



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

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(Received in revised form 5 June 1995)

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 $\delta 3.87$ (3H, s), a glucosyl anomeric

proton at $\delta 4.85$ (d, J=7.2 Hz), three aromatic proton signals at $\delta 6.85$ (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 $\delta 10.1$. 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 δ 2.02, 2.02, 2.03 and 2.06, an aromatic acetyl signal at δ 2.31 and three aromatic proton signals at δ 7.22 (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 (δ 6.85). 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 AlCl₃ 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-3methoxybenzoic acid glucose ester (1).

Alkaline hydrolysis of permethylated 1 yielded 3. The ¹H NMR spectrum of 3 indicated two methoxyl signals at δ 3.90 and 4.05, three aromatic proton signals at δ 7.14 (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 δ 10.1 (1H, bs). The ¹³C NMR spectrum showed two methoxyl carbons at δ 56.1 and 62.1, three secondary aromatic carbons at δ 122.2, 123.7 and 124.8, three tertiary aromatic carbons at δ 117.4, 148.2 and 152.1 and a carbonyl carbon at δ 165.8. The above NMR spectral data indicated that 3 was 2,3-dimethoxybenzoic acid [3]. Based on the above evidences, 1 was characterized as

^{*}Part 16 in the series 'studies on the Constituents of Formosan Gentianaceous Plants'. For part 15 see [1].

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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 1 H-decoupled spectra, DEPT pulse sequence, 1 H- 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 HNMR spectrum of 1, the aromatic proton signals at δ 7.35 and 7.40 were assigned to H-6 and H-4, respectively.

Compound 6, C₃₀H₂₆O₁₄, showed a UV absorption curve similar to that of isoorientin (4) [4]. The presence of bathochromic shifts induced by AlCl₃, NaOAc and NaOAc-H₂BO₄, 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-¹³C COSY, showed sugar proton signals at δ 4.40 (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 oneproton singlets at $\delta 6.65$ and 6.84, attributed to H-8 and H-3, respectively; and two pairs of ABX type signals at δ 7.24 (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 $\delta 6.49$ (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 cinnamovl 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 cinnamoul moiety was linked to the glucosyl C-6"-hydroxyl of isoorientin (4) [6]. The ¹³C NMR spectrum of 6 (Table 1) was assigned by ¹H-decoupled spectra, DEPT pulse sequence, ¹H-¹H and ¹³C-¹H COSY spectra, long-range ¹³C-¹HCOSY 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 13C-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 ¹³C-¹H COSY spectra, long-range

¹³C-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 CHCl₃ 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 CHCl₃ 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 CHCl₃-MeOH as eluant and then rechromatographed on Si gel, eluted with CHCl₃-MeOH-H₂O (5:1:0.5) and EtOAc-MeOH-H₂O (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-H₂O (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 $\nu_{\rm max}^{\rm KBr}$ cm⁻¹: 3530, 1670, 1620 and 1260. UV $\lambda_{\rm max}^{\rm MeOH}$ (log ε): 207 (4.39), 245 (4.08) and 314 (3.52); AlCl₃: 260 and 345. ¹H NMR (DMSO- d_6): see Text. ¹³C 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 C₁₄H₁₈O₉: 330.0951. Found (MS): 330.1016.

Compound 1 Peracetate (2). Needles, mp 151° . 1 H NMR (CDCl₃): see Text. 13 C NMR (CDCl₃): 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 Me₂CO (15 ml) was refluxed over dry K₂CO₃ (1.5 g) with dry Me₂SO₄ (15 ml) for 12 hr. The product was refluxed with 5% methanolic KOH (20 ml) and

Table 1. 13C and ¹H NMR chemical shifts of 4, 5 and 6 (pyridine-4₅)*

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75.5 5.24 d (10) 75.5 5.71 d (10) 75.5 5.85 72.8 5.20 t (86) 72.6 5.10 t (92) 72.5 5.31 80.6 4.47 m 80.3 4.37 m 80.6 4.40 71.9 4.47 m 71.7 4.37 m 80.0 4.40 82.9 4.18 m 70.3 4.83 d (9.6) 65.1 4.97 62.7 4.47 m 70.3 4.83 d (8.0) 126.8 7.04 62.7 4.47 m 70.3 4.83 d (8.0) 126.8 7.04 74.9 4.00 t (8.4) t (8.4) 115.8 7.04 74.9 4.00 t (8.4) m 147.5 7.17 71.3 4.18 m 150.3 7.04 78.3 3.84 m 165.6 7.07 78.9 115.6 7.07 7.07 7.07 <td>ور</td> <td>119.4</td> <td>7.49</td> <td>dd (8.3, 1.7)</td> <td>119.4</td> <td>7.43</td> <td>dd (8.3, 1.7)</td> <td>119.4</td> <td>7.39</td> <td>dd (8.1, 1.8)</td>	ور	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)
72.8 5.20 t (8.6) 72.6 5.10 t (9.2) 72.5 5.31 80.6 4.47 m 80.3 4.37 m 80.6 4.40 71.9 4.47 m 71.7 4.37 m 80.0 4.40 82.9 4.18 m 80.0 4.40 4.40 4.40 62.7 4.47 m 70.3 4.83 d (9.6) 65.1 4.97 62.7 4.47 m 70.3 4.83 d (9.6) 65.1 4.97 62.7 4.47 m 70.3 4.83 d (9.6) 65.1 4.97 62.7 4.47 m 74.9 4.00 t (8.4) 115.8 7.04 74.9 4.00 t (8.4) m 147.5 7.04 78.3 3.84 m 150.3 7.02 78.3 3.84 m 122.8 7.02 8.9 4.37 m 157.6	<u>"</u>	75.5	5.84	d (10)	75.5	5.71	d (10)	75.5	5.85	d (10)
80.6 4.47 m 80.3 4.37 m 80.6 4.40 71.9 4.47 m 71.7 4.37 m 80.6 4.40 82.9 4.18 m 80.0 4.37 m 80.0 4.40 62.7 4.47 m 70.3 4.83 d (9.6) 65.1 4.97 62.7 4.47 m 70.3 4.83 d (9.6) 65.1 4.97 105.1 4.94 d (8.0) 126.8 7.04 74.9 4.00 t (8.4) 115.8 7.04 78.3 4.18 m 147.5 7.17 78.3 3.84 m 150.3 7.02 78.3 3.84 m 122.8 7.02 145.8 7.83 145.8 7.89 115.0 6.49 167.6 6.49	7,,	72.8	5.20	t (8.6)	72.6	5.10	t (9.2)	72.5	5.31	t (8.0)
71.9 447 m 71.7 4.37 m 71.9 4.40 82.9 4.18 m 80.0 4.37 m 80.0 4.40 62.7 4.47 m 70.3 4.83 d (9.6) 65.1 4.97 62.7 4.47 m 70.3 4.83 d (8.0) 65.1 4.97 105.1 4.94 d (8.0) 126.8 7.04 74.9 4.00 t (8.4) 115.8 7.04 78.3 4.18 m 147.5 7.17 78.3 3.84 m 16.6 7.17 61.2 4.37 m 122.8 7.02 145.8 7.89 115.0 6.49 167.6	3"	90.6	4.47	E	80.3	4.37	W.	9.08	4.40	E
82.9 4.18 m 80.0 4.37 m 80.0 4.40 62.7 4.47 m 70.3 4.83 d (9.6) 65.1 4.97 105.1 4.94 d (8.0) 126.8 5.18 74.9 4.00 t (8.4) 115.8 7.04 78.3 4.18 m 147.5 7.17 78.3 3.84 m 166.0 7.17 61.2 4.37 m 122.8 7.02 115.0 6.49 115.0 6.49	, 4	71.9	4.47	E	71.7	4.37	u	71.9	4.40	E
62.7 4.47 m 70.3 4.83 d (9.6) 65.1 4.97 105.1 4.94 d (8.0) 126.8 74.9 4.00 t (8.4) 115.8 7.04 78.3 4.18 m 147.5 71.3 4.18 m 150.3 78.3 3.84 m 116.6 7.17 61.2 4.37 m 122.8 7.02 115.0 6.49 115.0 6.49	2,	82.9	4.18	E	80.0	4.37	E	80.0	4.40	w
105.1 4.94 d (8.0) 126.8 5.18 74.9 4.00 t (8.4) 115.8 7.04 78.3 4.18 m 147.5 71.3 4.18 m 150.3 7.17 8.3 3.84 m 116.6 7.17 61.2 4.37 m 122.8 7.02 145.8 7.89 115.0 6.49 115.0	ور	62.7	4.47	E	70.3	4.83	q (9.6)	65.1	4.97	dd (11.8, 6.0)
105.1 4.94 d (8.0) 126.8 74.9 4.00 t (8.4) 115.8 7.04 78.3 4.18 m 147.5 71.3 4.18 m 150.3 78.3 3.84 m 116.6 7.17 61.2 4.37 m 122.8 7.02 145.8 7.89 115.0 6.49 167.6									5.18	d (11.8)
74.9 4.00 t (8.4) 115.8 7.04 78.3 4.18 m 147.5 71.3 4.18 m 150.3 78.3 3.84 m 116.6 7.17 61.2 4.37 m 122.8 7.02 145.8 7.89 115.0 6.49 167.6	1,,,				105.1	4.94	q (8.0)	126.8		
78.3 4.18 m 147.5 71.3 4.18 m 150.3 78.3 3.84 m 116.6 7.17 61.2 4.37 m 122.8 7.02 145.8 7.89 115.0 6.49 167.6	5				74.9	4.00	t (8.4)	115.8	7.04	d (2.0)
71.3 4.18 m 150.3 78.3 3.84 m 116.6 7.17 61.2 4.37 m 122.8 7.02 145.8 7.89 115.0 6.49 167.6	3				78.3	4.18		147.5		
78.3 3.84 m 116.6 7.17 61.2 4.37 m 122.8 7.02 145.8 7.89 115.0 6.49 167.6	, "4				71.3	4.18	E	150.3		
61.2 4.37 m 122.8 7.02 145.8 7.89 115.0 6.49 167.6	2,,,				78.3	3.84	E	116.6	7.17	d (8.4)
145.8 7.89 115.0 6.49 167.6	,,,9				61.2	4.37	E E	122.8	7.02	dd (8.4, 2.0)
115.0 6.49 167.6	7,,,							145.8	7.89	d (15.6)
167.6	<u>*</u> 00							115.0	6.49	d (15.6)
	6″,							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. 13C 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
OCOCH ₃		164.5, 168.8, 169.3	
		170.0, 170.4	
OCOCH ₃		20.4, 20.5, 20.6	

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

yielded 3, mp 158°, IR $v_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3500–2800, ¹H NMR (CD₃OD): δ 3.90 (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), ¹³C NMR (CDCl₃): 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 $v_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3700-3050, 1650, 1320 and 850; UV $\lambda_{\text{max}}^{\text{MeOH}}$ (log ε): 210 (2.54), 245 (2.26), 275 (2.22), 300 (2.18)

and 325 (2.36); + AlCl₃: 210, 275, 300 and 375; + NaOAc: 210, 245, 275 and 410; H_3BO_3 : 210, 250, 270, 280 and 390; ¹H NMR (pyridine- d_5): see Table 2. ¹³C 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).

Acknowledgements—The work was supported, in part by a grant from the National Science Council of the Republic of China (NSC 84-2331-B037-075).

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[†]Spectra recorded in pyridine-d₅.

[‡]Spectra recorded in CDCl₃.