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PHENYL GLUCOSIDES FROM A HAIRY ROOT CULTURE OF *SWERTIA JAPONICA*

KANJI ISHIMARU, HIROSHI SUDO,* MOTOYOSHI SATAKE and KOICHIRO SHIMOMURA†

Tsukuba Medicinal Plant Research Station, National Institute of Hygienic Sciences, 1 Hachimandai, Tsukuba, Ibaraki, 305, Japan; *Kanebo Ltd, Biochemistry Lab., 3-28, 5-Chome, Kotobuki-cho, Odawara, Kanagawa, Japan

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Key Word Index—*Swertia japonica*; Gentianaceae; *Agrobacterium rhizogenes*; hairy root; phenyl glucosides; gentisic acid; 2,6-dimethoxy-4-hydroxyphenol; sinapinic acid.

Abstract—Two new phenyl glucosides, 5-(3′-glucosyl)benzoyloxygentisic acid and 2,6-dimethoxy-4-hydroxyphenol 1-glucoside were isolated together with 1-sinapoyl glucoside from the hairy roots of *Swertia japonica*. The structures were elucidated on the basis of chemical and spectroscopic evidence.

INTRODUCTION

Recently we reported the production of xanthone derivatives and the secoiridoids, amarogentin and amaroswerin, from a hairy root culture of *Swertia japonica* Makino [1]. In continuation of our studies on secondary metabolic constituents in this culture, we have now isolated two new phenyl glucosides (**1** and **2**), together with 1-sinapoyl glucoside (**3**) [2, 3]. This paper deals with the isolation and structural determination of these compounds.

RESULTS AND DISCUSSION

The aqueous solution of the hairy roots of *S. japonica* (prepared as described in our previous paper [1]) was separated by column chromatography on Sephadex LH-20 to give fractions 1 and 2. Fraction 1 was rechromatographed over Sephadex LH-20 and MCI-gel CHP-20P to give **2**. Fraction 2 was subsequently chromatographed with Sephadex LH-20 to afford a further three fractions 2.1–2.3. Fraction 2.1, which contained mainly highly polar constituents, was purified by MCI-gel CHP-20P and Bondapak C_{18} Porasil B to give **1** and **3**.

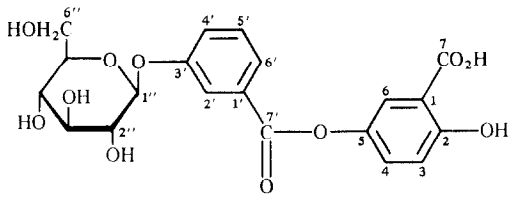
Compound **1**, gave a positive ferric chloride test and exhibited a prominent $[M+Na]^+$ ion peak at *m/z* 459 in the FAB mass spectrum. The ^1H NMR spectrum showed an anomeric [δ4.95 (*d*, $J=7.2$ Hz)], ABX-type aromatic [δ6.68 (1H, *d*, $J=8.8$ Hz), 7.04 (1H, *dd*, $J=8.8$, 3.0 Hz), 7.47 (1H, *d*, $J=3.0$)] and ABCD-type aromatic [δ7.40 (1H, *br dd*, $J=7.9$, 2.6 Hz), 7.52 (1H, *t*, $J=7.9$ Hz), 7.71 (1H, *br dd*, $J=2.6$, 1.6 Hz), 7.75 (1H, *br d*, $J=7.9$ Hz)] proton signals. The ^{13}C NMR spectrum (Table 1) showed signals due to one glucose moiety (δ60.5, 69.5, 73.2, 76.3, 77.0, 100.7), two aromatic rings (12 carbons), one carbonyl (δ164.7) and one carboxyl (δ170.4) carbon. Furthermore, the ^1H–^{13}C long-range shift correlation spectrum ($J_{CH}=10$ Hz) indicated the correlation of the carbonyl carbon at δ164.7 (C-7′) with the aromatic proton at δ7.71 (H-2′) through a three-bond coupling, suggesting that the carbonyl carbon was connected to C-1′ position (but the cross peak between C-7′ and H-6′ was not observed).

Methylation of **1** with diazomethane gave two methyl ethers **1a** and **1b**; the latter could not be isolated because of its small amount. Compound **1a** exhibited an intense $[M+Na]^+$ ion peak at *m/z* 337 in the FAB mass spectrum. The ^1H NMR spectrum of **1a** showed an anomeric [δ4.90 (*d*, $J=7.1$ Hz)], one methoxyl (δ3.85) and ABCD-type aromatic [δ7.33 (1H, *dd*, $J=2.2$, 8.0 Hz), 7.46 (1H, *t*, $J=8.0$ Hz), 7.57 (1H, *br d*, $J=2.2$ Hz), 7.61 (1H, *br d*, $J=8.0$ Hz)] proton signals. In this spectrum, the signals due to the ABX-type aromatic ring, shown in that of **1**, were absent. This suggested that **1**, on methylation with diazomethane, was readily cleaved at the ester linkage (C-7′ position). The ^1H–^1H NOESY spectrum of **1a** revealed NOE correlation cross peaks between the glucose C-1′ proton and two aromatic (H-2, H-4) protons and between methyl proton (H-8) and two aromatic (H-2, H-6) protons. This observation suggested that the glucose moiety was linked to the C-3 position through its C-1′ position and that the CO_2Me group was connected to the C-1 position. The configuration of the anomeric centre was concluded to be β from the *J* value (7.1 Hz) of the H-1′ signal in the ^1H NMR spectrum of **1a**. Therefore **1a** is characterized as 3-*O*-β-D-glucopyranosyl benzoic acid methyl ester.

Acid hydrolysis of **1** furnished two phenolic compounds, one of which was isolated and identified as gentisic acid by direct comparison of the spectral data with that of an authentic sample. In the ^1H NMR spectrum of **1**, the aromatic signals attributed to H-6 (δ7.47) and H-4 (δ7.04) were considerably shifted downfield compared with those of gentisic acid [δ7.15 (H-6), 6.96 (H-4)], thus indicating that in **1** the 3-glucosyl benzoic acid moiety was located at the C-5 position through its carbonyl carbon. From the chemical and spectral data above, **1** is concluded to be 5-(3′-*O*-β-D-glucopyranosyl)benzoyloxygentisic acid.

Compound **2** exhibited a prominent $[M+Na]^+$ ion peak at *m/z* 355 in the FAB mass spectrum. The ^1H and ^{13}C NMR (Table 1) spectra showed the presence of one glucose moiety (δ62.3, 70.8, 75.6, 77.5, 77.9, 106.3), a

†Author to whom correspondence should be addressed.



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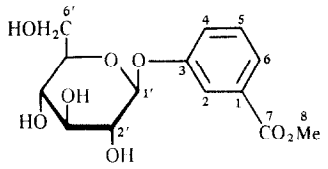

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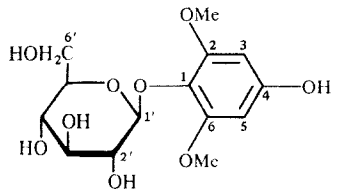

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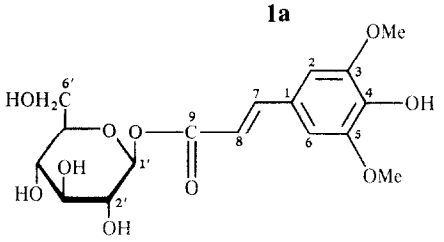

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Table 1. ^{13}C NMR spectral data of compounds **1**–**3** at 67.5 MHz (δ values)

C	1*	2†	3†
1	120.6	129.4	140.1
2	160.4	154.6	107.4
3	116.0	94.6	149.4
4	124.5	155.9	126.3
5	140.2	94.6	149.4
6	122.1	154.6	107.4
7	170.4		148.0
8			115.7
9			166.9
1′	130.5		
2′	117.4		
3′	157.5		
4′	121.5		
5′	130.0		
6′	123.0		
7′	164.7		
Glc-1	100.7	106.3	95.7
2	73.2	75.6	74.0
3	76.3	77.5	78.1
4	69.5	70.8	71.3
5	77.0	77.9	78.7
6	60.5	62.3	62.6
OMe		57.0	57.1

*In DMSO-d_6.
†In acetone-d_6 + D_2O.

symmetrically substituted aromatic ring [δ94.6, 129.4, 154.6, 155.9, δ6.10 (2H, s)] and two methoxyl groups [δ3.70 (6H, *s*)]. By comparing these spectral data with those of published data [4] (Saijo *et al.* obtained this compound by tannase hydrolysis of the gallate of **2**), **2** is concluded to be 2,6-dimethoxy-4-hydroxyphenol 1-*O*-β-D-glucopyranoside.

Compound **3** (FABMS *m/z*: 387 $[M+H]^+$, EIMS *m/z*: 386 $[M]^+$) gave ^{1}H and ^{13}C NMR (Table 1) spectra exhibiting the presence of a glucose moiety (δ62.6, 71.3, 74.0, 78.1, 78.7, 95.7), two methoxyl groups [δ3.83 (6H, s, OMe × 2)], one carbonyl (δ166.9) and one aromatic ring with a symmetrical substitution pattern [δ107.4, 126.3, 140.1, 149.4, δ6.99 (2H, s)]. Furthermore, two prominent doublets (1H each) at δ6.39 and δ7.62 with a large coupling constant (J = 16.1 Hz) showed the existence of two olefinic protons with a *trans* orientation. From these ^{1}H and ^{13}C NMR spectral data, the aglycone moiety of **3** was identified as sinapinic acid. In the ^{1}H NMR spectrum of **3** the glucose H-1 signal was observed downfield (δ5.53) suggesting that the sinapinic acid moiety was linked to this position through its carbonyl carbon. The configuration of the anomeric centre was confirmed to be β from the ^{1}H NMR coupling constant (J = 7.8 Hz) of the anomeric signal. Consequently, **3** is characterized as 1-*O*-sinapoyl-β-D-glucopyranoside [2, 3].

EXPERIMENTAL

^{1}H NMR spectra were measured at 270 and 400 MHz locked to the major deuterium resonance of the solvents (DMSO-d_6: δ2.50, Me_2CO-d_6: δ2.00). ^{13}C NMR spectra were recorded at 67.5 MHz in δ relative to the solvent signal (DMSO-d_6: δ39.5, Me_2CO-d_6: δ30.3). TLC was carried out on precoated Kieselgel 60 F_{254} plates and spots were detected by UV illumination and visualized by spraying with 10% H_2SO_4 and 2% ethanolic $FeCl_3$ reagents.

Plant material. The hairy roots of *S. japonica* Makino (2-year-old plant) were induced by direct inoculation of *Agrobacterium rhizogenes* strain 15834 harboring Ri plasmid (pRi 15834) and maintained on hormone free RC liquid medium [5] as in ref. [1]. Voucher specimens are deposited at the Herbarium of Breeding and Physiology Lab. in the Tsukuba Medicinal Plant Research Station.

Extraction and isolation. Lyophilized hairy roots (73 g dry wt), cultured in RC liquid medium for 6 weeks, were mashed, extracted and isolated to give an aq. layer as in ref. [1], which was subjected to Sephadex LH-20 CC using 60% MeOH to give frs 1 and 2. Fr. 1 was applied to MCI-gel CHP-20P (H_2O–MeOH) and Sephadex LH-20 (60% MeOH) column to give **2** (4.4 mg). Fr. 2 was rechromatographed over Sephadex LH-20 (60% MeOH) to afford a further three frs 2.1–2.3. Fr. 2.1

was purified by MCI-gel CHP-20P (H_2O–MeOH) and Bondapak C_{18} Porasil B (H_2O–30% MeOH) CC to furnish **1** (48 mg) and **3** (35 mg).

5-(3′-O-β-D-*glucopyranosyl*)*Benzoyloxygentisic acid* (**1**). White amorphous powder, $[\alpha]_D^{25}$ −38.5° (Me_2CO–MeOH 1:1; *c* 0.13), ^{1}H NMR (DMSO-d_6): δ3.20–3.40 (4H, *m*, Glc-2, 3, 4, 5), 3.49 (1H, *dd*, *J* = 5.4, 11.3 Hz, Glc-6), 3.69 (1H, *br d*, *J* = 11.3 Hz, Glc-6), 4.95 (1H, *d*, *J* = 7.2 Hz, Glc-1), 6.68 (1H, *d*, *J* = 8.8 Hz, H-3), 7.04 (1H, *dd*, *J* = 8.8, 3.0 Hz, H-4), 7.40 (1H, *br dd*, *J* = 7.9, 2.6 Hz, H-4′), 7.47 (1H, *d*, *J* = 3.0 Hz, H-6), 7.52 (1H, *t*, *J* = 7.9 Hz, H-5′), 7.71 (1H, *br t*, *J* = 2.6, 1.6 Hz, H-2′), 7.75 (1H, *br d*, *J* = 7.9 Hz, H-6′), ^{13}C NMR: see Table 1, FABMS *m/z* (rel. int.): 459 $[M+Na]^+$ (73), (Found: C, 49.30; H, 4.60. $C_{20}H_{20}O_{11}$ 5/2 H_2O requires: C, 49.90; H, 5.20).

Methylation of **1**. A soln of **1** (20 mg) in MeOH (2 ml) was treated with an ethereal soln of CH_2N_2 at room temp. for 30 min. The solvent was evapd off and the residue was purified by CC over silica gel (7 g) ($CHCl_3$–MeOH 1:0–7:1) to furnish **1a** (7.4 mg), an off-white amorphous powder, $[\alpha]_D^{25}$ −75.7° (MeOH; *c* 0.2), ^{1}H NMR (DMSO-d_6): δ3.15–3.50 (5H, *m*, Glc-2, 3, 4, 5, 6), 3.69 (1H, *dd*, *J* = 3.5, 11.5 Hz, Glc-6), 3.85 (3H, *s*, OMe), 4.90 (1H, *d*, *J* = 7.1 Hz, Glc-1), 7.33 (1H, *dd*, *J* = 2.2, 8.0 Hz, H-4), 7.46 (1H, *t*, *J* = 8.0 Hz, H-5), 7.57 (1H, *br d*, *J* = 2.2 Hz, H-2), 7.61 (1H, *br d*, *J* = 8.0 Hz, H-6), FABMS *m/z* (rel. int.): 337 $[M+Na]^+$ (58).

Acid hydrolysis of **1**. A mixture of **1** (5 mg) and 7% HCl (1 ml) was heated (90°) for 55 min. The reaction mixture, after cooling, was purified by Sephadex LH-20 (EtOH) CC to give gentisic acid (1 mg), ^{1}H NMR (DMSO-d_6): δ6.78 (1H, *d*, *J* = 8.7 Hz, H-3), 6.96 (1H, *dd*, *J* = 8.7, 3.3 Hz, H-4), 7.15 (1H, *d*, *J* = 3.3 Hz, H-6).

2,6-*Dimethoxy*-4-*hydroxyphenol* 1-O-β-D-*glucopyranoside* (**2**). An off-white amorphous powder, $[\alpha]_D^{25}$ −44.4° (Me_2CO–MeOH 1:1, *c* 0.23), ^{1}H NMR (Me_2CO-d_6 + D_2O): δ3.10–3.65 (6H, *m*, Glc-2, 3, 4, 5, 6), 3.70 (6H, *s*, OMe × 2), 4.56 (1H, *d*, *J* = 7.4 Hz, Glc-1), 6.10 (2H, *s*, H-3, 5), ^{13}C NMR: see Table 1, FABMS *m/z* (rel. int.): 355 $[M+Na]^+$ (100).

1-O-*Sinapoyl*-β-D-*glucopyranoside* (**3**). Needles (H_2O), mp 117°, $[\alpha]_D^{25}$ +34.8° (Me_2CO, *c* 0.12), ^{1}H NMR (Me_2CO-d_6 + D_2O): δ3.20–3.50 (4H, *m*, Glc-2, 3, 4, 5), 3.61 (1H, *dd*, *J* = 5.0, 15.0 Hz, Glc-6), 3.77 (1H, *dd*, 2.0, 15.0 Hz, Glc-6), 3.83 (6H, *s*, OMe × 2), 5.53 (1H, *d*, *J* = 7.8 Hz, Glc-1), 6.39 (1H, *d*, *J* = 16.1 Hz, H-8), 6.99 (2H, *s*, H-2, 6), 7.62 (1H, *d*, *J* = 16.1 Hz, H-7), ^{13}C NMR: see Table 1, FABMS *m/z* (rel. int.): 387 $[M+H]^+$ (89), EIMS *m/z* (rel. int.): 386 $[M]^+$ (28), (Found: C, 46.30; H, 6.04. $C_{17}H_{22}O_{10}$ 3H_2O requires: C, 46.36; H, 6.40%).

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