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IRIDOIDS AND SECOIRIDOIDS FROM *GENTIANA LINEARIS*

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Key Word Index—*Gentiana linearis*; Gentianaceae; iridoids; secoiridoids; flavonoids; 6‴″-*O*-glucosyltrifloroside; 7-*O*-coumaroyl-loganic acid; 7-*O*-(4″-*O*-glucosyl)coumaroyl-loganic acid; linearoside; 4″-*O*-glucosyllinearoside.

Abstract—Phytochemical investigation of the methanolic extract of *Gentiana linearis* roots led to the isolation of two novel iridoids (7-*O*-coumaroyl-loganic acid; 7-*O*-(4″-*O*-glucosyl)coumaroyl-loganic acid) and a new secoiridoid (6‴″-*O*-glucosyltrifloroside) together with trifloroside, gentiopicroside, isovitexin and isosaponarin. The structures have been elucidated by MS and NMR spectral data, enzymic and chemical degradation. The chemotaxonomic significance of the isolated secoiridoids and iridoids is briefly discussed. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

As a part of phytochemical studies of the Gentianaceae [1, 2], we have investigated *Gentiana linearis* Fröl. This gentian is found in Canada and is one of several species endemic to North America. Among these, *G. andrewsii* has furnished gentiobiose [3]; isoorientin, isoorientin-4′-*O*-glucoside and gentiopicroside have been isolated from both *G. affinis* and *G. calycosa* [4]. *Gentiana linearis* is a narrow-leaved perennial which grows to the north of the St Lawrence River on Laurentian Gneiss, or in peat bogs [5]. To our knowledge, no previous investigations have been done on this species. We report here the isolation and structural elucidation of two novel iridoids and a new secoiridoid.

RESULTS AND DISCUSSION

The powdered roots of *G. linearis* (300 g) were extracted successively with dichloromethane and methanol. The methanol extract (35 g) was analysed by HPLC coupled with diode-array detection, which allowed the identification of a secoiridoid (**1**), flavonoids (**2** and **3**), acyl-iridoids and -secoiridoids (**4**–**7**). Compounds **4** and **5** gave maxima at 314 and 245 nm in the UV spectra which suggested the presence of benzoyl derivatives, while the UV spectra of **6** and **7** gave peaks characteristic for cinnamoyl substituents at 296–313 and 226 nm. Fractionation of the methanolic extract by centrifugal partition chromatography (CPC), gel filtration on Sephadex and chromatography on silica gel afforded two new iridoids (**6**, **7**), a new (**5**) and two known secoiridoids gentiopicroside (**1**) and trifloroside (**4**), the flavone *C*-glycosides isovitexin (**2**) and isosaponarin (**3**). Gentianose was also identified by TLC comparison with an authentic sample.

The structures of **1**, **2** and **3** were deduced by comparison of their UV spectra, retention times on HPLC chromatograms (12.5, 19.5, 14.0 min, respectively), mass spectra and ^{13}C NMR data with those of reference samples [6, 7]. Enzymic hydrolysis of **3** with β-glucosidase gave isovitexin (**2**), and UV spectrometry using shift reagents (NaOMe, NaOAc) showed that the B ring was substituted at C-4′ by glucose [8]. This compound is known as isosaponarin [9], previously isolated from *G. asclepiadea* [10] and *G. cruciata* [11].

The UV spectra of **4** and **5** in MeOH were very similar with maxima at *ca* 314, 246 and 211 nm, typical of a secoiridoid linked to an aromatic ring like gelidoside, in which the secoiridoid-glycoside moiety is esterified with a 2,3-dihydroxybenzoic acid derivative [12]. The negative fast atom bombardment (FAB) mass spectrum of **4** exhibited 3 major peaks at *m/z* 781 $[M-H]^-$, 619 $[M-H-hexose]^-$ and 315. This latter peak corresponded to a dihydroxybenzoyl moiety bearing a hexosyl group. Enzymic hydrolysis of **4** with β-glucosidase gave deglucosyltrifloroside [13]. In conjunction with the ^{13}C NMR spectrum, the molecular formula was established as $C_{35}H_{42}O_{20}$. Comparison of ^{13}C NMR values (Table 1) with literature data allowed identification of **4** as trifloroside [1, 14] which was

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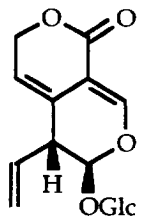

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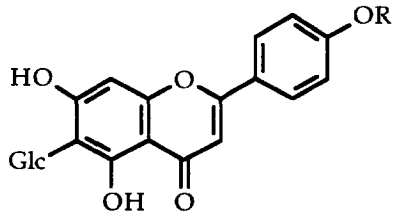

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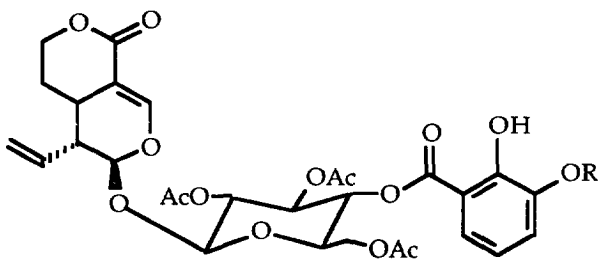
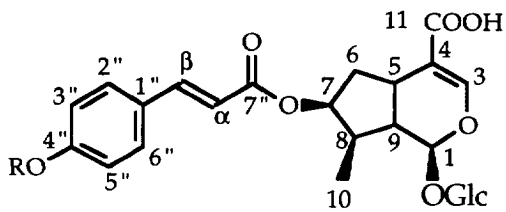
3 R=Glc



4 R=Glc'''
5 R=Glc''''(1''''->6''')Glc'''



6 R=H
7 R=Glc'''

previously isolated from *G. triflora* var. *japonica* [14], *G. scabra* var. *Buergeri* [15, 16], *G. septemfida* [17], *G. macrophylla* [1], *G. olivieri* [18], *G. algida* [19] and *G. gelida* [12].

Negative FAB-MS of **5** gave a molecular ion $[M-H]^-$ at *m/z* 943. The molecular weight was thus 162 amu higher than **4**, corresponding to the presence of an additional hexosyl moiety. The peak at *m/z* 477 was indicative of a dihydroxybenzoyl moiety bearing two hexoses. The sugars were identified as glucose by ^{13}C NMR spectroscopy (Table 2). The C-6 signal of the inner glucosyl (Glc''') moiety in the ^{13}C NMR spectrum of **5** was at 69.8 ppm, characteristic for a $1 \rightarrow 6$ interglycosidic linkage [20]. The corresponding C-6 signal of the second glucosyl moiety was at 62.7 ppm, indicating this to be a terminal sugar. Enzymic hydrolysis of **5** with β-glucosidase followed by HPLC analysis of the products showed that the loss of one glucosyl unit from **5** afforded **4** and a second loss resulted in the formation of deglucosyltrifloroside [13]. This hydrolysis confirmed that **5** possessed one glucosyl unit more than **4**. Compound **5** was thus 6''''-*O*-glucosyltrifloroside.

Compound **6** was obtained in amorphous form. Negative FAB-MS gave a pseudomolecular ion at *m/z* 521 $[M-H]^-$, consistent with the molecular formula of $C_{25}H_{30}O_{12}$. It showed UV maxima at 313 and 229 nm. The ^{13}C NMR spectrum of **6** (Table 2) was very similar to that of loganic acid [21] except for the C-7 signal which was shifted downfield. The downfield shift of C-7 ($\Delta\delta$ = 3.6 ppm) and highfield shifts of C-6 ($\Delta\delta$ = 2.0 ppm) and C-8 ($\Delta\delta$ = 0.9 ppm) were typical of esterification at this position as observed by Calis and Sticher [22]. The ^1H NMR spectrum of **6** showed that the esterification at C-7 was with a *p*-coumaroyl moiety (doublets at 7.47 and 6.81 ppm, and at 7.61 and 6.33 ppm). The value of the coupling constant (J = 16 Hz) of the double bond protons Hα and Hβ indicated *trans*-coumaric acid [23]. Final proof of structure **6** was confirmed by basic hydrolysis which afforded loganic acid and coumaric acid. These products were identified by co-HPLC with reference samples. Thus compound **6**, named linearoside, was shown to be 7-*O*-*p*-coumaroyl-loganic acid. A related compound, 7-*O*-*p*-coumaroyl-loganin, has been isolated from *Curtia tenuifolia* (Gentianaceae) [24].

The FAB-MS of **7** gave a pseudomolecular $[M-H]^-$ ion at *m/z* 683. An ion at *m/z* 521 corresponding to the loss of a hexosyl suggested that **7** had an additional glucose unit when compared with **6**. The ^{13}C NMR spectrum (Table 2) confirmed **7** to be a related derivative of loganic acid esterified at C-7 (78.7 ppm) with *p*-coumaric acid. The 4''-OH position of coumaric acid was substituted by a glucosyl moiety, resulting in a downfield shift of the ^{13}C NMR signal of C-1'' in the *para*-position [20]. Basic hydrolysis of **7** afforded loganic acid and *p*-glucosylcoumaric acid. Hydrolysis of this latter compound with β-glucosidase yielded coumaric acid and glucose. Thus **7** is the 4''-*O*-glucoside of linearoside.

The genus *Gentiana* is divided in 19 sections. *Gentiana linearis* belongs to the section *Pneumonanthe*. Only 12 of the 49 species of this section have been phytochemically investigated. Compounds isolated from gentians in the *Pneumonanthe* section are structurally similar to those present in the following sections: *Frigida*, *Aptera*, *Isomeria* and *Chondrophylla*. These sections do not possess xanthone-*O*-glycosides, with the exception of *Pneumonanthe* (*G. asclepiadea*). However, some species of *Pneumonanthe* and *Aptera* contain mangiferin and its *O*-glycosides. *Gentiana linearis* did not contain xanthones. Secoiridoids are pre-

Table 1. ^{13}C NMR data of compounds **4** and **5** (50 MHz in CD_3OD)

C	4	4*	5
C-1	98.6	98.6	98.6
C-3	153.3†	153.3	153.3†
C-4	106.8	106.8	106.7
C-5	28.7	28.7	28.7
C-6	25.8	25.8	25.8
C-7	70.0	70.0	70.0
C-8	132.7	132.8	132.8
C-9	43.4	43.4	43.4
C-10	121.4	121.4	121.4
C-11	168.0	168.0	168.0
C-1′	97.6	97.6	97.6
C-2′	72.9	73.0	73.0
C-3′	72.2	72.3	72.3
C-4′	71.3	71.3	71.3
C-5′	73.3	73.3	73.3
C-6′	63.3	63.2	63.3
C-1″	114.5	114.5	114.4
C-2″	153.3†	153.4	153.3†
C-3″	147.3	147.4	147.2
C-4″	124.9	124.9	124.8
C-5″	120.3	120.3	120.6
C-6″	124.2	124.2	124.2
C-7″	169.4	169.4	169.5
C-1‴	103.3	103.3	102.9
C-2‴	74.8	74.8	75.2
C-3‴	77.7	77.7	77.6†
C-4‴	70.8	70.9	71.6
C-5‴	78.3	78.3	77.6†
C-6‴	62.4	62.5	69.8
C-1⁗			104.8
C-2⁗			74.8
C-3⁗			78.0†
C-4⁗			71.6
C-5⁗			78.0†
C-6⁗			62.7
OAc	172.2	172.2	172.2
	171.3	171.3	171.4
	171.0	171.0	171.0
	20.6	20.6	20.6
	20.5	20.5	20.5
	20.4	20.4	20.4

* Values from [1].
† Signals interchangeable.

Table 2. Comparison of ^{13}C NMR data of compounds **6** and **7** with loganic acid (50 MHz in CD_3OD)

C	6	7	Loganic acid *
C-1	97.4	97.6	97.6
C-3	151.2	152.7	152.0
C-4	115.0	113.3	114.2
C-5	33.1	32.8	32.7
C-6	40.6	40.5	42.6
C-7	78.6	78.6	75.0
C-8	41.1	41.1	42.0
C-9	47.2	47.1	46.4
C-10	13.8	13.8	13.5
C-11	172.9	170.8	171.4
C-1′	100.1	100.2	99.9
C-2′	74.8	74.8	74.6
C-3′	78.0	77.9	77.9
C-4′	71.6	71.6	71.4
C-5′	78.4	78.3	78.1
C-6′	62.8	62.8	62.7
α	115.4	117.2	
β	146.5	145.9	
C-1″	127.1	129.9	
C-2″	131.2	130.9	
C-3″	116.8	118.0	
C-4″	161.3	160.9	
C-5″	116.8	118.0	
C-6″	131.2	130.9	
C-7″	168.9	168.6	
C-1‴		101.9	
C-2‴		74.8	
C-3‴		78.0	
C-4‴		71.3	
C-5‴		78.3	
C-6‴		62.5	

* Values from [21].

sent in all sections, and acylsecoiridoids such as trifloroside, scabraside, gentomoside and gelidoside have been found in *G. gelida* [12], *G. scabra* [15, 16], *G. triflora* [14], *G. septemfida* (*Pneumonanthe*) [17], *G. macrophylla* [1], *G. olivieri* (*Aptera*) [18], and *G. algida* (*Frigida*) [19]. Loganic acid has been isolated from *G. scabra* [16], *G. septemfida* (*Pneumonanthe*) [17], *G. pedicellata* (*Chondrophylla*) [25, 26] and *G. depressa* (*Isomeria*) [27] and benzoyl loganic acid derivatives from *G. depressa* [27] and *G. pedicellata* [25, 26].

EXPERIMENTAL

General. TLC: Silica gel $60F_{254}$ (Merck), detection UV (254, 366 nm) and Godin reagent. HPLC: Hewlett Packard 1050. UV: Shimadzu, UV-106A spectrophotometer. ^1H and ^{13}C NMR: Varian VXR 200 at 200.06 and 50.03 MHz, respectively, in DMSO-d_6, $CDCl_3$, CD_3OD; TMS as int. standard for ^1H and ^{13}C; multiplicities of ^{13}C were obtained by DEPT experiments. EI-MS, D/CI-MS, FAB: Finnigan MAT-TSQ-700 triple stage quadrupole instrument. Mp uncorr. Mettler-FT-80/82 hot stage apparatus. $[\alpha]_D$: Perkin-Elmer-241 polarimeter.

Plant material. The roots of *G. linearis* were collected in Forêt Montmorency, Quebec (Canada) in October 1995. Voucher specimens (95–628) are deposited at the Herbier Louis-Marie, Laval University, Quebec, Canada.

Extraction and isolation. Dried roots (300 g) were ground and extracted at room temp. successively with CH_2Cl_2 (1.5 l) and MeOH (1.5 l) to yield 12 g of CH_2Cl_2 and 55 g of MeOH extract.

A part of the methanol extract of roots (3 g) was fractionated by centrifugal partition chromatography (CPC) ($CHCl_3$–MeOH–H_2O; 9:12:8 using the lower phase as mobile phase) affording fractions (I–XII). Compound **1** (240 mg) was obtained from fraction VII. Fraction III by silica gel open column chro-

matography ($CHCl_3$–MeOH–H_2O; 9:12:8 (lower phase)) gave **4** (14 mg). Compound **2** (12 mg) was obtained by Sephadex LH-20 gel filtration ($CHCl_3$–MeOH; 1:1) of fraction X. Repeating the fractionation on a larger portion (10 g) of the methanol extract afforded fractions A–I. From C, **5** (14 mg) was obtained by silica gel open column ($CHCl_3$–MeOH–H_2O; 9:12:8 lower phase). Silica gel CC of fractions F and H ($CHCl_3$–MeOH–H_2O; 65:35:5) gave **3** (11 mg), **6** (12 mg) and **7** (9 mg). Compound **7** was treated by ion exchange chromatography on Amberlyst 15 (Fluka). The purity of compounds was checked by HPLC on a Nucleosil RP-18 column (7 μm; 250 × 4 mm i.d., Macherey-Nagel; MeCN–H_2O; 5:95 → 65:35 + 0.05% TFA in 50 min).

Enzymic hydrolysis of **3**. Compound **3** (1 mg) was hydrolysed by β-glucosidase (1 mg) in 1 ml NaOAc 0.5 M buffer (pH = 5) overnight at room temp. Extraction with BuOH and H_2O gave isovitexin and glucose.

4, $C_{35}H_{42}O_{20}$ UV λ_{max}^{MeOH} nm (log ε): 314 (3.48), 245 (3.61), 211 (3.80). FABMS *m/z*: 781 $[M-H]^-$, 619 $[M-H-Glc]^-$, 315 [M-triacylsweroside]$^-$; ^{13}C NMR (Table 1); ^1H NMR (200 MHz, CD_3OD): 7.59 (1H, *d*, *J* = 2.5 Hz, H-3), 7.48 (1H, *dd*, *J* = 7.8, 1.5 Hz, H-4″), 7.44 (1H, *dd*, *J* = 7.6, 1.4 Hz, H-6″), 6.88 (1H, *t*, *J* = 8.2 Hz, H-5″), 2.01 (3H, *s*, AcO), 1.96 (3H, *s*, AcO), 1.91 (3H, *s*, AcO).

Enzymic hydrolysis of **4**. Compound **4** (1 mg) was hydrolysed by β-glucosidase (1 mg) in 1 ml NaOAc 0.5 N buffer (pH = 5) overnight at room temp. The mixture analysed by HPLC showed deglucosyltrifloroside.

5, $C_{41}H_{52}O_{25}$ UV λ_{max}^{MeOH} nm (log ε): 314 (3.27), 246 (3.93), 211 (4.08); $[\alpha]_D$ −9.5° (MeOH, *c* 1.12); FABMS *m/z*: 943 $(M-H]^-$, 781 $[M-H-Glc]^-$, 619 $[M-2Glc]^-$, 577 $[M-2Glc-COCH_3]^-$, 477 [M-triacylsweroside]$^-$; ^{13}C NMR (Table 1); ^1H NMR (200 MHz, CD_3OD): 7.59 (1H, *d*, *J* = 2.5 Hz, H-3), 7.50 (1H, *dd*, *J* = 7.8, 1.2 Hz, H-4″), 7.48 (1H, *dd*, *J* = 7.4, 1.2 Hz, H-6″), 6.91 (1H, *t*, *J* = 8.0 Hz, H-5″), 2.01 (3H, *s*, AcO), 1.96 (3H, *s*, AcO), 1.90 (3H, *s*, AcO).

Enzymic hydrolysis of **5**. Compound **5** (1 mg) was hydrolysed by β-glucosidase (1 mg) in 1 ml NaOAc 0.5 N buffer (pH = 5) overnight at room temp. The mixture analysed by HPLC showed deglucosyltrifloroside.

6, $C_{25}H_{30}O_{12}$ UV λ_{max}^{MeOH} nm (log ε): 313 (4.17), 229 (4.07); FABMS *m/z*: 521 $(M-H]^-$; $[\alpha]_D$ −10.0° (MeOH, *c* 0.96); ^{13}C NMR (Table 2); ^1H NMR (200 MHz, CD_3OD): 7.61 (1H, *d*, *J* = 15.8 Hz, H-β), 7.47 (2H, *d*, *J* = 8.0 Hz, H-2″,6″), 7.44 (1H, *s*, H-3), 6.81 (2H, *d*, *J* = 7.8 Hz, H-3″,5″), 6.33 (1H, *d*, *J* = 15.9 Hz, H-α), 5.26 (2H, *d*, *J* = 4.9 Hz, H-1,7), 4.68 (1H, *d*, *J* = 7.5 Hz, H-1′), 3.91 (1H, *d*, *J* = 11.7 Hz, H-6′), 3.70–3.62 (1H, *m*, H-6′), 3.40–3.13 (4H, *m*, H-2′–H-5′), 2.40–2.30 (1H, *m*, H-9), 2.24–2.17 (1H, *m*, H-8), 2.13–2.02 (1H, *m*, H-6β), 1.88–1.74 (1H, *m*, H-6α), 1.09 (3H, *d*, *J* = 6.6 Hz, H-10).

Alkaline hydrolysis of **6**. A soln of **6** (1 mg) in 0.5 ml MeOH and 1 ml NaOH 0.5 N was kept 4 hr at room temp. and then acidified with H_2SO_4 to pH 5. Loganic acid and *p*-coumaric acid were identified in the solution by co-elution in HPLC with reference samples.

7, $C_{31}H_{40}O_{17}$ UV λ_{max}^{MeOH} nm (log ε): 296 (4.07), 226 (3.98), 211 (3.78); FABMS *m/z*: 683 $[M-H]^-$, 521 $[M-H-Glc]^-$. $[\alpha]_D$ −2.0° (MeOH, *c* 0.90); ^{13}C NMR (Table 2); ^1H NMR (200 MHz, CD_3OD): 7.64 (1H, *d*, *J* = 15.8 Hz, H-β), 7.58 (2H, *d*, *J* = 9.0 Hz, H-2″,6″), 7.33 (1H, *s*, H-3), 7.12 (2H, *d*, *J* = 8.8 Hz, H-3″,5″), 6.42 (1H, *d*, *J* = 15.8 Hz, H-α), 5.25 (2H, *m*, H-1,7), 4.97 (1H, *d*, *J* = 7.6 Hz, H-1‴), 4.68 (1H, *d*, *J* = 7.6 Hz, H-1′), 3.90 (1H, *d*, *J* = 7.6 Hz, H-6′,6‴), 3.79–3.59 (1H, *m*, H-6′,6‴), 3.50–3.13 (4H, *m*, H-2′–5′,2‴–5‴), 2.40–2.30 (1H, *m*, H-9), 2.24–2.17 (1H, *m*, H-8), 2.13–2.06 (1H, *m*, H-6β), 1.88–1.74 (1H, *m*, H-6α), 1.10 (3H, *d*, *J* = 6.8 Hz, H-10).

Hydrolysis of **7**. A soln of **7** (1 mg) in 0.5 ml MeOH and 1 ml NaOH 0.5 N was allowed to stand for 2 hr at room temp. The reaction mixt. was acidified to pH 5 with H_2SO_4. Loganic acid was identified by co-HPLC. The hydrolysate was further hydrolysed by β-glucosidase (1 mg) in 1 ml NaOAc 0.5 N buffer (pH = 5) kept overnight at room temp. The mixture was acidified to pH 5 and extracted with Et_2O to afford coumaric acid and glucose (co-HPLC, UV spectrum).

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