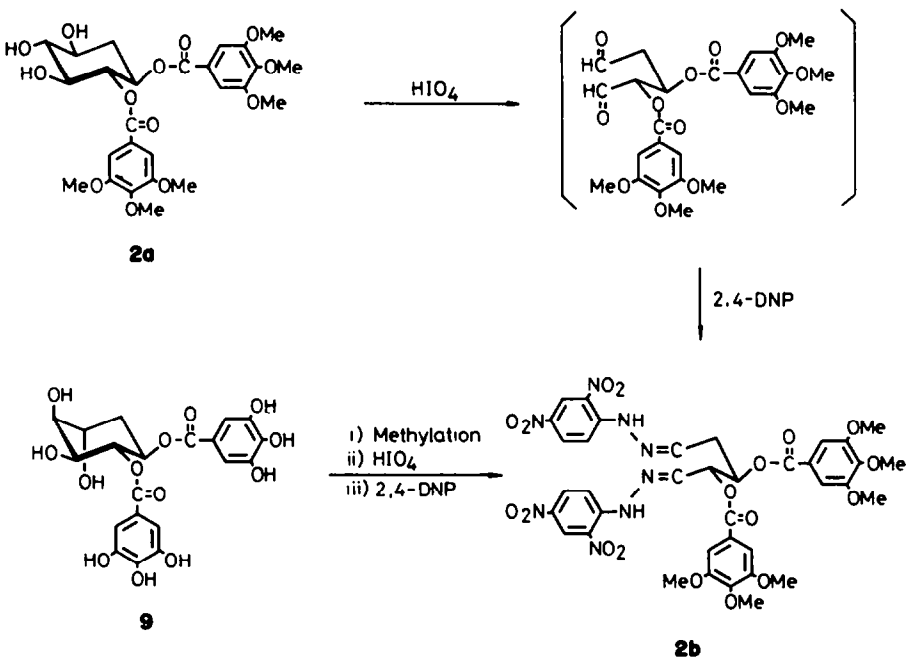


	R1	R2	R3	R4	R5
1	H	G	H	H	H
2	G	G	H	H	H
3	G	G	G	H	H
4	G	G	G	G	H
5	G	G	G	G	G
8	H	H	H	H	H



* Part 44 in the series "Tannins and Related Compounds". For Part 43 see Furuichi, E., Hayashi, K., Nonaka, G. and Nishioka, I., *Agric. Biol. Chem.* (in press).

†To whom correspondence should be addressed.



spectrum with that of an authentic sample [8] which had been prepared from an inositol derivative. In the ^{1}H NMR spectrum of **2**, the above-mentioned lowfield signals are assignable to H-2 and H-1 on the basis of their splitting patterns (t, J = 9 Hz and sextet, J = 4, 9 Hz, respectively), thus establishing the locations of two galloyl groups at the C-1 and C-2 positions.

The absolute configurations of the *scyllo*-quercitol moiety in **2** were determined as follows. The hexamethyl ether (**2a**) obtained by methylation of **2** was subjected to periodic acid oxidation. Without purification, the aldehyde was treated with 2,4-dinitrophenylhydrazine to yield a phenylhydrazone (**2b**) (Scheme 1), which was shown to be identical in all respects with the product similarly prepared from 4,5-di-*O*-galloyl-*proto*-quercitol (**9**) [3]. Accordingly, the C-1 and C-2 atoms in **2** have the same absolute configurations (1*R*, 2*S*) as those of the C-5 and C-4 atoms, respectively, in *proto*-quercitol whose absolute configuration was previously established to be 1*R*, 2*S*, 3*S*, 4*S* and 5*R* [9], and the structure of this compound, including absolute configuration, can be represented by **2**.

Similar enzymatic hydrolysis of **1**, **3**, **4** and **5** with tannase afforded, in all cases, gallic acid and *scyllo*-quercitol, the latter being identified by GC. The ^{1}H NMR spectrum of **1** showed the presence of one galloyl group, consistent with the observation of the $[M+H]^+$ peak at m/z 317 in the FAB-MS. The appearance of a lowfield triplet (δ4.99, J = 10 Hz) due to a methine bearing a galloyl group, suggested that the galloyl group is at either the C-2, C-3 or C-4 position, but because of the unsymmetrical signal pattern the galloyl group must be located at C-2 or C-4 (cf. C-2 and C-4 atoms are relatively equivalent). Thus **1** is either 2 (or 4)-*O*-galloyl-*scyllo*-quercitol.

The ^{1}H NMR spectra of **3**, **4** and **5** clearly indicate the occurrence of three, four and five galloyl groups, respectively. In addition, ^{1}H and ^{13}C NMR examination suggest that **3** and **4** are unsymmetrical and that **5** is symmetrical. The locations of galloyl groups in these compounds were determined as follows. The ^{1}H NMR spectrum (measured in pyridine-d_5) of **4** exhibited four lowfield signals (δ5.6–6.5, *m*), together with an isolated upfield signal (δ4.42) attributable to a methine bearing a hydroxyl group. Since spin-decoupling showed coupling between the upfield signal and C-6 methylene signals, the methine signal can be assigned to H-5 (or H-1). Thus, the galloyl groups in **4** are located at the C-1, C-2, C-3 and C-4 (or C-2, C-3, C-4 and C-5) positions. In the ^{1}H NMR spectrum of **3**, three lowfield and two upfield signals appeared as multiplets at δ5.1–5.7 and δ3.5–3.8, respectively. Irradiation of C-6 methylene signals at δ1.76 (*dd*, J = 12, 12 Hz) and 2.47 (*m*) caused a change in both these multiplets, indicating that there is one galloyl group either at the C-1 or C-5 position. These findings, in conjunction with the above results, indicated the location of three galloyl groups at the C-1, C-2 and C-3 or the C-1, C-2 and C-4 positions. Comparison of the ^{13}C NMR spectrum of **3** with that of **2** showed that in **3** the C-3 signal appeared at a lower field ($+\delta$2.9) than that in **2**, while the neighbouring C-2 and C-4 atoms were observed at higher field ($-\delta$4.0 and $-$2.1, respectively) (Table 1). In addition, when compared with the ^{13}C NMR spectrum of **4**, the C-4 resonance in **3** appeared at a higher field ($-\delta$4.1), and C-3 and C-5 at lower field ($+\delta$4.1 and $+$1.9, respectively). Thus the galloyl groups are attached to the C-1, C-2 and C-3 hydroxyls. The five galloyl groups in **5** were located at the C-1, C-2, C-3, C-4 and C-5 positions from the observed downfield shift of all five methine signals in the ^{1}H NMR spectrum and also from the symmetrical nature of the molecule.

The FAB-MS of **6** and **7** exhibit the same $[M+H]^+$ peak at m/z 771, consistent with the ^{1}H and ^{13}C NMR data which suggest the occurrence of a quercitol moiety and one hexahydroxydiphenoyl and two galloyl groups in each molecule. Tannase hydrolysis of **6** and **7** yielded

Table 1. ^{13}C NMR data for compounds **1–4**, **6–8** ($Me_2CO + D_2O$, TMS as int. standard)

Carbon No.	1	2	3	4	6	7	8*
Scyllo-quercitol							
1	67.8	70.7	70.5	70.1	72.0†	68.1†	69.3
2	79.8	78.2	74.2	74.2	78.3†	78.2†	77.6
3	73.4	73.2	76.1	72.0	77.8†	76.2†	74.8
4	78.2	76.6	74.5	76.8	72.3†	75.4†	77.6
5	69.4	69.1	69.1	67.2	68.2†	67.3†	69.3
6	38.3	35.3	35.2	35.5	32.8	35.7	37.5
Galloyl							
1	121.6	120.7	120.3	120.2(2C)	121.2	120.6	
		121.2	120.5	120.5	121.7	121.0	
			120.9	120.9			
2	110.1	109.9	110.0(3C)	110.0(4C)	109.9	110.0	
		110.0			110.1	110.1	
3	145.7	145.8(2C)	145.8(3C)	145.5(3C)	145.1	145.1	
				146.2	145.3	145.9	
4	138.8	139.1	139.4(3C)	139.1	138.9	139.2	
				139.3	139.2	139.4	
Hexahydroxydiphenoyl							
1					114.4	114.5(2C)	
					114.6		
2					127.0	126.3(2C)	
					127.2		
3					107.2	107.4(2C)	
					107.8		
4,6					144.3	144.3	
					146.1	146.0	
5					136.1	136.3(2C)	
					136.3		
–COO–	167.7	166.7	166.7	166.6(2C)	166.1	166.7	
		167.4	167.1	166.9(2C)	166.3	167.0	
					169.2(2C)	169.3	
						169.4	

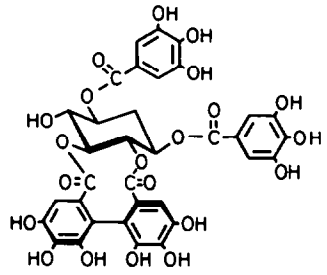
*Measured in D_2O with 1,4-dioxane (δ67.4) as int. standard.
†Assignments in any vertical column may be interchanged.

gallic acid and an identical hydrolysate (**6a**). The ^{1}H and ^{13}C NMR spectra of **6a** showed the presence of a hexahydroxydiphenoyl ester (δ6.70 and 6.71, each 1H, *s*) and no galloyl peak, agreeing with the observation of a $[M+H]^+$ peak at *m/z* 467 in its FAB-MS. Subsequent alkaline methanolysis of the methyl ether (**6b**) prepared from **6a** by methylation afforded *scyllo*-quercitol (**8**) and dimethyl-4,4′,5,5′,6,6′-hexamethoxydiphenoate (**10**). The negative sign of the optical rotation ($-27.3°$, $CHCl_3$) of **10** confirmed the chirality of the atropisomerism to be in the *S*-series.

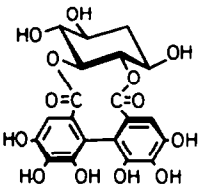
The locations of the hexahydroxydiphenoyl ester group were determined by ^{1}H NMR spectroscopy with spin-decoupling. Because of the broad signals in acetone-d_6 or acetone-$d_6 + D_2O$ and the overlapping of one of the *scyllo*-quercitol methylene signals with solvent peaks in **6a** and **6b**, the permethyl ether (**6c**), obtained by the Kuhn methylation [10], was chosen for study. In the spectrum (in $CDCl_3$) of **6c**, two methines bearing the ester groups appeared as symmetrical multiplets at δ5.30, suggesting that the esters are located at vicinal positions. In addition, since irradiation of each of the C-6 methylene signals (δ1.21, *dd*, J = 12, 13 Hz and δ2.16, *dt*, J = 13, 5 Hz) caused no change of these multiplets, the esters are considered to be present at the C-2 and C-3 (or C-3 and C-4) positions.

^{1}H NMR examination of **6** and **7** provided information concerning the allocations of the galloyl groups. Thus, in **6** the upfield signal (δ4.18, *t*, J = 10 Hz) due to a hydroxy-bearing methine was shown by spin-decoupling not to be coupled with the C-6 methylene signals, indicating that the galloyl groups are located at both the C-1 and C-5 positions and that **6** is therefore 1,5-di-*O*-galloyl-2,3- (or 3,4)-(*S*)-hexahydroxydiphenoyl-*scyllo*-quercitol. In contrast, the spin-decoupling of **7** showed coupling of the methylene protons and the hydroxy-bearing methine (δ4.20, *m*), indicating that the methine signal is assignable to either H-1 or H-5 and that one of the galloyl groups is attached at the C-5 or C-1 hydroxyl. From these observations, two structures (**7a** and **7b**) are possible for **7**, but there was not sufficient sample to allow complete characterization.

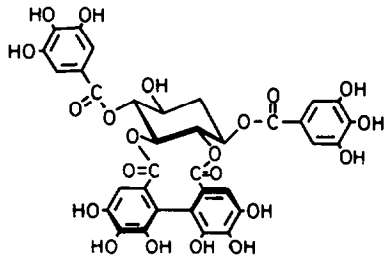
As far as we know, of the ten possible structural isomers of quercitols, only two compounds (*proto*- and *vibo*-quercitols) have hitherto been found in nature [11], and *scyllo*-quercitol therefore represents the third example of



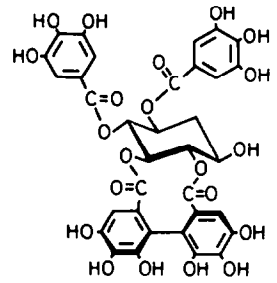
6



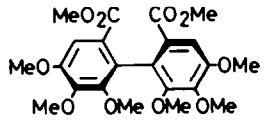
6a



7a



7b



10

a naturally occurring quercitol. In addition, compounds 1–7 can be classified as a new class of hydrolysable tannins containing a *scyllo*-quercitol core.

EXPERIMENTAL

Mps are uncorr. ^1H and ^{13}C NMR spectra were recorded at 100 and 25.05 MHz, respectively, with TMS as reference. FDMS were measured at 14–25 mA (emitter current) and 1.5–3 kV (accelerating voltage), while FAB-MS at 2–3 kV (accelerating voltage) and with DMSO–glycerol as matrix. GLC (with FID detector) was performed at 120° on glass columns (2 m × 3 mm) packed with 1.5% QF-1 and 5% 1,4-butanediol succinate, and the carrier gas, N_2, was set at a flow rate of 40 ml/min. TLC was conducted on silica gel and Avicel SF cellulose, and compounds visualized by $FeCl_3$ and by spraying 10% H_2SO_4 and heated. Plant material was collected in Tokushima Prefecture, Japan. A voucher specimen is deposited at the Herbarium, Faculty of Pharmaceutical Sciences, Kyushu University.

Extraction and isolation. Air-dried, ground material (4.74 kg) of *Q. stenophylla* was extracted × 5 with 80% aq. Me_2CO, and the concd extract re-extracted sequentially with Et_2O and EtOAc. The aq. layer was mixed with Celite 545 (1.2 kg) and air-dried. The EtOAc extract was concd, redissolved in EtOH and applied to a column of Sephadex LH-20. Elution with an EtOH–H_2O–Me_2CO solvent system as outlined previously [12] afforded six fractions; 1 (94.5 g), 2 (25.8 g), 3 (5.7 g), 4 (9.5 g), 5 (4.4 g) and 6 (4.2 g). Subsequent separation of fraction 2 on Sephadex LH-20 CC in H_2O with increasing amounts of MeOH and then in Me_2CO yielded **3** (0.001%). Fraction 4 was chromatographed on Sephadex LH-20 with H_2O–MeOH (1:4) to give five further fractions. Repeated CC of fraction 4-3 on MCI-gel CHP-20P with H_2O–MeOH (7:3) and on Avicel cellulose with 2% HOAc yielded **4** (0.0014%) and **6** (0.009%), while fraction 4-4 gave, on similar repeated CC, **7** (0.009%). Chromatography of fraction 5 on Sephadex LH-20 with H_2O–MeOH, followed by purification on MCI-gel CHP-20P CC, furnished **5** (0.0004%). The above air-dried aq. extract was packed in a glass column, and eluted with Me_2CO. The Me_2CO-soluble portion was subjected to Sephadex LH-20 CC. Elution with H_2O containing increasing amounts of MeOH gave seven fractions; 1 (296 g), 2 (122 g), 3 (198 g), 4 (41 g), 5 (138 g), 6 (24 g) and 7 (44 g). Chromatography of fraction 1 on MCI-gel CHP-20P with H_2O and then on Bondapak C_{18} Porasil B with H_2O afforded **1** (0.0016%). Similar CC of fraction 2 gave **2** (0.0026%).

2-O-*Galloyl*-scyllo-*quercitol* (**1**). Colourless needles ($CHCl_3$–EtOH), mp 254–257° (dec.), $[\alpha]_D^{23}$ +3.6° (Me_2CO; *c* 0.14). FAB-MS *m/z*: 317 $[M+H]^+$. ^1H NMR (Me_2CO-d_6): δ1.62 (1H, *dd*, *J* = 12, 12 Hz, H-6), 2.26 (1H, *dt*, *J* = 12, 4 Hz, H-6), 3.3–4.0 (4H, *m*, H-1, -3, -4 and -5), 4.99 (1H, *t*, *J* = 10 Hz, H-2), 7.15 (2H, *s*, galloyl H). (Found: C, 48.23; H, 5.14. $C_{13}H_{16}O_9 \cdot 1/2\ H_2O$ requires: C, 48.00; H, 5.27%.)

1,2-*Di*-O-*galloyl*-scyllo-*quercitol* (**2**). An off-white amorphous powder, $[\alpha]_D^{23}$ −95.6° (MeOH; *c* 0.31). FAB-MS *m/z* 469 $[M+H]^+$. ^1H NMR (Me_2CO-d_6 + D_2O): δ1.74 (1H, *dd*, *J* = 12, 12 Hz, H-6), 2.43 (1H, *dt*, *J* = 12, 4 Hz, H-6), 3.51, 3.73 (each 1H, *t*, *J* = 9 Hz, H-3 and H-4), 3.74 (1H, *m*, H-5), 5.10 (1H, sextet, *J* = 4, 9 Hz, H-1), 5.36 (1H, *t*, *J* = 10 Hz, H-2), 7.04, 7.10 (each 2H, *s*, galloyl H). (Found: C, 46.16; H, 4.81. $C_{20}H_{20}O_{13} \cdot 3\ H_2O$ requires: C, 45.98; H, 5.02%.)

Tannase hydrolysis of **2**. A soln of **2** (50 mg) in H_2O (10 ml) was incubated with tannase at 37° for 30 min. The solvent was concd under red. pres., and the residue treated with MeOH. The MeOH-soluble portion was CC on Sephadex LH-20 with H_2O and then on silica gel with $CHCl_3$–MeOH–H_2O (7:3:0.5) to yield gallic acid and *scyllo*-quercitol (**8**, 10 mg), colourless needles, mp 233–237°.

Methylation of **2**. A mixture of **2** (30 mg), Me_2SO_4 (0.5 ml) and K_2CO_3 (1 g) in dry Me_2CO (8 ml) was refluxed for 3 hr. After filtration of the inorganic ppt, the soln was concd to a syrup which was CC on silica gel. Elution with C_6H_6–Me_2CO (2:1) afforded the hexamethyl ether (**2a**, 15 mg) as a white amorphous powder, $[\alpha]_D^{16}$ −56.9° ($CHCl_3$; *c* 0.37). FDMS *m/z*: 552 $[M]^+$. ^1H NMR ($CDCl_3$): δ1.78 2.54 (each 1H, *m*, H-6), 3.5–4.9 (3H, *m*, H-3, -4 and -5), 5.19 (1H, *m*, H-1), 5.40 (1H, *t*, *J* = 9 Hz, H-2), 7.12, 7.16 (each 2H, *s*, aromatic H).

Periodic acid oxidation of **2b**, *followed by formation of phenylhydrazone*. A mixture of **2b** (15 mg) and HIO_4 (20 mg) in MeOH was kept at room temp. for 2 hr. The solvent was removed by blowing N_2 gas, and the residue was extracted with H_2O–$CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried and evaporated and the residue treated with 2,4-dinitrophenylhydrazine (15 mg) in 1 N HCl–MeOH at room temp. for 48 hr. After removal of solvent the product was purified by silica gel CC using C_6H_6–Me_2CO (19:1) to give the phenylhydrazone (**2b**) as a yellow amorphous powder, $[\alpha]_D^{24}$ +56.2° ($CHCl_3$; *c* 0.6). FDMS *m/z*: 880 $[M]^+$. ^1H NMR ($CDCl_3$): δ3.08 (2H, *m*, H-4), 3.8–3.95 (18H, 6 × OMe), 6.11 (2H, *m*, H-2 and H-3), 7.27 (4H, *s*, aromatic H), 7.60 (2H, *m*, H-1 and H-5), 7.82, 7.92 (each 1H, *d*, *J* = 7 Hz, aromatic H), 8.23, 8.33 (each 1H, *dd*, *J* = 7, 2 Hz, aromatic H), 9.07, 9.10 (each 1H, *d*, *J* = 2 Hz, aromatic H), 11.06, 11.20 (each 1H, *s*, –NH–, exchangeable on addition of D_2O). ^{13}C NMR ($CDCl_3 + D_2O$): δ34.4 (C-4), 56.3 (4 × C, OMe), 60.9 (2 × C, OMe), 70.3, 73.4 (C-2 and C-3), 165.0, 165.4 (–COO–).

Methylation of **9**. Compound **9** (0.3 g) was methylated with Me_2SO_4 (2 ml) and K_2CO_3 (3 g) in dry Me_2CO (15 ml). The reaction mixture was worked up in the same way as described above to yield the hexamethyl ether (0.19 g) as a white amorphous powder, $[\alpha]_D^{16}$ −47.6° ($CHCl_3$; *c* 0.21). FDMS *m/z*: 552 $[M]^+$. ^1H NMR ($CDCl_3$): δ2.30 (2H, *m*, H-6), 3.8–3.9 (OMe), 4.1–4.3 (3H, *m*, H-1, -2 and -3), 5.54 (2H, *m*, H-4 and H-5), 7.20, 7.23 (each 2H, *s*, aromatic H).

Periodic acid oxidation, followed by formation of phenylhydrazone. A mixture of the methyl ether (0.15 g) of **9** and HIO_4 (0.19 g) in MeOH (3 ml) was kept at room temp. for 30 min. The reaction mixture was worked up as described above to give a dialdehyde, which was dissolved in 1 N HCl–MeOH (20 ml) and treated with 2,4-dinitrophenylhydrazine (0.14 g) at room temp. for 2 hr. Purification of the product as above afforded the phenylhydrazone, which was shown to be identical with **2b** in respects of $[\alpha]_D$ and ^1H and ^{13}C NMR spectra.

1,2,3-*Tri*-O-*galloyl*-scyllo-*quercitol* (**3**). An off-white amorphous powder, $[\alpha]_D^{18}$ +15.6° (MeOH; *c* 0.27). ^1H NMR (MeOH-d_4): δ1.76 (1H, *dd*, *J* = 12, 12 Hz, H-6), 2.47 (1H, *m*, H-6), 3.5–3.8 (2H, *m*, H-4 and H-5), 5.1–5.7 (3H, *m*, H-1, -2 and -3), 6.87, 6.96, 7.00 (each 2H, *s*, galloyl H). (Found: C, 51.14; H, 4.55. $C_{27}H_{24}O_{17} \cdot H_2O$ requires: C, 50.79; H, 4.10%.)

1,2,3,4-*Tetra*-O-*galloyl*-scyllo-*quercitol* (**4**). An off-white amorphous powder, $[\alpha]_D^{17}$ −38.0° (Me_2CO; *c* 0.48). FDMS *m/z*: 773 $[M+H]^+$, 795 $[M+NA]^+$. ^1H NMR (Me_2CO-d_6): δ2.02 (1H, *dd*, *J* = 12, 12 Hz, H-6), 2.58 (1H, *m*, H-6), 4.24 (1H, *m*, H-5), 5.2–6.5 (4H, *m*, H-1, -2, -3 and -4), 6.92, 6.95, 7.04, 7.05 (each 2H, *s*, galloyl H). ^1H NMR (C_5D_5N): δ2.24 (1H, *dd*, *J* = 12, 12 Hz, H-6), 2.90 (1H, *m*, H-6), 4.42 (1H, *m*, H-5), 5.6–6.5 (4H, *m*, H-1, -2, -3 and -4), 7.82 (6H, *s*, 3 × galloyl H), 7.85 (2H, *s*, galloyl H). (Found: C, 50.81; H, 3.90. $C_{34}H_{28}O_{21} \cdot 2H_2O$ requires: C, 50.50; H, 3.99%.)

1,2,3,4,5-*Penta*-O-*galloyl*-scyllo-*quercitol* (**5**). An off-white amorphous powder. ^1H NMR (Me_2CO-d_6): δ2.1–3.0 (2H, *m*, H-6), 5.4–6.0 (5H, *m*, H-1, -2, -3, -4 and -5), 6.97 (2H, *s*, galloyl H), 7.00, 7.06 (each 4H, *s*, 2 × galloyl H).

Tannase hydrolysis of **1**, **3**, **4** *and* **5**. Each sample (3 mg) in H_2O (1 ml) was shaken with tannase at 37° for 30 min. TLC analysis (C_6H_6–HCOOEt–HCO_2H, 2:7:1) gave gallic acid (R_f 0.74), while GC gave the peaks [R_t 10.2 min (1.5% QF-1) and R_t 10.9 (5% 1,4-butanediol succinate)] which coincide with those of *scyllo*-quercitol (**8**).

1,5-*Di*-O-*galloyl*-2,3-(S)-*hexahydroxydiphenoyl*-scyllo-*quercitol* (**6**). Colourless needles (H_2O), mp 253–255°, $[\alpha]_D^{20}$ −25.5° (Me_2CO; *c* 0.85). FAB-MS *m/z*: 771 $[M+H]^+$ $[M+Na]$. ^1H NMR (Me_2CO-d_6): δ1.94 (1H, *dd*, *J* = 12, 12 Hz, H-6), 2.66 (1H, *m*, H-6), 4.18 (1H, *t*, *J* = 10 Hz, H-4), 5.0–5.6 (4H, *m*, H-1, -2, -3 and -5), 6.39, 6.72 (each 1H, *s*, hexahydroxydiphenoyl H), 7.09, 7.16 (each 2H, *s*, galloyl H). (Found: C, 52.39; H, 3.77. $C_{34}H_{26}O_{21} \cdot 1/2\ H_2O$ requires: C, 52.38; H. 3.49%.)

Tannase hydrolysis of **6**. A soln of **6** (15 mg) in H_2O (1 ml) was treated with tannase in H_2O (1 mg/ml), and the mixture was incubated at 37° for 3 hr. The reaction products were separated by CC over Sephadex LH-20. Elution with H_2O yielded gallic acid and **6a** as an off-white amorphous powder, $[\alpha]_D^{19}$ +30.3° (MeOH; *c* 0.32). FAB-MS *m/z*: 467 $[M+H]^+$, 489 $[M+Na]^+$. ^1H NMR (Me_2CO-d_6): δ1.62 (1H, *dd*, *J* = 12, 12 Hz, H-6), 2.35 (1H, *m*, H-6), 3.6–4.1 (3H, *m*, H-1, -4 and -5), 4.80 (2H, m, H-2 and H-3), 6.70, 6.71 (each 1H, *s*, aromatic H).

Methylation of **6a**. Compound **6a** (85 mg) was methylated in the usual way to furnish the hexamethyl ether (**6b**, 50 mg) as colourless plates ($CHCl_3$–EtOAc), mp 261–264°, $[\alpha]_D^{24}$ −40.8° (Me_2CO; *c* 0.35). FDMS *m/z*: 505 $[M]^+$. ^1H NMR (Me_2CO-d_6 + D_2O): δ1.62 (1H, *dd*, *J* = 12, 12 Hz, H-6), 2.30 (1 H, *m*, H-6), 3.60, 3.85, 3.88 (each 6H, *s*, 2 × OMe), 3.5–4.0 (3H, *m*, H-1, -4 and -5), 4.82 (2H, *m*, H-2 and H-3), 7.06, 7.08 (each 1H, *s*, aromatic H).

Alkaline methanolysis of **6b**. A soln of **6b** (8 mg) in 2% NaOMe–MeOH was kept at room temp. for 2 hr. The reaction mixture was neutralized with Dowex 50W-X8 resins, and the products separated by silica gel CC. Elution with EtOAc–hexane (1:3) yielded dimethyl (*S*)-hexamethoxydiphenoate (**10**, 2 mg) as a colourless oil, $[\alpha]_D^{20}$ −27.3° ($CHCl_3$; *c* 0.11). Further elution with $CHCl_3$–MeOH–H_2O (7:3:0.5) gave *scyllo*-quercitol (**8**) which was identified by GLC examination.

Permethylation of **6b**. A mixture of **6b** (30 mg), MeI (2.5 ml) and freshly prepared Ag_2O (150 mg) in DMF (0.5 ml) was left at room temp. with stirring for 6 hr. After filtration of inorganic salts, the filtrate was subjected to silica gel CC. Elution with C_6H_6–Me_2CO (23:2) gave the permethyl ether (**6c**) as colourless needles (hexane–$CHCl_3$), mp 210°, $[\alpha]_D^{17}$ −2.9° ($CHCl_3$; *c* 0.76). EIMS *m/z*: 592 $[M]^+$. ^1H NMR (C_6D_6): δ1.21 (1H, *dd*, *J* = 12, 13 Hz, H-6), 2.16 (1H, *dt*, *J* = 5, 13 Hz, H-6), 2.8–3.3 (3H, *m*, H-1, -4 and -5), 3.18, 3.20, 3.34, 3.56, 3.76, 3.77, 3.78, 3.79 (each 3H, *s*, OMe), 5.30 (2H, *m*, H-2 and H-3), 6.77, 6.81 (each 1H, *s*, aromatic H). CD (MeOH; *c* 3.2 × 10^{-4}): $[\theta]_{232} + 1.46 \times 10^5$, $[\theta]_{250} - 1.76 \times 10^4$, $[\theta]_{260} + 0.93 \times 10^4$, $[\theta]_{300} - 1.39 \times 10^4$. (Found: C, 58.62; H, 6.23. $C_{29}H_{36}O_{13}$ requires: C, 58.78; H, 6.12%.)

1,4- (*or* 4,5)-*Di*-O-*galloyl*-2,3-(S)-*hexahydroxydiphenoyl* scyllo-*quercitol* (**7**). Colourless needles (H_2O), mp 248–250°, $[\alpha]_D^{20}$ +3.1° (Me_2CO; *c* 0.45). FAB-MS *m/z*: 771 $[M+H]^+$. ^1H NMR (Me_2CO-d_6): δ2.02, 2.60 (each 1H, *m*, H-6), 4.20 (1H, *m*, H-1), 5.0–5.6 (4H, m, H-1, -2, -3 and -4), 6.36, 6.40 (each 1H, *s*, aromatic H), 7.13 (4H, *s*, 2 × galloyl H).

Acknowledgements— The authors are grateful to Prof. T. Nohara (Kumamoto University) and Dr. K. Murakami (Tokushima University) for collection of plant material, and Dr. T. Tanaka (Sankyo Co., Ltd.) for provision of tannase. They are also indebted to Mr. Y. Tanaka, Miss K. Soeda and Mr. K. Isobe (Kyushu University) for measurements of ^{13}C NMR, ^1H NMR and MS, respectively. This work was supported in part by a grant from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1. Nonaka, G., Nishimura, H. and Nishioka, I. (1982) *Chem. Pharm. Bull.* **30**, 2061.
2. Nishimura, H., Nonaka, G. and Nishioka, I. (1984) *Chem. Pharm. Bull.* **32**, 1735.
3. Nishimura, H., Nonaka, G. and Nishioka, I. (1984) *Chem. Pharm. Bull.* **32**, 1741.
4. Nishimura, H., Nonaka, G. and Nishioka, I. (1984) *Chem. Pharm. Bull.* **32**, 1750.
5. Nishimura, H., Nonaka, G. and Nishioka, I. (1984) *Phytochemistry* **23**, 2621.
6. Nonaka, G., Nishimura, H. and Nishioka, I. (1985) *J. Chem. Soc. Perkin Trans.* 1, 163.
7. MacCasland, G. E., Furta, S., Johnson, L. F. and Shoolery, J. N. (1961) *J. Am. Chem. Soc.* **20**, 2335.
8. Angyal, S. J. and Odier, L. (1982) *Carbohydr. Res.* **100**, 43.
9. MacCasland, G. E., Naumann, M. O. and Durham, L. J. (1968) *J. Org. Chem.* **33**, 4420.
10. Kuhn, R., Löw, I. and Trischmann, H. (1960) *Chem. Ber.* **88**, 1492.
11. Plouvier, V. (1963) in *Chemical Plant Taxonomy* (Swain, T., ed.). Academic Press, New York.
12. Nishizawa, M., Yamagishi, T., Nonaka, G. and Nishioka, I. (1982) *J. Chem. Soc. Perkin Trans.* 1, 2525.