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A FLAVONE *C*-GLYCOSIDE AND AN AROMATIC GLUCOSIDE FROM *GENTIANA* SPECIES*

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Key Word Index—Gentianaceae; *Gentiana formosana*; *G. arisanensis*; aromatic glucoside; 2-hydroxy-3-methoxybenzoic acid glucose ester; flavone *C*-glycosides; isoorientin 6″-*O*-caffeate.

Abstract—A new aromatic glucoside, 2-hydroxy-3-methoxybenzoic acid glucose ester, was isolated from the whole plant of *Gentiana formosana* while a new flavone *C*-glycoside, isoorientin 6″-*O*-caffeate, and isoorientin 6″-*O*-glucoside and isoorientin were identified from *G. arisanensis*. The new compounds were characterized by spectral methods and chemical reactions.

INTRODUCTION

In a search for active constituents of Formosan *Gentiana* species, a mixture of α- and β-amyrin palmitate, uvaol palmitate, sitosterol, sitosterol-β-D-glucoside, ursolic acid, uvaol, 2α-hydroxyursolic acid, sweroside, and a new acylated secoiridoid, 3′-acetyl sweroside have been reported [1]. In a continuation of this research a new aromatic glucoside, 2-hydroxy-3-methoxybenzoic acid glucose ester (**1**) and four known compounds, mangiferin, rutin, quercetin, and quercetin 3-galactoside, and a mixture of sweroside and gentiopicroside were isolated from *Gentiana formosana* Hayata. Two known compounds, isoorientin (**4**) and isoorientin 6″-*O*-glucoside (**5**), and a new flavone *C*-glycoside, isoorientin 6″-*O*-caffeate (**6**), were isolated from *G. arisanensis* Hayata. The characterization of **1** and **6** and the assignment of the NMR spectra of **4** and **5** are reported in this paper.

RESULTS AND DISCUSSION

Compound **1** showed UV absorption maxima typical of an aromatic ring [2]. Its IR spectrum exhibited absorption bands at 3350 (OH), 1670, 1620 and 1260 cm^{-1} (aromatic ester). On alkaline hydrolysis it afforded glucose, as detected by TLC. In the FABMS spectrum (positive mode) of **1**, peaks at *m/z* 353 $[M + Na]^+$ and 169 $[M - 162 + H]^+$ indicated an $[M]^+$ at *m/z* 330.

The ^1H NMR spectrum of **1** indicated the presence of a methoxyl signal at δ3.87 (3H, *s*), a glucosyl anomeric proton at δ4.85 (*d*, *J* = 7.2 Hz), three aromatic proton signals at δ6.85 (*t*, *J* = 8.0 Hz, H-5), 7.35 (*dd*, *J* = 8.0, 1.5 Hz, H-4 or H-6) and 7.40 (*dd*, *J* = 8.0, 1.5 Hz, H-4 or H-6) and a phenolic hydroxyl signal at δ10.1. Based on the above evidence, **1** was concluded to be a glucosyl ester of a 1,2,3-trisubstituted aromatic compound.

The ^1H NMR spectrum of the peracetate of **1** (**2**) indicated four aliphatic acetyl signals at δ2.02, 2.02, 2.03 and 2.06, an aromatic acetyl signal at δ2.31 and three aromatic proton signals at δ7.22 (*dd*, *J* = 6.0, 1.0 Hz, H-4 or H-6), 7.25 (*dd*, *J* = 6.0, 1.0 Hz, H-4 or H-6) and 7.66 (*t*, *J* = 6.0 Hz, H-5). In the ^1H NMR of **2**, H-5 was shifted downfield in comparison with that of the corresponding proton of **1** (δ6.85). In addition to the above evidence, **1** also showed a chelated carbonyl ester group IR absorption at 1670 cm^{-1} and a bathochromic shift with $AlCl_3$ in its UV spectrum, suggesting that it possessed an aromatic *ortho*-hydroxycarbonyl moiety. The data also indicated the presence of a phenolic substituent at C-2 of **1** and a glucosyl moiety attached by an ester linkage at C-1. The EIMS of **2** showed no molecular ion peak but characteristic peaks at *m/z* 498 $[M - 42]^+$, 331 and 169. Therefore, **1** was characterized as 2-hydroxy-3-methoxybenzoic acid glucose ester (**1**).

Alkaline hydrolysis of permethylated **1** yielded **3**. The ^1H NMR spectrum of **3** indicated two methoxyl signals at δ3.90 and 4.05, three aromatic proton signals at δ7.14 (1H, *dd*, *J* = 6.2, 3.1 Hz), 7.16 (1H, *dd*, *J* = 6.2, 3.1 Hz) and 7.66 (1H, *t*, *J* = 6.2 Hz) and a carboxylic proton signals at δ10.1 (1H, bs). The ^{13}C NMR spectrum showed two methoxyl carbons at δ56.1 and 62.1, three secondary aromatic carbons at δ122.2, 123.7 and 124.8, three tertiary aromatic carbons at δ117.4, 148.2 and 152.1 and a carbonyl carbon at δ165.8. The above NMR spectral data indicated that **3** was 2,3-dimethoxybenzoic acid [3]. Based on the above evidences, **1** was characterized as

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2-hydroxy-3-methoxybenzoic acid glucose ester (**1**). The NOESY spectrum showed intense interactions among H-4, H-5, H-6 and H-1', supporting the characterization of **1**. The ^{13}C NMR spectrum of **1**, **2** and **3** were assigned by ^1H-decoupled spectra, DEPT pulse sequence, ^1H–^{13}C COSY spectrum and NOESY experiment. The ^{13}C NMR spectra of **1**, **2** and **3** also supported the characterization of **1**. Therefore, in the ^1H NMR spectrum of **1**, the aromatic proton signals at δ7.35 and 7.40 were assigned to H-6 and H-4, respectively.

Compound **6**, $C_{30}H_{26}O_{14}$, showed a UV absorption curve similar to that of isoorientin (**4**) [4]. The presence of bathochromic shifts induced by $AlCl_3$, NaOAc and NaOAc–H_2BO_4, in the UV spectrum and an ester and a chelated carbonyl group absorptions at 1720 and 1640 cm^{-1} in the IR spectrum suggested that **6** was an ester of a 5,7,3',4'-tetrahydroxyflavone derivative. The ^1H NMR spectrum of **6**, analysed with the aid of ^1H–^1H and ^1H–^{13}C COSY, showed sugar proton signals at δ4.40 (3H, *m*), 4.97 (1H, *dd*, J = 11.8, 6.0 Hz), 5.18 (1H, *d*, J = 11.8 Hz), 5.31 (1H, *t*, J = 8.0 Hz), and 5.85 (1H, *d*, 10 Hz), attributed to the H-3″, H-4″ and 5″; H-6‴; H-6‴; H-2″ and anomeric proton H-1″, respectively; two one-proton singlets at δ6.65 and 6.84, attributed to H-8 and H-3, respectively; and two pairs of ABX type signals at δ7.24 (*d*, J = 8.4 Hz), 7.44 (*dd*, J = 8.4, 2.0 Hz), 7.83 (*d*, J = 8.4 Hz) and 7.02 (*dd*, J = 8.4, 2 Hz), 7.17 (*d*, J = 8.4 Hz), 7.49 (*d*, J = 2 Hz), attributed to H-5', H-6', H-2' and H-6‴, H-5‴, H-2‴, respectively. The ^1H NMR spectrum also indicated the presence of a cinnamoyl moiety by the presence of doublets at δ6.49 (H-8‴) and 7.89 (H-7‴) (J = 15.6 Hz). Based on the above evidences, **6** was concluded to be a 3‴,4‴-dioxygenated cinnamoyl ester of isorientin (**6**) [4, 5]. Because the chemical shift values of the two glucosyl H-6 signals indicated a downfield shift, compared to those of corresponding data for isoorientin (**4**) (Table 1), it was clear that the cinnamoyl moiety was linked to the glucosyl C-6″-hydroxyl of isoorientin (**4**) [6]. The ^{13}C NMR spectrum of **6** (Table 1) was assigned by ^1H-decoupled spectra, DEPT pulse sequence, ^1H–^1H and ^{13}C–^1H COSY spectra, long-range ^{13}C–^1H COSY spectra (Fig. 1), comparison of chemical shifts with those of corresponding data for isoorientin (**4**) (Table 1) and reported data in literature [3]. The chemical shift values of glucosyl C-6″ and C-5″ of **6** indicated a downfield and an upfield shift, respectively, compared to those of corresponding data for isoorientin (**4**) (Table 1). It was further supported that **6** was an isoorientin 6″-*O*-dioxygenated cinnamoate [7]. Alkaline hydrolysis of **6** afforded caffeic acid (3,4-dihydroxycinnamic acid) and isoorientin (**4**), identified by comparison of melting point and spectral data with those of authentic samples. Therefore, compound **6** was characterized as isoorientin 6″-*O*-caffeate (**6**) (6″-*O*-caffeoyl isoorientin). The characterization of **6** was also supported by the mass spectrum and the long-range ^{13}C–^1H COSY spectrum (Fig. 1).

The NMR spectrum of **4** and **5** (Table 1) were assigned by ^1H NMR, ^1H-decoupled spectra, DEPT pulse sequence, ^1H–^1H and ^{13}C–^1H COSY spectra, long-range ^{13}C–^1H COSY spectrum and comparison of chemical shift values with those of corresponding literature data [4, 8]. The chemical shift values of glucosyl C-1″ to C-4″ of **4** and **5**, and C-5 and C-9 of **4**, reported in literature [8] should be revised as shown in Table 2.

EXPERIMENTAL

Plant material, extraction and isolation. Plants of *G. formosana* (1.5 kg) were collected at Yu Shien, Chiayi Hsieh, Taiwan, during July 1992 and a voucher specimen deposited in the authors' laboratory. Whole plants were extracted with hot MeOH. The water-soluble fraction of concd MeOH extract was successively partitioned with $CHCl_3$ and EtOAc, respectively. The EtOAc extract was chromatographed on Si gel. Elution with EtOAc yielded **1**. Elution with EtOAc–MeOH (9:1) yielded a mixture of sweroside and gentiopicroside. Elution with EtOAc–MeOH (4:1) yielded mangiferin, while elution with EtOAc–MeOH (1:1) and (2:1) yielded quercetin 3-galactoside and rutin. Known compounds were identified by UV, IR, NMR, and comparison of mmps and spectral data with those of authentic samples.

Plants of *G. arisanensis* (1.5 kg) were collected at Yu Shieh, Chiayi Hsieh, Taiwan, during April 1994 and a voucher specimen deposited in the authors' laboratory. Whole plants were extracted successively ×3 with $CHCl_3$ and MeOH at room temp. in a closed container. The combined MeOH extracts were evaporated and the water soluble fraction of the MeOH extract was partitioned first with EtOAc and then *n*-BuOH. The EtOAc extract was chromatographed on Si gel, using $CHCl_3$–MeOH as eluant and then rechromatographed on Si gel, eluted with $CHCl_3$–MeOH–H_2O (5:1:0.5) and EtOAc–MeOH–H_2O (9:1:1). The eluates were further chromatographed on Sephadex LH-20. Elution with MeOH yielded isoorientin (**4**). The *n*-BuOH extract was also chromotographed on Si gel eluted with EtOAc–MeOH–H_2O (4:1:1). After rechromatography on Sephadex LH-20 with MeOH, the 50% MeOH eluates gave isoorientin 6″-*O*-β-D-glucoside (**5**) and **6**. Compounds **4** and **5** were identified by UV, IR, NMR, MS and chemical reactions [4, 8].

2-Hydroxy-3-methoxybenzoic acid glucose ester (**1**). Plates. mp 186°. IR ν_{max}^{KBr} cm^{-1}: 3530, 1670, 1620 and 1260. UV λ_{max}^{MeOH} (log ε): 207 (4.39), 245 (4.08) and 314 (3.52); $AlCl_3$: 260 and 345. ^1H NMR (DMSO-d_6): see Text. ^{13}C NMR (DMSO-d_6): see Table 2. FABMS (positive mode) *m/z*: 353 (47) $[M + Na]^+$, 179 (25), 169 (100) $[M - 162 + H]^+$, 137 (41). Cald for $C_{14}H_{18}O_9$: 330.0951. Found (MS): 330.1016.

Compound 1 *Peracetate* (**2**). Needles, mp 151°. ^1H NMR ($CDCl_3$): see Text. ^{13}C NMR ($CDCl_3$): see Table 2. EIMS (direct melt) 70 eV, *m/z* (rel. int.) 498 (3) $[M - 42]^+$, 331 (75), 169 (100).

2,3-Dimethoxybenzoic acid (**3**). Compound **1** (100 mg) in dry Me_2CO (15 ml) was refluxed over dry K_2CO_3 (1.5 g) with dry Me_2SO_4 (15 ml) for 12 hr. The product was refluxed with 5% methanolic KOH (20 ml) and

Table 1. ^{13}C and ^{1}H NMR chemical shifts of **4**, **5** and **6** (pyridine-d_5)*

No C	4			5			6		
	δ^{13}Cppm	δ^{1}Hppm	mult (*J*, Hz)	δ^{13}Cppm	δ^{1}Hppm	mult (*J*, Hz)	δ^{13}Cppm	δ^{1}Hppm	mult (*J*, Hz)
2	164.6			164.5			164.5		
3	103.8	6.88	*s*	103.8	6.83	*s*	104.0	6.65	*s*
4	182.8			182.8			182.9		
5	162.0			161.8			162.2		
6	110.0			109.6			109.8		
7	164.7			164.5			164.7		
8	94.5	6.69	*s*	94.6	6.66	*s*	94.4	6.51	*s*
9	157.4			157.3			157.5		
10	104.8			104.7			104.8		
1′	122.8			122.7			123.0		
2′	114.5	7.87	*d* (1.7)	114.5	7.84	*d* (1.7)	114.5	7.42	*d* (1.8)
3′	147.7			147.6			147.7		
4′	151.6			151.5			151.6		
5′	116.8	7.27	*d* (8.3)	116.8	7.24	*d* (8.3)	116.8	6.88	*d* (8.1)
6′	119.4	7.49	*dd* (8.3, 1.7)	119.4	7.43	*dd* (8.3, 1.7)	119.4	7.39	*dd* (8.1, 1.8)
1″	75.5	5.84	*d* (10)	75.5	5.71	*d* (10)	75.5	5.85	*d* (10)
2″	72.8	5.20	*t* (8.6)	72.6	5.10	*t* (9.2)	72.5	5.31	*t* (8.0)
3″	80.6	4.47	*m*	80.3	4.37	*m*	80.6	4.40	*m*
4″	71.9	4.47	*m*	71.7	4.37	*m*	71.9	4.40	*m*
5″	82.9	4.18	*m*	80.0	4.37	*m*	80.0	4.40	*m*
6″	62.7	4.47	*m*	70.3	4.83	*d* (9.6)	65.1	4.97	*dd* (11.8, 6.0)
								5.18	*d* (11.8)
1‴				105.1	4.94	*d* (8.0)	126.8		
2‴				74.9	4.00	*t* (8.4)	115.8	7.04	*d* (2.0)
3‴				78.3	4.18	*m*	147.5		
4‴				71.3	4.18	*m*	150.3		
5‴				78.3	3.84	*m*	116.6	7.17	*d* (8.4)
6‴				61.2	4.37	*m*	122.8	7.02	*dd* (8.4, 2.0)
7‴							145.8	7.89	*d* (15.6)
8‴							115.0	6.49	*d* (15.6)
9‴							167.6		

*The number of protons directly attached to each carbon was verified with DEPT pulse sequence.

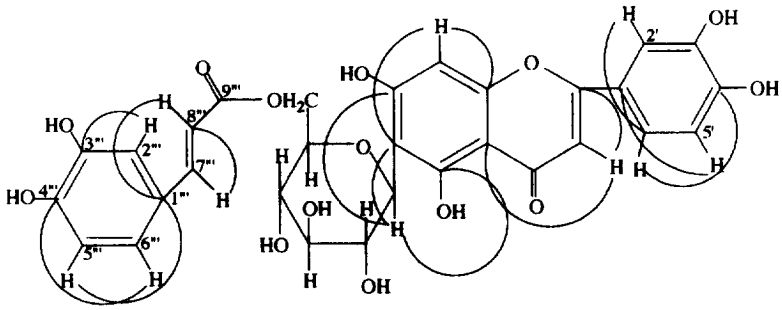

Fig. 1. Long range HETCOR of **6**.

Table 2. ^{13}C NMR chemical shifts of **1**, **2** and **3***

No C	1†	2†	3‡
1	114.8	119.7	117.4
2	146.2	140.6	148.2
3	150.1	149.3	152.1
4	123.0	126.1	124.7
5	119.0	125.3	122.2
6	121.0	124.8	123.7
1′	101.4	98.9	
2′	73.5	70.5	
3′	76.6	72.0	
4′	69.9	68.1	
5′	77.4	72.4	
6′	60.9	61.7	
COO	169.0	169.4	165.8
OMe	52.6	52.3	56.1, 62.1
$O\underline{C}OCH_3$		164.5, 168.8, 169.3 170.0, 170.4	
$OCO\underline{C}H_3$		20.4, 20.5, 20.6	

*The number of protons directly attached to each carbon was verified with DEPT pulse sequence.

†Spectra recorded in pyridine-d_5.

‡Spectra recorded in $CDCl_3$.

yielded **3**, mp 158°, IR ν_{max}^{KBr} cm^{-1}: 3500–2800, ^{1}H NMR (CD_3OD): δ3.90 (3H, *s*, OMe), 4.05 (3H, *s*, OMe), 7.15 (2H, *m*, H-4 and H-6), 7.66 (1H, *m*, H-5), 10.1 (1H, *m*, carboxylic proton), ^{13}C NMR ($CDCl_3$): see Table 2. EIMS (direct melt), 70 eV, *m/z* (rel. int.): 182 (70) $[M]^+$, 137 (25), 107 (100).

Isoorientin 6″-O-caffeate (**6**). Pale yellow powder, mp 228°, IR ν_{max}^{KBr} cm^{-1}: 3700–3050, 1650, 1320 and 850; UV λ_{max}^{MeOH} (log ε): 210 (2.54), 245 (2.26), 275 (2.22), 300 (2.18) and 325 (2.36); + $AlCl_3$: 210, 275, 300 and 375; + NaOAc: 210, 245, 275 and 410; H_3BO_3: 210, 250, 270, 280 and 390; ^{1}H NMR (pyridine-d_5): see Table 2. ^{13}C NMR (pyridine-d_5): see Table 1. FABMS (positive mode) *m/z* 633 (3) $[M + Na]^+$, 611 (1) $[M + 1]^+$, 463 (2), 413 (5), 311 (3), 301 (5), 273 (3), 207 (8), 163 (3), 147 (7), 115 (100).

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