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SECOIRIDOIDS FROM *GENTIANA SIPHONANTHA*

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Key Word Index—*Gentiana siphonantha*; Gentianaceae; secoiridoids; 6′-gentisoyl 8-epi-kingiside; 2′-gentisoyl gelidoside; sterols; triterpene.

Abstract—Repeated fractionations of the methanol extract of the subterranean parts (rhizomes and roots) of *Gentiana siphonantha* afforded two new and five known secoiridoids, in addition to the widespread plant constituents β-sitiosterol, daucosterol and oleanolic acid. The structures of the new acyl secoiridoid glycosides were elucidated as 6′-gentisoyl 8-epikingiside and 2′-gentisoyl gelidoside mainly by a combination of high field NMR techniques. The known secoiridoids were identified as gentiolactone, gentiopicroside, sweroside, gelidoside and trifloroside. None of these constituents was active against human pathogenic fungi (*Candida albican*, *Aspergillus flavus* and *Trichoderma viride*). The chemotaxonomic significance of the isolates is discussed briefly. © 1997 Elsevier Science Ltd

INTRODUCTION

In the preceding paper [1], we reported on the isolation of acyl secoiridoids and antifungal constituents from roots of *Gentiana macrophylla* Pall., a well-known and widely used traditional Chinese drug. With regard to the underground parts of *G. siphonantha* Maxim., a perfect substitute for roots of *G. macrophylla*, little is known about its chemical constituents except for a report of the detection of alkaloids by HPLC [2]. Renewed pharmacological interest in gentiopicroside (the most predominant and widespread secoiridoid glycoside in the Gentianaceae family) has suggested its potential use for the suppression of chemically and immunologically induced hepatic injuries [3]. In continuation of our phytochemical investigation of Chinese *Gentiana* species [1, 4], these observations prompted us to analyse the chemical constituents of *G. siphonantha* for the presence of gentiopicroside and providing a comparison between the constituents *G. siphonantha* and *G. macrophylla*.

RESULTS AND DISCUSSION

Careful separation of the methanol extract of the subterranean part of *G. siphonantha* afforded two new (**6** and **7**) and five known (**1**–**5**) secoiridoids, along with two sterols (β-sitosterol and daucosterol) and a triterpene (oleanolic acid) which were identified by direct comparisons with authentic samples (co-TLC, ^{1}H NMR).

The EIMS, ^{1}H and ^{13}C NMR spectra of secoiridoid **1** indicated that it was gentiolactone, a constituent characterized earlier from roots of *G. purpurea* [5]. The well-assigned ^{1}H and ^{13}C NMR data are given in the Experimental section. Based on the ^{1}H and ^{13}C NMR data, compounds **2** and **3** were identified as gentiopicroside and sweroside, both being widespread in Gentianaceae family [6, 7]. The FAB mass spectrum, ^{1}H and ^{13}C NMR spectral data of acyl secoiridoid glycosides **4** and **5** established their identities as trifloroside and gelidoside (= rindoside), respectively [8, 9]. Secoiridoides **2**–**5** were re-isolated recently from roots of *G. macrophylla* [1] and *G. algida* [4].

The FAB mass spectrum, ^{1}H and ^{13}C NMR spectra of compound **6** suggested that it was also an acylated secoiridoid glycoside. In its FAB mass spectrum a quasimolecular ion appeared at m/z 541 with two typical fragment ions at m/z 405 and 243 produced presumably through successive eliminations of dihydroxybenzoyl and glucosyl residues from the parent ion [1, 4]. The ^{13}C NMR spectrum and DEPT experiments gave a total of 24 resonance lines (Table 1) of which 17 could be assigned to an 8-epikingiside moiety acylated at the C-6′ position. This was seen by the downfield shift of this carbon atom (1.8 ppm) and the upfield shift of C-5′ (−2.8 ppm) when compared to the spectrum of 8-epikingiside [10], the signals being almost superimposable. The remaining seven signals of **6** (Table 1) fitted well with a gentisoyl group and this was confirmed by the ^{1}H NMR spectrum (Exper-

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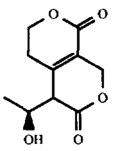

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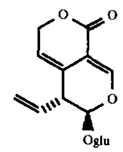

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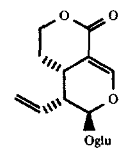

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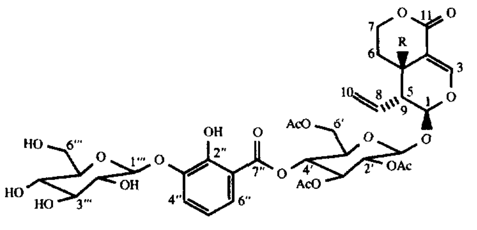

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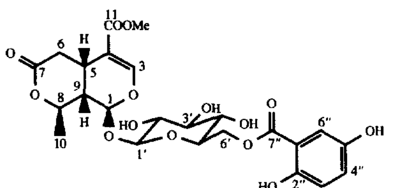

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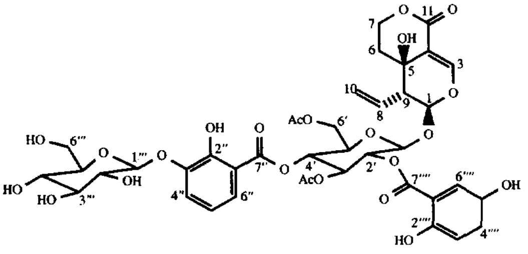

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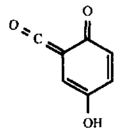

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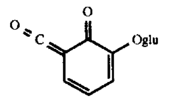

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Table 1. ^{13}C NMR spectral data of secoiridoid 6 (125 MHz, CD$_3$OD)

C	8-Epikingiside*	6	DEPT
1	96.3	96.5	CH
3	154.5	154.2	CH
4	109.6	109.5	C
5	28.2	28.6	CH
6	34.6	34.6	CH$_2$
7	174.7	174.5	C
8	75.8	75.5	CH
9	41.9	41.8	CH
10	21.7	21.4	CH$_3$
11	168.3	168.2	C
OMe	51.8	51.9	CH$_3$
1′	100.7	101.0	CH
2′	74.2	74.6	CH
3′	78.0	77.7	CH
4′	71.7	71.7	CH
5′	78.5	75.7	CH
6′	62.8	64.6	CH$_2$
1″	—	113.3	C
2″	—	155.9	C
3″	—	115.3	CH
4″	—	119.3	CH
5″	—	150.9	C
6″	—	125.2	CH
7″	—	170.7	C

* Cited from ref. [10]

imental) [11]. The methyl group on C-8 was found to be β-oriented as evident from the C-8 signal at δ 21.4 (against δ 17.7 for kingiside [12]) and a coupling constant value $J_{8,9} = 7.1$ Hz for 6, strikingly different from that for kingiside ($J_{8,9} = 2.6$ Hz) [13] but close to that of 8-epikingiside [14]. Thus, the structure of 6 was elucidated as 6′-gentisoyl 8-epikingiside.

The FAB mass spectrum, ^{1}H and ^{13}C NMR spectra of compound 7 demonstrated that it was an acyl secoiridoid glycoside, too. The intense quasimolecular ions at m/z 893 $[M+H]^+$, 915 $[M+Na]^+$ and 931 $[M+K]^+$ in the FAB mass spectrum of 7 were identical to those for macrophylloside B [1]. However, the ^{1}H and ^{13}C NMR spectral data were different from those of macrophylloside B. A subsequent scrutiny of the ^{1}H and ^{13}C NMR spectra of compound 7 and macrophylloside B indicated that both possessed a 2-hydroxy-3-(1-β-D-glucopyranosyl) oxybenzoyl moiety whereas the proton and carbon resonances attributed to the 2,3-dihydroxybenzoyl group in the ^{1}H and ^{13}C NMR spectra of macrophylloside B were replaced by a set of signals ascribable to a gentisoyl group in those of 7. Spin decouplings confirmed this assumption and allowed the unequivocal assignment of all proton signals. Comparisons of the ^{1}H NMR spectrum of compound 7 with those of peracetylated glucosides [10, 15] showed that the signals arising from H-1′ through H-5′ (Experimental) were shifted downfield by *ca* 0.4, 0.3, 0.5, 0.3 and 0.4, respectively. This set of downfield deviations could only be explained by assuming benzoylations on C-2′ and C-4′, which exerted more paramagnetic effects on H-1′ through H-3′ and H-3′ through H-5′ than acetylations thereon did on those protons [4, 7, 16]. The proposed acylation pattern of 7 was reinforced by chemical shifts of H-1′ through H-6′ which were very close to those of macrophylloside A and B with 2′,4′-bisbenzoyl-like functions [1]. In the COLOC spectrum of 7, the long-range correlations of C-7″ with H-4′ and H-6″ demonstrated that the 2-hydroxy-3-(β-D-glucopyranosyl)oxybenzoyl group was situated on C-4′. The gentisoyl function was attached to C-2′ as evidenced from the long-range couplings of C-7⁗ with H-6⁗ and H-2′. In conclusion, acyl secoiridoid 7 was 2′-gentisoyl gelidoside.

Using *Candida albicans*, *Aspergillus flavus* and *Trichoderma viride* as test microorganisms, all constituents mentioned above were subjected to antifungal bioassays as outlined [17, 18], but none showed any activity.

This phytochemical investigation of *G. siphonantha* showed its close relationship to *G. macrophylla* as the constituents isolated from both plants are quite similar and therefore it is reasonable that it can be used as a substitute for *G. macrophylla*. However, the minor antifungal chromene derivatives characterized in *G. macrophylla* [1] and *G. algida* [4] were not detected in the extract of *G. siphonantha* due to either their presence in a hardly detectable amount or a different fractionation strategy. In addition, the underground parts of *G. siphonantha* have been shown to be another rich source of gentiopicroside (Experimental).

EXPERIMENTAL

General. NMR: 500 MHz (^{1}H) and 125 MHz (^{13}C) with TMS as an int. standard; MS: ZAB-HS mass spectrometer; CC: silica gel (200–300 mesh); TLC: silica GF_{254} (10–20 μ).

Plant material. The subterranean parts of *G. siphonantha* were collected in July 1995 in Pingliang County, Gansu Province, People's Republic of China. The material was identified by Dr L. X. Zhang and a voucher specimen (ZL-PL 950704) was preserved in the Herbarium of the Department of Biological Sciences & Technology, Nanjing University.

Extraction and isolation. The pulverized air-dried underground parts (0.7 kg) of *G. siphonantha* were extracted twice with MeOH at room temp. Evapn of the solvent *in vacuo* afforded a brown gum (36 g) which was dissolved in MeOH containing 5% H_2O. The soln was kept at $-10°$ for 24 h to ppt the less polar gummy substance. The 'de-gummed' MeOH filtrate was concd to dryness (28 g) under vacuum and subsequently chromatographed by CC (silica gel, 800 g) eluting with $CHCl_3$ containing gradually increased amounts of MeOH. Using TLC monitoring, six frs were collected (F-1: 8 g, F-2: 2.1 g, F-3: 3.4 g, F-4: 2.6 g, F-5: 3.2 g and F-6: 3.5 g). CC of F-1 over silica gel (400 g) with a gradient of petrol (60–90°)–Me_2CO (30:1 → 1:7) gave β-sitosterol (163 mg) and an UV-active mixt. which afforded, by gel filtration over a Sephadex LH-20 column in $CHCl_3$–MeOH (1:1), oleanolic acid (32 mg) and gentiolactone (**1**, 21 mg). CC of F-2 over silica gel with a $CHCl_3$–MeOH gradient (50:1 → 1:2) yielded 2 frs (F-2-1 and F-2-2). Gel filtration of F-2-1 over Sephadex LH-20 with $CHCl_3$–MeOH (1:1) yielded again oleanolic acid (38 mg) and pigments. Gentiopicroside (**2**, 530 mg) was obtained from F-2-2 by chromatographing over silica gel using $CHCl_3$–MeOH mixts of increasing polarity. CC of F-3 over silica gel using a $CHCl_3$–MeOH gradient afforded again **2** (120 mg) and two frs (F-3-1 and F-3-2). Compound **4** (13 mg) was afforded by a gel filtration of F-3-1 over Sephadex LH-20 with MeOH while work-up of F-3-2 in the same manner gave a mixt. of **4** and **5**. (*ca.* 2:1). CC of F-4 over silica gel with a $CHCl_3$–MeOH gradient yielded further **2** (125 mg) and 3 frs (F-4-1, F-4-2 and F-4-3). F-4-1 contained mainly compound **5**. Repeated gel filtration of F-4-2 over Sephadex LH-20 with $CHCl_3$–MeOH (1:1) gave 6′-gentisoyl 8-epikingiside (**6**, 9 mg). CC of F-4-3 afforded a pigment mixt. and a fr. which yield 2′-gentisoyl gelidoside (**7**, 15 mg) by repeated gel filtration over Sephadex LH-20 with MeOH. CC of F-5 over silica gel with a $CHCl_3$–MeOH gradient gave again **2** (89 mg) and a brown gum which afforded finally a mixt. (85 mg) of secoiridoid glycosides **2** and **3** (*ca* 3:1). F-6 contained mainly saccharides as indicated by the spraying agents.

Gentiolactone (**1**). Gum; ^{1}H NMR (500 MHz, CD_3OD) δ: 5.13 (1H, *dt*, $J = 16.3$, 1.6 Hz, H-3A), 5.04 (1H, *dt*, $J = 16.3$, 2.9 Hz, H-3B), 2.67 (2H, *m*, H-6), 4.50 (1H, *ddd*, $J = 11.2$, 6.8, 5.4 Hz, H-7A), 4.45 (1H, *ddd*, $J = 11.2$, 7.5, 5.3 Hz, H-7B), 1.92 (1H, *dq*, $J = 14.2$, 7.5 Hz, H-8A), 1.78 (1H, *dq*, $J = 14.2$, 7.5 Hz, H-8B), 0.95 (3H, *t*, $J = 7.5$ Hz, H-10); ^{13}C NMR (125 MHz, CD_3OD, multiplicities by DEPT pulse sequences) δ: 173.53 (*s*, C-1), 67.05 (*t*, C-3), 121.10 (*s*, C-4), 155.15 (*s*, C-5), 31.38 (*t*, C-6), 67.85 (*t*, C-7), 23.38 (*t*, C-8), 73.30 (*s*, C-9), 7.97 (*q*, C-10), 163.57 (*s*, C-11).

6′-Gentisoyl 8-epikingiside (**6**). Gum; $[\alpha]_D^{20}$ $-3°$ (MeOH, *c* 0.002); IR ν_{max}^{KBr} cm^{-1}: 3550–3230 (OH), 1690 (α,β-unsaturated ester); FAB MS (positive) *m/z*: 541 $[M+H]^+$, 405 $[M+H-a]^+$, 243 $[M+H-a-glucosyl]^+$. ^{1}H NMR (500 MHz, CD_3OD) δ: 5.28 (1H, *d*, $J = 7.4$ Hz, H-1), 7.52 (1H, *d*, $J = 1.4$ Hz, H-3), 2.99 (1H, *m*, H-5), 2.18 (1H, *dd*, $J = 16.5$, 11.4 Hz, H-6α), 2.77 (1H, *dd*, $J = 16.5$, 4.2 Hz, H-6β), 4.27 (1H, *dq*, $J = 7.1$, 6.3 Hz, H-8), 2.06 (1H, *ddd*, $J = 7.4$, 7.3, 7.1 Hz, H-9), 1.36 (3H, *d*, $J = 6.3$ Hz, H-10), 3.71 (3H, *s*, OMe), 4.76 (1H, *d*, $J = 7.8$ Hz, H-1′), 3.22 (1H, *dd*, $J = 9.0$, 7.8 Hz, H-2′), 3.42 (1H, *dd*, $J = 9.0$, 9.0 Hz, H-3′), 3.40 (1H, *dd*, $J = 9.0$, 9.0 Hz, H-4′), 3.62 (1H, *m*, H-5′), 4.73 (1H, *dd*, $J = 11.9$, 2.4 Hz, H-6′A), 4.60 (1H, *dd*, $J = 11.9$, 5.9 Hz, H-6′B), 6.78 (1H, *d*, $J = 8.6$ Hz, H-3″), 6.98 (1H, *dd*, $J = 8.6$, 2.8 Hz, H-4″), 7.24 (1H, *d*, $J = 2.8$ Hz, H-6″); ^{13}C NMR: Table 1.

2′-Gentisoyl gelidoside (**7**). Gum; $[\alpha]_D^{20}$ $-8°$ (MeOH, *c* 0.005); IR ν_{max}^{KBr} cm^{-1}: 3580–3240 (OH), 1680 (α,β-unsaturated ester); FAB MS (positive) *m/z*: 931 $[M+K]^+$, 915 $[M+Na]^+$, 893 $[M+H]^+$, 875 $[M+H-H_2O]^+$, 757 $[M+H-a]^+$, 595 $[M+H-b]^+$; ^{1}H NMR (500 MHz, CD_3OD) δ: 5.56 (1H, *d*, $J = 1.2$ Hz, H-1), 7.08 (1H, *s*, H-3), 1.78 (1H, *ddd*, $J = 13.0$, 12.5, 5.1 Hz, H-6α), 1.71 (1H, *br d*, $J = 13.0$ Hz, H-6β), 4.26 (1H, *m*, H-7α), 4.68 (1H, *ddd*, $J = 12.5$, 11.6, 3.2 Hz, H-7β), 5.38 (1H, *ddd*, $J = 17.0$, 10.2, 8.5 Hz, H-8), 2.93 (1H, *dd*, $J = 8.5$, 1.2 Hz, H-9), 5.32 (1H, *dd*, $J = 10.2$, 1.9 Hz, H-10A), 5.27 (1H, *dd*, $J = 17.0$, 1.9 Hz, H-10B), 5.25 (1H, *d*, $J = 8.0$ Hz, H-1′), 5.29 (1H, *dd*, $J = 9.3$, 8.0 Hz, H-2′), 5.79 (1H, *dd*, $J = 9.3$, 9.3 Hz, H-3′), 5.45 (1H, *dd*, $J = 9.3$, 9.3 Hz, H-4′), 4.19 (1H, *m*, H-5′), 4.31 (1H, *dd*, $J = 12.3$, 4.7 Hz, H-6′A), 4.22 (1H, *dd*, $J = 12.3$, 1.8 Hz, H-6′B), 7.48 (1H, *dd*, $J = 8.1$, 1.4 Hz, H-4″), 6.86 (1H, *d*, $J = 8.1$ Hz, H-5″), 7.46 (1H, *dd*, $J = 8.1$, 1.4 Hz, H-6″), 4.84 (1H, *d*, $J = 7.5$ Hz, H-1‴), 3.37 (1H, *dd*, $J = 9.0$, 7.5 Hz, H-

2‴), 3.49 (1H, *dd*, *J* = 9.0, 9.0 Hz, H-3‴), 3.43 (1H, *dd*, *J* = 9.0, 9.0 Hz, H-4‴), 3.38 (1H, *m*, H-5‴), 3.85 (1H, *dd*, *J* = 12.1, 1.5 Hz, H-6‴A), 3.67 (1H, *dd*, *J* = 12.1, 4.9 Hz, H-6‴B), 6.76 (1H, *d*, *J* = 9.0 Hz, H-3⁗), 6.99 (1H, *dd*, *J* = 9.0, 3.0 Hz, H-4⁗), 7.11 (1H, *d*, *J* = 3.0 Hz, H-6⁗), 2.00 (3H, *s*, OAc), 1.86 (3H, *s*, OAc); ^{13}C NMR: Table 2.

Table 2. ^{13}C NMR spectral data of secoiridoid **7** (125 MHz, CD$_3$OD)

C	Macrophylloside*	7	DEPT†
1	100.1	102.2	CH
3	153.1	153.3	CH
4	109.5	109.5	C
5	64.2	64.2	C
6	33.5	33.6	CH$_2$
7	65.8	65.7	CH$_2$
8	133.1	133.1	CH
9	51.8	51.9	CH
10	121.8	121.7	CH$_2$
11	166.8	166.9	C
1′	98.7	98.9	CH
2′	72.6	73.0	CH
3′	70.7	70.4	CH
4′	71.7	72.6	CH
5′	73.3	73.1	CH
6′	63.2	63.1	CH$_2$
1″	114.5	114.6	C
2″	152.6	153.3	C
3″	147.5	147.4	C
4″	124.9	124.9	CH
5″	120.5	120.3	CH
6″	124.2	124.2	CH
7″	169.3	169.2	C
1‴	103.3	103.3	CH
2‴	74.8	74.6	CH
3‴	77.7	77.4	CH
4‴	71.2	71.0	CH
5‴	78.3	78.1	CH
6‴	62.4	62.4	CH$_2$
1⁗	112.7	112.6	C
2⁗	151.7	156.2	C
3⁗	147.3(C)	114.9	CH
4⁗	121.0	119.9	CH
5⁗	120.4(CH)	151.0	C
6⁗	122.8	126.5	CH
7⁗	171.1	170.8	C
OAc	172.2	172.2	C
	171.4	171.1	C
	20.6	20.3	CH$_3$
	20.5	20.1	CH$_3$

* Cited from ref. [1].

† Unless indicated otherwise.

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