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Tannins and Related Compounds. XVI.[1] Isolation and Characterization of Six Methyl Glucoside Gallates and a Gallic Acid Glucoside Gallate from *Sanguisorba officinalis* L.

TAKASHI TANAKA, GEN-ICHIRO NONAKA and ITSUO NISHIOKA*

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

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Six methyl glucoside gallates (I—VI) were isolated, together with gallic acid 3-*O*-β-D-(6′-*O*-galloyl)-glucopyranoside (VII), from the underground part of *Sanguisorba officinalis* L. On the basis of chemical and spectroscopic evidence, the structures of these compounds were established to be 6-*O*-gallate (**1**), 6-*O*-digallate (**2**), 4,6-di-*O*-gallate (**3**), 2,3,6-tri-*O*-gallate (**4**), 3,4,6-tri-*O*-gallate (**5**) and 2,3,4,6-tetra-*O*-gallate (**6**) of methyl β-D-glucopyranoside.

Keywords——*Sanguisorba officinalis*; Rosaceae; tannin; methyl β-D-glucoside gallate; gallic acid glucoside gallate

In the course of studies on tannins and related compounds in *Sanguisorba officinalis* L. (Rosaceae), we have so far reported the isolation and structure elucidation of five ellagitannins (eugeniin and sanguiins H-1, H-2, H-3[2] and H-6[3]), four galloylglucoses (1,2,6-tri-, 1,2,3,6-tetra-, 2,3,4,6-tetra- and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucoses),[4] and several flavan-3-ol derivatives [(+)-catechin, (+)-gallocatechin, 7-*O*-galloyl-(+)-catechin, procyanidin B-3, 3′-*O*-galloylprocyanidin B-3, procyanidin C-2, and gambiriins A-1 and B-3].[4] Further chemical examination of phenolic constituents in this plant has resulted in the isolation of six methyl glucoside gallates (I—VI) and a gallic acid glucoside gallate (VII). This paper describes the isolation and structure elucidation of these compounds.

The aqueous acetone extract of the underground parts of *Sanguisorba officinalis* L. was partitioned between EtOAc and H_2O. From the H_2O-soluble portion, compounds I—III and VII were isolated by a combination of Sephadex LH-20 and Diaion HP-20 chromatography. The EtOAc-soluble portion was repeatedly chromatographed over Sephadex LH-20, cellulose and Diaion HP-20 to afford compounds IV—VI.

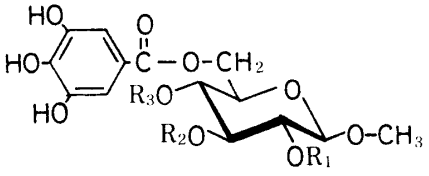
Compound I (**1**), a pale yellow amorphous powder, $C_{14}H_{18}O_{10}$, $[\alpha]_D$ −18.6° (H_2O), was strongly positive (a dark blue color) to the ferric chloride reagent. The occurrence of a galloyl function in I was easily deduced from the proton nuclear magnetic resonance (^{1}H-NMR) [δ 7.14, (2H), s] and carbon-13 nuclear magnetic resonance (^{13}C-NMR) [δ 110.0 (2C), 121.7, 138.8, 146.0 (2C) and 166.9] spectra. The ^{13}C-NMR spectrum shows aliphatic carbon signals including an anomeric carbon signal [δ 104.8 (C_1), 77.6 (C_3), 74.8, 74.6, 71.3 (C_4), and 64.4 (C_6)], together with a methoxyl signal (δ 56.8), suggesting the presence of a methyl hexoside moiety. This was further supported by the appearance of the signals at δ 3.44 (3H, s, OCH_3) and δ 4.24 (1H, d, J=8 Hz, C_1–H) in the ^{1}H-NMR spectrum. Treatment of I with tannase in aqueous solution yielded gallic acid and a colorless hydrolysate, mp 111—112 °C, $[\alpha]_D$ −32.8° (H_2O), which was identified as methyl β-D-glucopyranoside by comparison of the physical and spectral data with those of an authentic sample. The location of the galloyl group in I was determined to be at the C_6 position of the methyl glucoside moiety on the basis of ^{1}H- and ^{13}C-NMR spectra, which show downfield shifts of the C_6–H signals (δ 4.57, dd, J=2, 13 Hz; 4.34, dd, J=6, 13 Hz) and the C_6 signal (δ 64.4) as compared with those of methyl β-D-

glucoside. Consequently, the structure of I was concluded to be methyl 6-*O*-galloyl-*β*-D-glucopyranoside (**1**).

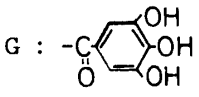
Compound II (**2**), an off-white amorphous powder, $C_{21}H_{22}O_{14}\cdot 1/2H_2O$, $[\alpha]_D -16.0°$ (acetone), gave a ^{1}H-NMR spectrum similar to that of I except for additional *meta*-coupled signals at δ 7.38 and 7.49 ($J=2$ Hz). Methanolysis of II with aqueous methanol (acetate buffer; pH 5.5) gave methyl gallate and I. This result suggests that the additional galloyl function is linked depsidically to I. The ^{13}C-NMR spectrum of II provided further support for the presence of a digalloyl group at the C_6-position of the glucose moiety; it shows sugar carbon signals analogous to those observed in I, but the C_6 signal appeared at 0.3 ppm downfield as compared with that of I. This downfield shift is analogous with those previously reported in structural studies on depsidically linked galloylglucoses in Turkish[5] and Chinese[6] galls. On the basis of these results, compound II was characterized as methyl 6-*O*-digalloyl-*β*-D-glucopyranoside. Compound II was shown to be a mixture of products having the depside galloyl group at the *m*- and *p*-positions by analysis of the ^{13}C-NMR spectrum, which exhibits duplicated galloyl carbon signals similar to those found in a mixture of methyl *m*- and *p*-digallates.[6]

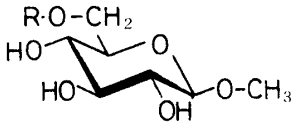
Compound III (**3**), a tan amorphous powder, $C_{21}H_{22}O_{14}$, $[\alpha]_D +22.0°$ (acetone), gave a dark blue color with ferric chloride reagent. In the ^{1}H-NMR spectrum, the appearance of a four-proton singlet signal at δ 7.14 indicated the occurrence of two galloyl groups in the molecule. On enzymatic hydrolysis with tannase, III gave gallic acid and methyl *β*-D-glucopyranoside. The location of the galloyl groups were determined to be at the C_4- and C_6-hydroxyls in the methyl glucoside moiety because signals due to C_4–H (δ 5.12, t, $J=9$ Hz) and C_6–H (δ 4.44, dd, $J=2$, 12 Hz; 4.16, dd, $J=6$, 12 Hz) were observed at lower field in the ^{1}H-NMR spectrum. Accordingly, compound III was characterized as methyl 4,6-di-*O*-galloyl-*β*-D-glucopyranoside (**3**).

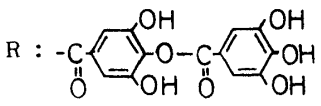
Compound IV, (**4**), a tan amorphous powder, $C_{28}H_{26}O_{18}$, $[\alpha]_D +84.3°$ (acetone), shows in the ^{1}H-NMR spectrum the presence of three galloyl groups (δ 7.02, 7.05 and 7.17, each 2H, s) and a methyl glucoside moiety [δ 3.44 (3H, s, OCH_3), δ 4.75 (1H, d, $J=8$ Hz, C_1–H)]. Treatment of IV with tannase afforded gallic acid and methyl *β*-D-glucopyranoside. In the ^{1}H-NMR spectrum of IV, signals assignable to C_2–, C_3– and C_6–H in the glucose moiety were shifted downfield (δ 5.49, t, $J=8$ Hz; 5.17, t, $J=8$ Hz and 4.57, br s, respectively), indicating



	R_1	R_2	R_3
1 :	H	H	H
3 :	H	H	G
4 :	G	G	H
5 :	H	G	G
6 :	G	G	G







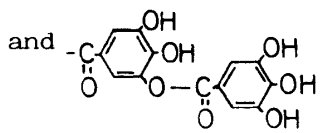


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Chart 1

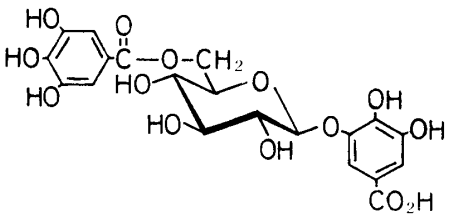

Chart 2

TABLE I. ^{1}H-NMR Chemical Shifts for Compounds I—VI
(δ Values, J Values in Hz)[a]

	I	II	III[b]	IV[b]	V[b]	VI
C_1–H	4.24 (d, J=8)	4.24 (d, J=8)	4.37 (d, J=8)	4.75 (d, J=8)	4.58 (d, J=8)	4.95 (d, J=8)
C_2–H	3.14—3.80 (m)	3.12—3.78 (m)	3.36 (t, J=9)	5.17 (t, J=8)	3.69 (t, J=8)	5.55 (t, J=8)
C_3–H	3.14—3.80 (m)	3.12—3.78 (m)	3.81 (t, J=9)	5.49 (t, J=8)	5.57 (t, J=9)	5.85 (t, J=8)
C_4–H	3.14—3.80 (m)	3.12—3.78 (m)	5.12 (t, J=9)	3.84—4.00 (m)	5.37 (t, J=9)	5.35 (t, J=8)
C_5–H	3.14—3.80 (m)	3.12—3.78 (m)	3.90 (m)	3.84—4.00 (m)	4.04—4.50 (m)	4.20—4.65 (m)
C_6–H	4.34 (dd, J=6, 13) 4.57 (dd, J=2, 13)	4.40 (dd, J=5, 12) 4.61 (dd, J=2, 12)	4.16 (dd, J=6, 12) 4.44 (dd, J=2, 12)	4.57 (br s)	4.04—4.50 (m)	4.20—4.65 (m)
OCH_3	3.44	3.40	3.46	3.44	3.52	3.50
Galloyl-H	7.14 (2H, s)	7.27 (s), 7.38, 7.49 (each d, J=2)	7.14 (4H, s)	7.02, 7.05, 7.17 (each 2H, s)	7.05, 7.07, 7.20 (each 2H, s)	6.96, 7.07, 7.09, 7.23 (each 2H, s)

a) Measured in acetone-d_6 at 100 MHz with tetramethylsilane as an internal standard.
b) Assignments of the glucose signals were made using spin-decoupling techniques.

that the galloyl groups are located at these positions. Thus, the structure of IV was confirmed to be methyl 2,3,6-tri-*O*-galloyl-β-D-glucopyranoside (**4**).

Compound V (**5**), a tan amorphous powder, $C_{28}H_{26}O_{18}$, $[\alpha]_D$ $-52.7°$ (acetone), gave gallic acid and methyl β-D-glucopyranoside on enzymatic hydrolysis with tannase. The ^{1}H-NMR spectrum of V revealed the occurrence of three galloyl groups in the molecule [δ 7.05, 7.07 and 7.20 (each 2H, s)]. The downfield shifts of the signals due to C_3–, C_4– and C_6–H (Table I), which were assigned by using the spin-decoupling technique, indicated that galloyl groups were attached to these positions. Thus, the structure of V was established as methyl 3,4,6-tri-*O*-galloyl-β-D-glucopyranoside (**5**).

Compound VI (**6**), a tan amorphous powder, $C_{35}H_{30}O_{22}\cdot 5/2H_2O$, $[\alpha]_D$ $+33.8°$ (acetone), contains four galloyl groups in the molecule as indicated by the ^{1}H-NMR spectrum [δ 7.23, 7.09, 7.07 and 6.96 (each 2H, s)]. The presence of a methyl β-D-glucopyranoside core was confirmed by hydrolysis of VI with tannase. Since the ^{1}H-NMR spectrum shows downfield shifts of C_2–, C_3–, C_4– and C_6–H (Table I), VI should be methyl 2,3,4,6-tetra-*O*-galloyl-β-D-glucopyranoside (**6**).

Compound VII (**7**), colorless needles, mp 261—263° (dec.), $[\alpha]_D$ $-19.4°$ (acetone), $C_{20}H_{20}O_{14}\cdot 3/2H_2O$, was shown by ^{1}H and ^{13}C-NMR analyses to be identical with gallic acid 3-*O*-β-D-(6′-*O*-galloyl)-glucopyranoside.[7] This compound was recently isolated from rhubarb

in our laboratory.

These methyl β-D-glucopyranoside gallates are not artifacts formed in the processes of extraction and separation, because these compounds were isolated without the use of MeOH. Methyl glucopyranoside has rarely been found in the plant kingdom. It is therefore of interest from the viewpoint of plant physiology that methyl glucoside occurs as its gallates in this plant.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were taken with a JASCO DIP-4 digital polarimeter (cell length: 0.5 dm). ^{1}H- and ^{13}C-NMR spectra were measured with JEOL PS-100 and JEOL FX-100 spectrometers at 100 and 25.5 MHz, respectively, with tetramethylsilane as an internal standard, and chemical shifts are given in δ (ppm). Thin-layer chromatography (TLC) was conducted on precoated Kieselgel 60 F_{254} plates (Merck, 0.2 mm thick) with benzene–ethyl formate–formic acid (1 : 7 : 1) and precoated cellulose F_{254} plates (Merck, 0.1 mm thick) with 2% AcOH, and spots were detected by spraying 2% ethanolic ferric chloride or by spraying 5% H_2SO_4 followed by heating. Column chromatography was carried out with Sephadex LH-20 (Pharmacia Fine Chemicals, 25—100 μ) and Diaion HP-20 AG (Mitsubishi Chemical Industries Ltd., 75—150 μ) and Avicel microcrystalline cellulose (Funakoshi). All solvent ratios are given in v/v.

Extraction and Isolation——The fresh underground part of *Sanguisorba officinalis* L. (16 kg) was extracted with 80% aqueous acetone at room temperature. The aqueous solution, after removal of the acetone under reduced pressure, afforded precipitates which were removed by filtration. The filtrate was extracted with EtOAc. The aqueous layer was mixed with Celite-545 (1 kg) and air-dried. A brown powder thus obtained was packed in a glass column and eluted with acetone. The eluate was evaporated to dryness and the residue (863 g) was chromatographed over Sephadex LH-20 in an EtOH–H_2O–acetone solvent system (10 : 0 : 0–9 : 1 : 0–8 : 2 : 0–7 : 3 : 0–6 : 4 : 0–54 : 36 : 10–48 : 32 : 20–0 : 1 : 1) to give six fractions: fr. I (300 g), fr. II (125 g), fr. III (21 g), fr. IV (90 g), fr. V (80 g) and fr. VI (84 g). Fr. I was rechromatographed over Sephadex LH-20 using H_2O to afford compound I (1.4 g). Repeated chromatography of fr. II over Sephadex LH-20 using EtOH and 60% aqueous MeOH and over Diaion HP-20 using 20% EtOH gave compounds II (120 mg), III (13 mg) and VII (67 mg). A part (100 g) of the above EtOAc-soluble portion (500 g) was chromatographed over Sephadex LH-20 using EtOH to give six fractions; fr. I (36 g), fr. II (91.5 g), fr. III (2.2 g), fr. IV (3.2 g), fr. V (2.8 g), and fr. VI (2.8 g). Fr. II was further separated by Sephadex LH-20 chromatography using EtOH into two fractions; fr. II-1 (1.6 g) and fr. II-2 (3.7 g). Fr. II-1 was chromatographed over cellulose using 2% AcOH, followed by rechromatography on Diaion HP-20 in 30% aqueous EtOH to furnish compounds IV (16 mg) and V (108 mg). Rechromatography of fr. II-2 over cellulose using 2% AcOH and over Diaion HP-20 using 30% aqueous EtOH afforded compound VI (280 mg).

Compound I (1)——A pale yellow amorphous powder, $[\alpha]_D^{22}$ −18.6° (c=1.3, H_2O). *Anal.* Calcd for $C_{14}H_{18}O_{10}$: C, 48.56; H, 5.24. Found: C, 49.09; H, 5.48. ^{1}H-NMR: Table I. ^{13}C-NMR (acetone-d_6): 56.8 (OCH_3), 64.4 (C_6), 71.3 (C_4), 74.6, 74.8 ($C_{2,5}$), 77.6 (C_3), 104.8 (C_1), 110.0 (galloyl $C_{2,6}$), 121.7 (galloyl C_1), 138.8 (galloyl C_4), 146.0 (galloyl $C_{3,5}$), 166.9 (–COO–).

Hydrolysis of I with Tannase——A solution of I (600 mg) in H_2O (5 ml) was incubated with tannase at 37 °C for 1.5 h. The reaction mixture was evaporated to dryness, and the residue was treated with EtOH. The EtOH-soluble portion was subjected to Sephadex LH-20 chromatography using EtOH to furnish gallic acid (264 mg), mp 265—269 °C and a hydrolysate (95 mg), colorless plates (MeOH), mp 111—112 °C, $[\alpha]_D^{22}$ −32.8° (c=1.2, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3340, 2850, 1448, 1401, 1220, 1095, 1079, 1030, 992, 884. This compound was shown to be identical with methyl β-D-glucopyranoside by comparison of the physical and spectral data with those of an authentic sample.

Compound II (2)——An off-white amorphous powder, $[\alpha]_D^{25}$ −16.0° (c=0.72, acetone). *Anal.* Calcd for $C_{21}H_{22}O_{14}\cdot 1/2H_2O$: C, 49.71; H, 4.57. Found: C, 49.47; H, 4.82. ^{1}H-NMR: Table I. ^{13}C-NMR (acetone-d_6): 56.8 (OCH_3), 64.7 (C_6), 71.3 (C_4), 74.6 (2C) ($C_{2,5}$), 77.6 (C_3), 104.8 (C_1), 109.8, 110.6, 121.6, 128.7, 132.4, 139.4, 146.1, 151.3, 164.2, 166.5 (*p*-digalloyl group), 110.6, 114.7, 117.4, 120.5, 121.6, 139.6, 139.8, 143.6, 146.1, 146.9, 165.0, 166.3 (*m*-digalloyl group).

Methanolysis of II——A solution of II (2 mg) in 0.05 M acetate buffer (1 ml) and MeOH (9 ml) was kept at 40 °C for 24 h. The reaction mixture was examined by silica gel TLC (benzene–ethyl formate–formic acid (1 : 7 : 1)). The chromatogram showed three spots whose *Rf* values were the same as those of methyl gallate (*Rf* 0.87), compound I (*Rf* 0.26) and compound II (*Rf* 0.24).

Compound III (3)——A tan amorphous powder, $[\alpha]_D^{24}$ +22.0° (c=0.4, acetone). *Anal.* Calcd for $C_{21}H_{22}O_{14}$: C, 50.06; H, 4.45. Found: C, 50.46; H, 4.91. ^{1}H-NMR: Table I.

Hydrolysis of III with Tannase——A solution of III (2 mg) in H_2O was incubated with tannase at 37 °C for 1 h. The reaction mixture was examined by silica gel TLC (benzene–ethyl formate–formic acid (2 : 7 : 1)), and showed two

spots. The upper spot (*Rf* 0.80, dark blue color with $FeCl_3$) coincided with that of gallic acid and the lower spot (*Rf* 0.15, brown color with 2% H_2SO_4) corresponded to that of methyl β-D-glucopyranoside.

Compound IV (4)——A tan amorphous powder, $[\alpha]_D^{23}$ +84.3° (*c*=1.0, acetone). *Anal.* Calcd for $C_{28}H_{26}O_{18}$: C, 51.70; H, 4.03. Found: C, 51.73; H, 4.31. ^{1}H-NMR: Table I. ^{13}C-NMR (acetone-d_6): 56.8 (OCH_3), 64.0 (C_6), 69.6 (C_4), 72.5 (C_2), 74.8 (C_5), 76.1 (C_3), 102.2 (C_1), 109.7 (galloyl $C_{2,6}$), 120.4, 120.5, 120.9 (galloyl C_1), 138.8 (galloyl C_4), 145.5, 145.7 (galloyl $C_{3,5}$), 165.8, 166.6, 166.9 (–COO–). Hydrolysis of IV with tannase in a manner similar to that described for III gave gallic acid and methyl β-D-glucopyranoside.

Compound V (5)——A tan amorphous powder, $[\alpha]_D^{32}$ −52.7° (*c*=0.6, acetone). *Anal.* Calcd for $C_{28}H_{26}O_{18}$: C, 51.70; H, 4.03. Found: C, 51.71; H, 4.44. ^{1}H-NMR: Table I. Hydrolysis of V with tannase as for III furnished gallic acid and methyl β-D-glucopyranoside.

Compound VI (6)——A tan amorphous powder, $[\alpha]_D^{29}$ +33.8° (*c*=0.9, acetone). *Anal.* Calcd for $C_{35}H_{30}O_{22}\cdot 5/2H_2O$: C, 49.59; H, 4.16. Found: C, 49.45; H, 4.39. ^{1}H-NMR: Table I. Tannase hydrolysis of VI in the same way as described for III gave gallic acid and methyl β-D-glucopyranoside.

Compound VII (7)——Colorless needles, mp 261—263 °C (dec.) with blackening at about 240 °C. $[\alpha]_D^{26}$ −19.4° (*c*=0.48, acetone). *Anal.* Calcd for $C_{20}H_{20}O_{14}\cdot 3/2H_2O$: C, 46.97; H, 4.53. Found: C, 47.00; H, 4.60. ^{1}H-NMR (acetone-d_6 + D_2O): 4.25 (1H, dd, *J*=6, 12 Hz, $C_{6'}$–H), 4.78 (1H, d, *J*=12 Hz, $C_{6'}$–H), 4.99 (1H, d, *J*=8 Hz, $C_{1'}$–H), 7.07 (2H, s, galloyl H), 7.34, 7.57 (each 1H, d, *J*=2 Hz, $C_{2,6}$–H). ^{13}C-NMR (acetone-d_6): 64.9 ($C_{6'}$), 71.1 ($C_{4'}$), 74.4 ($C_{2'}$), 75.6 ($C_{5'}$), 76.9 ($C_{3'}$), 104.0 ($C_{1'}$), 110.0 (galloyl $C_{2,6}$), 111.6 (C_6), 113.6 (C_2), 121.3, 121.5 (C_1, galloyl C_1), 138.8 (galloyl C_4), 141.2 (C_4), 145.9 (galloyl $C_{3,5}$), 146.3, 146.5 ($C_{3,5}$), 166.9 (–COO–), 169.1 (–COOH). Hydrolysis of VII with 2 N H_2SO_4 for 3 h furnished gallic acid and glucose, which were identified by thin-layer co-chromatography with authentic samples.

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