Phytochemistry, Vol. 26, No. 5, pp. 1501–1504, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00

GALLIC ACID ESTERS OF *PROTO*-QUERCITOL, QUINIC ACID AND (−)-SHIKIMIC ACID FROM *QUERCUS MONGOLICA* AND *Q. MYRSINAEFOLIA**

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(*Received* 22 *September* 1986)

Key Word Index—*Quercus mongolica*; *Q. myrsinaefolia*; Fagaceae; gallotannins; *proto*-quercitol; quinic acid; (−)-shikimic acid; gallic acid.

Abstract—Six new gallotannins: 1-*O*- and 1,4-di-*O*-galloyl *proto*-quercitols, 1,4-di-*O*- and 1,3,4-tri-*O*-galloylquinic acids and 4-*O*- and 5-*O*-galloyl(−)-shikimic acids have been isolated from *Quercus mongolica* and *Q. myrsinaefolia* and their structures elucidated on the basis of chemical and spectroscopic evidence.

INTRODUCTION

Recent chemical studies of polyphenolic constituents in fagaceous plants have revealed the occurrence of many gallotannins containing a variety of polyalcohol cores such as D-glucose [1], D-hamamelose [2–4], *proto*-quercitol [5], *scyllo*-quercitol [6], quinic acid [7], (−)-shikimic acid [8] and simple phenolic glycosides (salidroside, etc.) [9, 10]. In continuing our systematic chemical examinations of fagaceous plants, we have now isolated six additional gallic acid esters with *proto*-quercitol (**1**, **2**), quinic acid (**3**, **4**) and (−)-shikimic acid (**5**, **6**), together with previously reported compounds (**7**–**12**), from acorns of *Quercus mongolica* Fisch. ex Turcz. and leaves of *Q. myrsinaefolia* Blume.

RESULTS AND DISCUSSION

The aqueous acetone extract of fresh acorns of *Quercus mongolica* was subjected to a combination of Sephadex LH-20 and MCI-gel CHP-20P chromatography using various solvent systems to afford compounds **1**, **5**, **6** and **12**. Extraction of the fresh leaves of *Quercus myrsinaefolia* with aqueous acetone, followed by similar chromatographic separation, gave compounds **2**–**4** and **7**–**11**. Among these compounds, **7**–**12** were identified as 3-*O*-galloyl *proto*-quercitol (**7**) [5], 3-*O*-, 4-*O*-, 3,4-di-*O*- and 3,5-di-*O*-galloylquinic acids (**8**–**11**) [7] and 3-*O*-galloyl-(−)-shikimic acid (**12**) [8] by comparison of their physical and spectral data with those of authentic samples.

Compound **1** gave a blue colouration (characteristic of gallotannins) with ferric chloride. The ^{1}H and ^{13}C NMR spectra indicated the presence of a galloyl group [δ7.13 (2H, *s*). δ110.1 (2C), 120.8, 139.4, 1459 (2C), 166.6] and a polyalcohol moiety with one methylene (δ32.3) and five methines carrying an oxygen function (δ70.1, 70.8, 72.4, 72.7, 75.5). On enzymatic hydrolysis with tannase, **1** gave gallic acid and a polyalcohol which was shown to be identical with *proto*-quercitol [5] by comparison of its physical and spectral data with those of an authentic sample. The location of the galloyl group was determined as follows. In the ^{1}H NMR spectrum of **1** a signal due to a methine bearing the galloyl group appeared considerably downfield [δ5.19 (1H, *dt*, J = 3, 4 Hz)] and this signal was considered to be assignable to the C-1 proton from its coupling pattern. This assignment was confirmed by a spin-decoupling experiment; on irradiation at the frequency of the C-6 methylene signal (δ2.10), this double–triplet signal changed into a doublet having a small J-value (4 Hz). Since the C-5 proton, which was also coupled with the C-6 methylene, has an axial orientation, this methine signal was assignable to the C-1 proton. On the basis of these results **1** was characterized as 1-*O*-galloyl *proto*-quercitol.

Compound **2** liberated gallic acid and *proto*-quercitol on treatment with tannase. The ^{1}H NMR spectrum of **2** showed the occurrence of two galloyl groups [δ7.15, 7.16 (each 2H, *s*)] in the molecule. In addition, two methine signals were observed in the lowfield [δ5.26 (1H, *dt*, J = 3, 4 Hz), 5.36 (1H, *t*, J = 9 Hz)]; the former double–triplet signal could be assigned to the C-1 proton based on the coupling pattern similar to that of **1**, and the latter to the C-4 proton from its large coupling constant (the neighbouring C-3 and C-5 protons both possess axial orientations). From these spectral data the locations of the galloyl groups were concluded to be at the C-1 and C-4 positions. Accordingly **2** was characterized as 1,4-di-*O*-galloyl *proto*-quercitol.

The ^{1}H and ^{13}C NMR spectra of compound **3** showed the existence of two galloyl groups [δ7.16, 7.18 (each 2H, *s*)] and a polyalcohol moiety with two methylenes (δ35.7, 40.4), three methine carbons bearing an oxygen function (δ65.1, 67.2, 78.9), a quarternary carbon (δ80.6) and a carboxylic acid (δ173.5). On enzymatic hydrolysis with tannase, **3** gave gallic acid and a hydrolysate, which was identified as quinic acid by direct comparison. The ^{1}H NMR spectrum of **3** showed a lowfield methine signal [δ4.96 (1H, *dd*, J = 9, 3 Hz)], ascribable to the C-4 axial

* Part 57 in the series "Tannins and Related Compounds". For Part 56 see Hashimoto, F., Nonaka, G. and Nishioka, I. *Chem. Pharm. Bull.* (submitted for publication).

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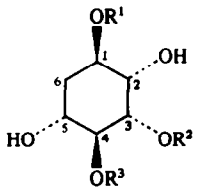

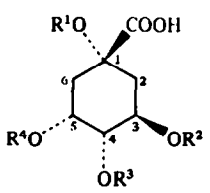

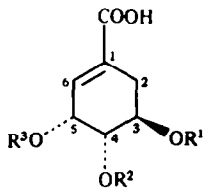

	R^1	R^2	R^3
1	G	H	H
2	G	H	G
7	H	G	H

	R^1	R^2	R^3	R^4
3	G	H	G	H
4	G	G	G	H
8	H	G	H	H
9	H	H	G	H
10	H	G	G	H
11	H	G	H	G

	R^1	R^2	R^3
5	H	G	H
6	H	H	G
12	G	H	H

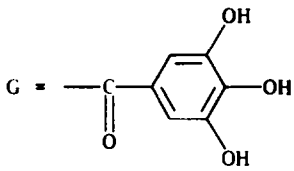

Table 1. ^{13}C NMR spectral data of compounds **1**–**6** at 25.05 MHz (δ values)

	1*	2†	3†	4†	5†	6†
Polyalcohol						
C-1	72.7‡	72.0‡	80.6	80.1	129.9	132.4
C-2	72.4‡	71.6‡	40.4	37.7	31.8	31.5
C-3	70.8‡	71.6‡	65.1	67.4	65.3‡	68.3
C-4	75.5	78.4	78.9	75.4	74.5	70.4‡
C-5	70.1	68.4	67.2	68.2	65.6‡	70.6‡
C-6	32.3	33.2	35.7	35.5	138.9	134.5
COOH			173.5	172.8	168.1	167.9
Galloyl						
C-1′	120.8	121.5	122.0 (×2)	121.2	121.9	121.6
		122.2		121.3		
				121.8		
C-2′	110.1	109.9	110.2 (×2)	110.0	110.1	110.2
		110.2		110.1		
				110.3		
C-3′	145.9	145.8	145.8 (×2)	145.8 (×3)	146.0	146.0
		146.1				
C-4′	139.4	138.6	138.8 (×2)	138.9 (×3)	138.9	138.9
		139.1				
–COO–	166.6	165.7	165.9	165.9	166.5	166.4
		167.3	166.7	166.3		
				166.4		

*In Me_2CO-d_6 + D_2O.
†In Me_2CO-d_6.
‡Signals may be interchanged in each column.

proton. On the other hand, the ^{13}C NMR spectrum of **3** showed lowfield shifts of the C-1 and C-4 signals (δ80.6, 78.9) as compared with those (δ76.5, 77.9) in **9**. From these spectral data, the locations of two galloyl groups were concluded to be at the C-1 and C-4 positions. Thus, **3** was determined to be 1,4-di-*O*-galloylquinic acid.

Compound **4** afforded gallic acid and quinic acid on enzymatic hydrolysis with tannase. The ^{1}H NMR spectrum of **4** exhibited the presence of three galloyl groups [δ7.08, 7.12, 7.27 (each 2H, *s*)] and two methine carbons bearing a galloyl group [δ5.32 (1H, *dd*, J = 9, 3 Hz), 5.92 (1H, *m*)]; the former double-doublet methine signal could be assigned to the C-4 axial proton, and the latter multiplet signal to the C-3 proton owing to its large halfwdith value ($J_{W_{h/2}}$ = 20 Hz). Furthermore, the ^{13}C NMR spectrum of **4** showed the lowfield shift of the C-1 signal

(δ80.1) analogous to that (δ80.6) observed in **3**. From these results, **4** was concluded to be 1,3,4-tri-*O*-galloylquinic acid.

The ^{1}H and ^{13}C NMR spectra of **5** revealed the presence of a galloyl group [δ7.10 (2H, *s*)] and a polyalcohol moiety with a methylene (δ31.8), three methines carrying an oxygen function (δ65.3, 65.6, 74.5), a trisubstituted double bond (δ129.9, 138.9) and a carboxylic acid (δ168.1). On enzymatic hydrolysis with tannase, **5** gave gallic acid and a hydrolysate, which was identified as (−)-shikimic acid by direct comparison. The location of the galloyl group was determined by analysis of the ^{1}H NMR spectrum of **5**; a lowfield signal [δ5.16 (1H, *dd*)], corresponding to the methine proton geminal to the galloyl group, was assignable to the C-4 proton on the basis of its *J*-value (7, 4 Hz). Thus, **5** was characterized as 4-*O*-galloyl-(−)-shikimic acid.

Compound **6** yielded gallic acid and (−)-shikimic acid on enzymatic hydrolysis with tannase. The ^{1}H and ^{13}C NMR spectra of **6** were similar to those of **5** showing the presence of a galloyl group. In addition, the ^{1}H NMR spectrum gave a lowfield methine signal [δ5.80 (1H, *t*, *J* = 4 Hz)], which was assignable to the C-5 proton, indicating the location of the galloyl group at this position. Consequently, **6** was concluded to be 5-*O*-galloyl-(−)-shikimic acid.

Several gallic acid esters with *proto*-quercitol [5] and quinic acid [7, 11] are known, but this is the first reported isolation of gallic acid esters substituted at the C-1 positions of *proto*-quercitol and quinic acid.

EXPERIMENTAL

Mps are uncorr. ^{1}H and ^{13}C NMR spectra were recorded at 100 and 25.05 MHz, respectively, with TMS as reference. TLC was performed on silica gel and Avicel SF cellulose, and the spots were detected by spraying $FeCl_3$, 10% H_2SO_4 and NH_4OH–$AgNO_3$ reagents. Plant materials were collected at Fukuoka prefecture, Japan. Voucher specimens are deposited at the Herbarium, Faculty of Pharmaceutical Sciences, Kyushu University.

Extraction and isolation. (a) From acorns of *Quercus mongolica*: fresh acorns (12.3 kg) were mashed and extracted × 5 at room temp. with 80% aq. Me_2CO. The combined extracts, after concn under red. pres. to *ca* 3 l, were subjected to Sephadex LH-20 CC using H_2O with increasing amounts of MeOH to afford 6 fractions: frs. 1 (6.1 g), 2 (*ca* 130 g), 3 (2.99) 4 (*ca* 315 g), 5 (20 g) and 6 (29 g). Fraction 2 was rechromatographed over MCI-gel CHP-20P [H_2O–MeOH (1:0–1:9)] and Sephadex LH-20 (EtOH, 60% aq. MeOH) to yield **1** (86 mg), **5** (12 mg), **6** (25 mg) and **12** (12 mg). (b) From leaves of *Q. myrsinaefolia*: fresh leaves (7.9 kg) were extracted × 4 at room temp. with 80% aq. Me_2CO. The combined extracts were concd under red. pres., and the ppt. was filtered off. The filtrate (*ca* 1 l.) was subjected to CC over Sephadex LH-20 using H_2O–MeOH (1:0–0:1) to give 5 fractions (frs. 1–5). Fraction 1 (4.8 g) was rechromatographed over MCI-gel CHP-20P (H_2O–MeOH) and Fuji-gel ODS-G3 (H_2O–MeOH) to afford **7** (55 mg) and **9** (498 mg). Fractions 3 (32 g) and 4 (6.1 g) were separately purified by CC over Sephadex LH-20 (EtOH, 60% aq. MeOH), MCI-gel CHP-20P (H_2O–MeOH) and Bondapak C_{18} Porasil B (H_2O–MeOH) to give **2** (87 mg), **3** (1 g), **8** (55 mg), **10** (198 mg) and **11** (1 g) (from fr. 3) and **4** (458 mg) (from fr. 4).

General procedure for enzymatic hydrolysis. A solution of the sample (5–30 mg) in H_2O (2 ml) was treated with tannase at room temp. for 1 hr. The reaction mixture was filtered, and the filtrate concd to dryness *in vacuo*. The residue was subjected to Sephadex LH-20 CC using H_2O–MeOH (1:0–0:1) to furnish gallic acid and a hydrolysate.

1-O-*Galloyl* proto-*quercitol* (**1**). Colourless needles, mp 282–284°, $[\alpha]_D^{17}$ −10.6° (MeOH; *c* 0.4). ^{1}H NMR (Me_2CO-d_6 + D_2O): δ2.0–2.2 (2H, *m*, H-6), 3.6–3.9 (3H, *m*, H-3, -4 and -5), 4.0–4.1 (1H, *m*, H-2), 5.19 (1H, *dt*, *J* = 3, 4 Hz, H-1), 7.13 (2H, *s*, galloyl-H). ^{13}C NMR: see Table 1. (Found: C, 49.40; H, 5.24. $C_{13}O_9H_{16}$ requires: C, 49.37; H, 5.10%.)

1,4-*Di*-O-*galloyl* proto-*quercitol* (**2**). Colourless needles, mp 185–187°, $[\alpha]_D^{23}$ −10.1° (Me_2CO; *c* 0.8). ^{1}H NMR (Me_2CO-d_6): δ2.0–2.4 (2H, *m*, H-6), 3.8–4.2 (3H, *m*, H-2, -3 and -5), 5.26 (1H, *dt*, *J* = 3, 4 Hz, H-1), 5.36 (1H, *t*, *J* = 9 Hz, H-4), 7.15, 7.16 (each 2H, *s*, galloyl-H). ^{13}C NMR: see Table 1. (Found: C, 48.30; H, 4.71. $C_{20}H_{20}O_{13}\cdot 3/2\ H_2O$ requires: C, 48.49; H, 4.68%.)

1,4-*Di*-O-*galloylquinic* acid (**3**). Colourless needles, mp 213–215°, $[\alpha]_D^{23}$ −18.9° (Me_2CO; *c* 1.1). ^{1}H NMR (Me_2CO-d_6): δ2.0–2.9 (4H, *m*, H-2 and -6), 4.4–4.7 (2H, *m*, H-3 and -5), 4.96 (1H, *dd*, *J* = 9, 3 Hz, H-4), 7.16, 7.18 (each 2H, *s*, galloyl-H). ^{13}C NMR: see Table 1. (Found: C, 49.41; H, 4.51. $C_{21}H_{20}O_{14}\cdot H_2O$ requires: C, 49.03; H, 4.31%.)

1,3,4-*Tri*-O-*galloylquinic acid* (**4**). Colourless needles, mp 220–225° (dec.). $[\alpha]_D^{23}$ −5.2° (Me_2CO; *c* 0.9). ^{1}H NMR (Me_2CO-d_6): δ2.0–3.0 (4H, *m*, H-2 and -6), 4.60 (1H, m, $J_{W\,h/2}$ = 8 Hz, H-5), 5.32 (1H, *dd*, *J* = 9, 3 Hz, H-4), 5.92 (1H, *m*, $J_{W\,h/2}$ = 20 Hz, H-3), 7.08, 7.12, 7.27 (each 2H, *s*, galloyl-H). ^{13}C NMR: see Table 1. (Found: C, 48.38; H, 4.21. $C_{28}H_{24}O_{18}\cdot 5/2\ H_2O$ requires: C, 48.49; H, 4.22%.)

4-O-*Galloyl*-(−)-*shikimic acid* (**5**). An amorphous powder, $[\alpha]_D^{24}$ −138.9° (Me_2CO; *c* 0.4). ^{1}H NMR (Me_2CO-d_6): δ2.36 (1H, *dd*, *J* = 6, 18 Hz, H-2), 2.74 (1H, *dd*, *J* = 6, 18 Hz, H-2), 4.30 (1H, *m*, H-3), 4.72 (1H, *t*, *J* = 4 Hz, H-5), 5.16 (1H, *dd*, *J* = 4, 7 Hz, H-4), 6.88 (1H, *d*, *J* = 4 Hz, H-6), 7.10 (2H, *s*, galloyl-H). ^{13}C NMR: see Table 1. (Found: C, 48.61; H, 4.56. $C_{14}H_{14}O_9\cdot H_2O$ requires: C, 48.84; H, 4.68%.)

5-O-*Galloyl*-(−)-*shikimic acid* (**6**). An amorphous powder, $[\alpha]_D^{24}$ −142.0° (Me_2CO; *c* 0.5). ^{1}H NMR (Me_2CO-d_6): δ2.36 (1H, *dd*, *J* = 6, 18 Hz, H-2), 2.81 (1H, *dd*, *J* = 6, 18 Hz, H-2), 4.0–4.3 (2H, *m*, H-3 and -4), 5.80 (1H, *t*, *J* = 4 Hz, H-5), 6.80 (1H, *d*, *J* = 4 Hz, H-6), 7.16 (2H, *s*, galloyl-H). ^{13}C NMR: see Table 1. (Found: C, 48.59; H, 4.65. $C_{14}H_{14}O_9\cdot H_2O$ requires: C, 48.84; H, 4.68%.)

Acknowledgements—We thank Mr. T. Tanaka of Sankyo Co., Ltd. for the supply of tannase and Mr. Y. Tanaka and Miss. K. Soeda for ^{1}H and ^{13}C NMR measurements.

REFERENCES

1. Haddock, E. A., Gupta, R. K., Al-Shafi, S. M. K. and Haslam, E. (1982) *J. Chem. Soc. Perkin Trans.* 1 2515.
2. Mayer, W., Kunz, W. and Loebich, F. (1965) *Justus Liebig's Ann. Chem.* **688**, 232.
3. Nonaka, G., Ishimaru, K., Tanaka, T. and Nishioka, I. (1984) *Chem. Pharm. Bull.* **32**, 483.
4. Ozawa, T., Kobayashi, S., Seki, R. and Imagawa, H. (1984) *Agric. Biol. Chem.* **48**, 1411.
5. Nishimura, H., Nonaka, G. and Nishioka, I. (1984) *Chem. Pharm. Bull.* **32**, 1741.
6. Nishimura, H., Nonaka, G. and Nishioka, I. (1987) *Phytochemistry* (in press).
7. Nishimura, H., Nonaka, G. and Nishioka, I. (1984) *Phytochemistry* **23**, 2621.
8. Nonaka, G., Ageta, M. and Nishioka, I. (1985) *Chem. Pharm. Bull.* **33**, 96.

9. Nonaka, G., Nishimura, H. and Nishioka, I. (1982) *Chem. Pharm. Bull.* **30**, 2061.
10. Nishimura, H., Nonaka, G. and Nishioka, I. (1984) *Chem. Pharm. Bull.* **32**, 1735.
11. Haslam, E., Haworth, R. D. and Keen, P. C. (1962) *J. Chem. Soc. C* 3814.