Phytochemistry, Vol 23, No 11, pp 2621–2623, 1984
Printed in Great Britain

0031-9422/84 $3 00+0 00

SEVEN QUINIC ACID GALLATES FROM *QUERCUS STENOPHYLLA**

Hiroaki Nishimura, Gen-Ichiro Nonaka and Itsuo Nishioka

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

(*Received* 2 *April* 1984)

Key Word Index—*Quercus stenophylla*, Fagaceae, quinic acid gallates, gallotannins, tannase

Abstract—A chemical investigation of the bark of *Quercus stenophylla* has led to the isolation and characterization of all of the possible structural isomers of quinic acid gallates, 3-*O*-, 4-*O*-, 5-*O*-, 3,4-di-*O*-, 3,5-di-*O*-, 4,5-di-*O*- and 3,4,5-tri-*O*-galloylquinic acids Evidence for the structures of these compounds was obtained from analysis of the ^1H and ^{13}C NMR spectra, and hydrolytic studies

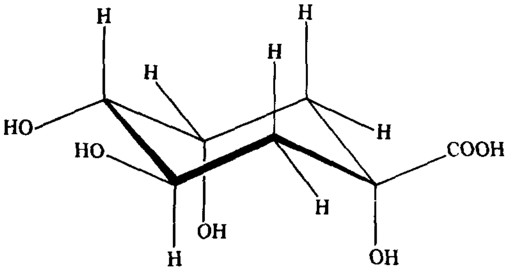
INTRODUCTION

Previous chemical examinations of polyphenolic constituents in the bark of *Quercus stenophylla* (Fagaceae) have revealed the occurrence of fifteen gallotannins based on a simple phenolic glucoside (salidroside) core [1] and a pentahydroxycyclohexane (proto-quercitol) core [2], and of four ellagitannins containing a salidroside [3] and a proto-quercitol moiety [4] Our continuing work on the polyphenols occurring in the same plant source has now resulted in the isolation and characterization of seven gallotannins based on a quinic acid core, and in this paper detailed evidence leading to the structures **1–7** is described

RESULTS AND DISCUSSION

The water-soluble part, after removal of the ether and ethyl acetate soluble portions of the 80 % aqueous acetone extract, was subjected to a combination of Sephadex LH-20 and MCI GEL CHP-20P chromatography to afford compounds **1–7**

Compound **1**, $C_{21}H_{20}O_{14}$ H_2O, gave a dark blue colour with ferric chloride characteristic of gallotannins It gave an $[M+H]^+$ ion peak at m/z 497 in the field-desorption mass spectrum (FDMS) The occurrence of two galloyl functions in the molecule was easily deduced from analysis of the ^1H NMR (see Experimental) and ^{13}C NMR (see Table 1) spectra The ^{13}C NMR spectrum showed six aliphatic signals due to two methylenes (δ35 7, 37 5, each *t*), three methine carbons bearing an oxygen function (δ70 1, 72 0, 72 1, each *d*) and a quaternary carbon atom (δ74 2, *s*), together with a carboxyl signal (δ175 8) On enzymatic hydrolysis with tannase, **1** gave gallic acid and a crystalline hydrolysate (**1a**), mp 255–259°, $[\alpha]_D - 48$ 2° (methanol) The IR spectrum of **1a** exhibited absorption bands of hydroxyl groups (3350 cm^{-1}, broad) and a carboxyl group (1680 cm^{-1}) These physical and spectral data agreed with those of quinic acid The locations of the galloyl groups on the quinic acid nucleus were determined from ^1H NMR analysis, two multiplet signals (δ5 54, 2H in total) due to methines bearing a galloyl group were observed in the lowfield, while a methine (δ4 14) free from a galloyl group appeared in the upfield Since the latter signal could be assigned to the C-4 axial proton on the basis of its coupling constant ($J = 9$, 3 Hz) (The neighboring C-3 and C-5 protons possess axial and equatorial orientations, respectively), the locations of the two galloyl groups were concluded to be at the C-3 and C-5 positions Accordingly, **1** was shown to be 3,5-di-*O*-galloylquinic acid



1a

Compound **2**, m/z 497 ($[M+H]^+$, FABMS) and compound **3**, $C_{21}H_{20}O_{14}$, m/z 497 ($[M+H]^+$, FDMS), both afforded gallic acid and quinic acid on enzymatic hydrolysis with tannase The ^1H and ^{13}C NMR spectra of these compounds were very similar to that of **1**, showing the presence of two galloyl groups In the ^1H NMR spectra, the appearance of two lowfield signals due to methines bearing a galloyl group was consistent, in both cases, with a digalloylquinic acid A double doublet appearing in the lowfield (δ5 17, $J = 9$, 3 Hz in **2** and δ5 24, $J = 9$, 3 Hz in **3**) could be assigned to the C-4 protons, indicating in both cases the presence of the galloyl group at this position Another lowfield signal appeared as a multiplet at δ5 72 in **2** and δ5 80 in **3** The multiplet in **2** was considered to be due to the C-5 proton from its small half-width value ($J_{w\,h/2} = 8$ Hz), while that of **3** was to the C-3 proton owing to a large half-width value ($J_{w\,h/2} = 20$ Hz) On the basis of these findings, **2** and **3** were concluded to be 4,5-di-*O*-galloylquinic acid [5] and 3,4-di-*O*-galloylquinic acid, respectively

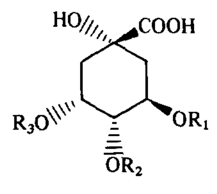
---

*Part 24 in the series "Tannins and Related Compounds" For Part 23 see Kashiwada, Y, Nonaka, G and Nishioka, I, *Chem Pharm Bull* (*Tokyo*) (in press)

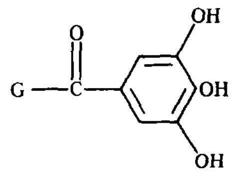
Table 1 ^{13}C NMR spectral data of compounds 1–7 at 25 05 MHz in Me_2CO-d_6 + D_2O

Carbon no	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Quinic acid							
1	74 2	74 6	75 5	76 9	76 5	75 2	74 1
2	37 5	41 6	39 0	39 4	40 0	40 2	38 1
3	72 0	65 5	68 5	73 7*	66 5†	68 1‡	68 8
4	70 1	75 9	75 2	72 6*	77 9†	73 8‡	72 1
5	72 1	69 7	68 7	71 8*	67 8†	72 8‡	69 7
6	35 7	36 7	38 0	38 2	39 6	36 4	36 2
COOH	175 8	176 5	175 5	179 3	181 1	169 5	176 2
Galloyl							
1	121 4 121 9	121 3 121 7	120 6 (2C)	121 5	121 5	121 7	120 4 (2C) 121 1
2	109 8 (2C)	109 9 (2C)	109 7 109 8	110 1	110 2	110 2	109 8 (3C)
3	145 5 (2C)	145 5 (2C)	145 5 (2C)	145 8	145 9	145 6	145 5 (3C)
4	138 3 138 6	138 3 138 5	138 7 (2C)	139 1	139 1	138 9	138 7 139 0 (2C)
–COO–	166 1 (2C)	165 8 166 1	166 3 166 6	167 7	167 4	167 5	166 2 (3C)

*†‡Signals in columns may be interchanged



	R_1	R_2	R_3
1	G	H	G
2	H	G	G
3	G	G	H
4	G	H	H
5	H	G	H
6	H	H	G
7	G	G	G



Compounds **4**–**6** exhibited, in the FABMS, the same $[M+Na]^+$ ion peak at *m/z* 367 corresponding to a monogalloylquinic acid structure Tannase hydrolysis of these compounds confirmed their constitution The ^{1}H NMR spectrum showed, in each case, a lowfield methine signal (δ5 43, *m*, $J_{w\,h/2}$ = 20 Hz in **4**, δ5 04, *dd*, *J* = 9, 3 Hz in **5** and δ5 46, *m*, $J_{w\,h/2}$ = 8 Hz in **6**) These signals were respectively assigned to the C-3, C-4 and C-5 protons on the basis of their coupling constants and half-width values, thus characterizing them as the 3-*O*-gallate [5, 6], 4-*O*-gallate [5] and 5-*O*-gallate [5] of quinic acid

Compound **7**, *m/z* 649 ($[M+H]^+$, FABMS), was shown to be a trigalloylquinic acid by ^{1}H and ^{13}C NMR and MS examinations and a similar hydrolytic study Since the ^{1}H NMR spectrum exhibited three methine signals shifted fairly downfield [δ5 51, *dd*, *J* = 9, 3 Hz and δ5 81 (2H), *m*], the galloyl groups were concluded to be at the C-3, C-4 and C-5 positions, thus confirming its structure as 3,4,5-tri-*O*-galloylquinic acid [5, 7, 8]

Gallic acid esters with quinic acid are known as the constituents of Tara gallotannin [7] which is obtained from the fruit pods of the South American shrub, *Caesalpinia spinosa*, but their structures were not fully characterized because of the difficulties in isolating individual components Our systematic isolation and unequivocal structural characterization of all of the possible structural isomers of quinic acid gallates is thus noteworthy

EXPERIMENTAL

Mps are uncorr ^{1}H and ^{13}C NMR spectra were measured at 100 and 25 05 MHz, respectively, with TMS as int standard TLC was conducted on silica gel and Avicel SF cellulose, and spots were visualized by $FeCl_3$ reagent and by spraying 5% H_2SO_4 followed by heating

Extraction and isolation The air-dried bark (4 74 kg) of *Quercus stenophylla*, collected in Takushima Prefecture, Japan (authentic sample lodged in Herbarium, Kyushu University), was extracted at room temp with 80% aq Me_2CO The aq soln, after removal of the Me_2CO by concn under red pres, afforded a ppt which was removed by filtration The filtrate was shaken with Et_2O and then with EtOAc The aq layer was mixed with Celite-545 (1 2 kg) and air-dried A brown powder thus obtained was packed in a glass column and eluted with Me_2CO The eluate was evapd under red pres and subjected to Sephadex LH-20 CC with an increasing amount of MeOH in H_2O (0 1–1 0) to give seven fractions, 1 (296 g), 2 (122 g), 3 (198 g), 4 (41 g), 5 (138 g), 6 (24 g) and 7 (44 g) Subsequent separation of fr 1 on Sephadex LH-20

CC with H_2O afforded a further three fractions, 1A (130 g), 1B (113 g) and 1C (15 g) A part (55 g) of fr 1B was rechromatographed over MCI GEL CHP-20P with H_2O to furnish compounds **4** (9 1 mg), **5** (218 mg) and **6** (350 mg) Fr 2 was separated by MCI GEL CHP-20P CC with a mixture of H_2O–MeOH (19 1–7 3) into four fractions, 2-1 (3 g), 2-2 (26 g), 2-3 (30 g) and 2-4 (18 g) Fr 2-2 was rechromatographed over Sephadex LH-20 with 50% aq MeOH and MCI GEL CHP-20P with an increasing amount of MeOH in H_2O (0 1–1 3) to yield compounds **2** (226 mg) and **3** (185 mg), Frs 3 and 4 were chromatographed over MCI GEL CHP-20P with a mixture of H_2O–MeOH (1 0–7 3) and Sephadex LH-20 with 60% aq MeOH to yield compounds **1** (99 mg) and **7** (588 mg)

3,5-*Di-O-galloylquinic acid* (**1**) An off-white amorphous powder, $[\alpha]_{\mathrm{D}}^{26}$ −63 0° (Me_2CO, *c* 0 20) FDMS *m/z* 497 $[M+H]^+$, 519 $[M+Na]^+$, 535 $[M+K]^+$ ^1H NMR (Me_2CO-d_6) δ2 0–2 6 (4H, *m*, H-2 and H-6), 4 14 (1H, *dd*, *J* = 9, 3 Hz, H-4), 5 54 (2H, *m*, H-3 and H-5), 7 14, 7 19 (each 2H, *s*, galloyl H) (Found C, 49 03, H, 4 31 $C_{21}H_{20}O_{14}$ H_2O requires C, 48 98, H, 4 52%)

Enzymatic hydrolysis of **1** A soln of **1** (134 mg) in H_2O (5 ml) was incubated with tannase at 37° for 6 hr The reaction mixture was concd to dryness under red pres and the residue treated with MeOH The MeOH-soluble portion was chromatographed over silica gel ($CHCl_3$–MeOH–H_2O, 6 4 1) to furnish gallic acid (97 mg) and a hydrolysate (**1a**) Pale yellow granules, mp 255–259°, $[\alpha]_{\mathrm{D}}^{20}$ −48 2° (H_2O, *c* 1 63) IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1} 3350 (OH), 1680 (COOH), no aromatic ring absorption

4,5-*Di-O-galloylquinic acid* (**2**) [5] An off-white amorphous powder, $[\alpha]_{\mathrm{D}}^{23}$ −35 5° (Me_2CO, *c* 0 20) FABMS *m/z* 497 $[M+H]^+$ ^1H NMR (Me_2CO-d_6) δ2 0–2 6 (4H, *m*, H-2 and H-6), 4 52 (1H, *m*, $J_{\mathrm{w\,h/2}}$ = 20 Hz, H-3), 5 17 (1H, *dd*, *J* = 9, 3 Hz, H-4), 5 72 (1H, *m*, $J_{\mathrm{w\,h/2}}$ = 8 Hz, H-5), 7 08, 7 13 (each 2H, *s*, galloyl H)

3,4-*Di-O-galloylquinic acid* (**3**) An off-white amorphous powder, $[\alpha]_{\mathrm{D}}^{27}$ −102 8° (Me_2CO, *c* 0 32) FDMS *m/z* 497 $[M+H]^+$ ^1H NMR (Me_2CO-d_6) δ2 1–2 5 (4H, *m*, H-2 and H-6), 4 50 (1H, *m*, $J_{\mathrm{w\,h/2}}$ = 8 Hz, H-5), 5 24 (1H, *dd*, *J* = 9, 3 Hz, H-4), 5 80 (1H, *m*, $J_{\mathrm{w\,h/2}}$ = 20 Hz, H-3), 7 05, 7 11 (each 2H, *s*, galloyl H) (Found C, 50 81, H, 4 06 $C_{21}H_{20}O_{14}$ requires C, 50 76, H, 4 58%)

3-O-*Galloylquinic acid* (**4**) [5,6] An off-white amorphous powder, $[\alpha]_{\mathrm{D}}^{18}$ −36 4° (H_2O, *c* 0 37) FABMS *m/z* 345 $[M+H]^+$, 367 $[M+Na]^+$ ^1H NMR (Me_2CO-d_6 + D_2O) δ1 8–2 4 (4H, *m*, H-2 and H-6), 3 85 (1H, *dd*, *J* = 9, 3 Hz, H-4), 4 0–4 3 (H-5), 5 43 (1H, *m*, $J_{\mathrm{w\,h/2}}$ = 20 Hz, H-3), 7 15 (2H, *s*, galloyl H)

4-O-*Galloylquinic acid* (**5**) [5] An off-white amorphous powder, $[\alpha]_{\mathrm{D}}^{17}$ −30 4° [H_2O–MeOH (1 1), *c* 0 24] FABMS *m/z* 345 $[M+H]^+$, 367 $[M+Na]^+$ ^1H NMR (Me_2CO-d_6 + D_2O) δ1 9–2 3 (4H, *m*, H-2 and H-6), 3 9–4 3 (H-3), 4 36 (1H, *m*, $J_{\mathrm{w\,h/2}}$ = 8 Hz, H-5), 5 04 (1H, *dd*, *J* = 9, 3 Hz, H-4), 7 20 (2H, *s*, galloyl H)

5-O-*Galloylquinic acid* (**6**) [5] An off-white amorphous powder, $[\alpha]_{\mathrm{D}}^{17}$ −13 7° (H_2O, *c* 0 36) FABMS *m/z* 345 $[M+H]^+$, 367 $[M+Na]^+$ ^1H NMR (Me_2CO-d_6 + D_2O) δ1 8–2 4 (4H, *m*, H-2 and H-6), 3 81 (1H, *dd*, *J* = 9, 3 Hz, H-4), 4 1–4 5 (H-3), 5 46 (1H, *m*, $J_{\mathrm{w\,h/2}}$ = 8 Hz, H-5), 7 17 (2H, *s*, galloyl H)

3,4,5-*Tri-O-galloylquinic acid* (**7**) [5, 7, 8] An off-white amorphous powder, $[\alpha]_{\mathrm{D}}^{21}$ −116 5° (Me_2CO, *c* 0 32) FABMS *m/z* 649 $[M+H]^+$ ^1H NMR (Me_2CO-d_6) δ2 2–2 8 (4H, *m*, H-2 and H-6), 5 51 (1H, *dd*, *J* = 9, 3 Hz, H-4), 5 81 (2H, *m*, H-3 and H-5), 7 05, 7 08, 7 16 (each 2H, *s*, galloyl H)

Enzymatic hydrolysis of **2–7** A soln of each sample in H_2O (3 mg/0 5 ml) was incubated with tannase at 37° for 1 hr The solvent was evapd under red pres and the residue subjected to TLC examination R_f 0 93 (gallic acid), 0 21 (quinic acid) [silica gel, C_6H_6–HCO_2Et–HCO_2H (1 5 2)], R_f 0 91 (gallic acid), 0 52 (quinic acid) [silica gel, EtOH–H_2O (5 1)]

Methylation of **7** Compound **7** (50 mg) was methylated for 2 5 hr with Me_2SO_4 (2 ml) and K_2CO_3 (2 g) in dry Me_2CO (10 ml) After filtration of the inorganic ppt, the soln was concd to a syrup which was purified by CC over silica gel Elution with C_6H_6–Me_2CO (1 0–17 3) furnished the decamethylate (**7a**), a white amorphous powder, $[\alpha]_{\mathrm{D}}^{16}$ −97 1° ($CHCl_3$, *c* 0 26) EIMS *m/z* 788 $[M]^+$ ^1H NMR ($CDCl_3$) δ2 3–2 7 (4H, *m*, H-2 and H-6), 3 6–3 9 (OMe × 10), 5 56 (1H, *dd*, *J* = 9, 3 Hz, H-4), 5 94 (1H, *m*, $J_{\mathrm{w\,h/2}}$ = 8 Hz, H-5), 6 06 (1H, *m*, $J_{\mathrm{w\,h/2}}$ = 20 Hz, H-3), 7 06, 7 17, 7 40 (each 2H, *s*, galloyl H)

Acknowledgements—The authors thank Prof T Nohara (Kumamoto University) and Dr K Murakami (Tokushima University) for providing the plant material, and Dr H Okazaki (Sankyo Co, Ltd) for tannase The help given by Mr Y Tanaka, Miss K Soeda and Mr K Isobe (Kyushu University) in obtaining the spectral data is gratefully acknowledged

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 Haslam, E, Haworth, R D and Lawton, D A (1963) *J Chem Soc* 2173

6 Roberts, E A H and Mayers, M (1958) *J Sci Food Agric* **11**, 701

7 Haslam, E, Haworth, R D and Keen, P C (1962) *J Chem Soc* 3815

8 Haslam, E (1967) *J Chem Soc* (*C*) 1734