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IRIDOIDS AND FLAVONES FROM *GENTIANA DEPRESSA*

ALBERT J. CHULIA,* JOSEPH VERCAUTEREN† and ANNE MARIE MARIOTTE‡

Biomolécules, Laboratoire de Pharmacognosie et de Phytochimie, UFR de Pharmacie, 2 rue du Docteur Marcland, Université de Limoges, 87025 Limoges Cédex, France; †Laboratoire de Pharmacognosie UFR des Sciences Pharmaceutiques, Bat. 3A zone nord, 141 rue Léo-Saignat, Université de Bordeaux II, 33076 Bordeaux Cédex, France; ‡Laboratoire de Pharmacognosie, UFR de Pharmacie, Université J. Fourier Grenoble I, Domaine de la Merci, 38706 La Tronche Cédex, France

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Key Word Index—*Gentiana depressa*; Gentianaceae; iridoid; secoiridoid; bis-iridoid glucoside; C-glucosylflavones; loganin; depressine; 3″-glucosyl depresteroside; isoscoparin; 2″-glucosyl isovitexin; 2″-glucosyl isoorientin.

Abstract—The dried aerial parts of *Gentiana depressa* were found to contain the known flavones, isoscoparin, 2″-glucosyl isovitexin and 2″-glucosyl isoorientin, the known iridoid loganin, and two new iridoid glucosides: 3″-glucosyl depresteroside and depressine. The last two compounds were characterized at 7-[7-(3-β-glucopyranosyloxy-2-hydroxy-benzoyl)-loganoyl] secologanol and 7-(3-β-glucopyranosyloxy-2-hydroxy-benzoyl) secologanol, respectively, by spectroscopy and chemical transformations.

INTRODUCTION

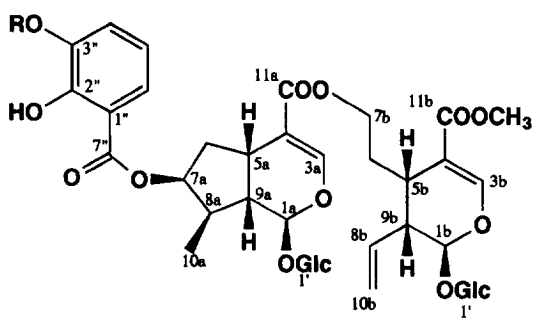
Many species of the Gentianaceae have been investigated chemically and shown to contain C-glucosylflavones, xanthones, and iridoid and secoiridoid glycosides. *Gentiana depressa* D. Don. has been shown to contain depresteroside [1], a bis-iridoid glucoside unusual to the family, in addition to the C-glucosyl 6-flavones isovitexin and isoorientin [2] and the iridoid depressoside [3].

Further chromatographic analysis of extracts from the same plant indicated the presence of several additional flavonoid and iridoid glucosides. In the present paper, we report on the characterization of loganin, isoscoparin, isovitexine 2″-glucoside and isoorientine 2″-glucoside, together with the isolation and the structural elucidation of 3″-glucosyl depresteroside (**1**) and depressine (**2**), two new iridoid and secoiridoid glucosides.

RESULTS AND DISCUSSION

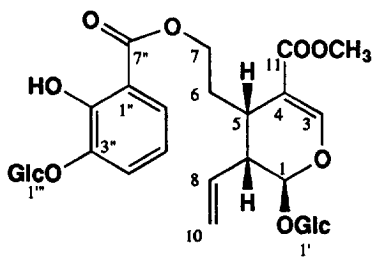
Fractionation of the *n*-butanol-soluble part of the methanol extract of the dried aerial parts of *G. depressa* by column chromatography on Sephadex LH20, gave a mixture of iridoid and flavone glycosides. Purification by LC, CCTLC and HPLC yielded the iridoids loganin, 3″-glucosyl depresteroside (**1**) and depressine (**2**) in the more polar fractions and the flavones 2″-glucosyl isoorientin, 2″-glucosyl isovitexine and isoscoparine in the intermediary polar fractions (see Experimental). The known compounds isoorientin 2″-glucoside, isovitexine 2″-glucoside, isoscoparin and loganin, were identified by their TLC mobilities and their UV, ^{1}H and ^{13}C NMR spectra, in comparison with authentic samples [4–12].

Compound **1** gave a positive sulphuric-vanillin reaction, like depresteroside (**3**), but was more polar (TLC: cellulose and silica gel; reverse-phase HPLC: C18) than **3**. Its molecular formula $C_{46}H_{62}O_{27}$ was based on the FABMS, ^{13}C NMR and ^{1}H NMR spectra. The close similarity between the ^{1}H and ^{13}C NMR data of **1** and depresteroside (**3**), established the presence of an analogous bis-iridoid structure in both products (Tables 1 and 2). Moreover, alkaline hydrolysis of **1** yielded loganic acid, secologanol and the bis-iridoid **4**, as reported for **3** [1]. Acid hydrolysis of **1** yielded glucose and 2,3-dihydroxybenzoic acid. All these compounds were identified by TLC and by their UV and NMR data. When compared with depresteroside (**3**), compound **1** showed one more glucosyl unit which was associated with the aromatic part of the molecule. These structural differences were evident from the UV, MS and NMR data. Thus, on FABMS the quasi-molecular ions at m/z 1069 $[M + Na]^+$, 1047 $[M + H]^-$ and 1045 $[M - H]^-$ were 160 amu higher than those of **3** and there were ions at m/z 561 $[M - 3Glc + H]^+$ and 543 $[M - 3Glc - H_2O + H]^+$. The additional



1 R = Glc(‴)

3 R = H



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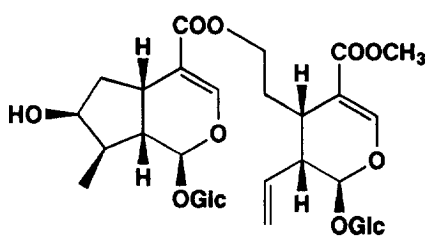

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2) [15, 16]. Furthermore, the lowfield positions of these anomeric nuclei proved its attachment to the aromatic part of the molecule [10]. This was also supported by the presence in the FAB^- mass spectrum, the ion at *m/z* 315 (3-glucosyloxy 2-hydroxy benzoate, base peak). The attachment of the glucosyl to the 3″-position of the benzoyl part was deduced from (i) the deshielding of all the aromatic protons, particularly those in *ortho* ($\Delta\delta_{H\text{-}4''}$ + 0.40) and *para* ($\Delta\delta_{H\text{-}6''}$ + 0.22) positions (Table 1), and by the expected downfield shifts of the C-4″ (+2.8 ppm) and C-6″ (+2.9 ppm) signals (Table 2) relative to those of **3**, (ii) the UV spectrum of **1** and the bathochromic shifts produced by $AlCl_3$ and NaOAc ($\Delta\lambda$ 317 = +33 and +13 nm, respectively), all 7 - [7 - (3 - β - glucopyranosyloxy - 2 - hydroxy - benzoyl) - loganoyl] secologanol, i.e. the 3″-glucosyl depresteroside.

Compound **2** gave a red colour characteristic of a secologanin type structure when sprayed with H_2SO_4-vanillin reagent. It was more polar on TLC than **1**, but exhibited a similar UV spectrum (see Experimental). Acid hydrolysis of **2** yielded glucose and 2,3-dihydroxybenzoic acid, both identified by TLC. The ^1H (^1H–^1H COSY) and ^{13}C NMR (J-mod) spectra of compound **2** (Tables 1 and 2) indicated two mono-substituted β-glucopyranosyl units, one aromatic part and a seco-oiridoid moiety. The two glucosyl groups were in a β configuration (δ 4.91, *d*, *J* = 7.5 Hz, H-1‴; δ 4.70, *d*, *J* = 8 Hz, H-1′) [13, 14]. Comparison of the NMR spectra of **1** and **2** (Tables 1 and 2) showed identical magnetic constants for the aromatic part, establishing the presence of a 3-glucosyloxy-2-hydroxy-benzoyl moiety in **2**. In the ^1H and ^{13}C NMR spectra of **2**, the signals relative to the terpenoid part revealed a secologanol-like structure. The point of esterification in the secologanol moiety was shown to be at the C-7 oxygen by the downfield shift of the C-7 protons (+0.87 ppm) and of the C-7 signal (+4.9 ppm), when compared with those of secologanol [20, 21]. From the evidence above, **2** was identified as 7-(3-β-glucopyranosyloxy-2-hydroxy-benzoyl) secologanol, a new natural product, and named depressine.

EXPERIMENTAL

Plant material. See ref. [1].

General procedures. See ref. [1]. ^1H NMR: 400 and 500 MHz; ^{13}C NMR: 100 and 125 MHz. *J* (Hz) of the different ^1H multiplets were determined after selective irradiation.

Extraction and purification. The air-dried aerial parts of *G. depressa* (100 g) were first defatted with *n*-hexane and then extracted as reported in [2]. The *n*-BuOH ext. (11 g) was fractionated by CC on Sephadex LH20 eluting with MeOH [1]. The frs were monitored by TLC on silica gel ($CHCl_3$–MeOH, 17:3). Seven frs were collected, A_1–A_3 (4.6 g.), A_4 (1.4 g.), A_5 (1.9 g.), A_6 (1.5 g.) and A_7 (0.4 g). Loganin and compounds **1** and **2** were localized in fr. A_4. This fr. was subjected to silica gel CCTLC ($CHCL_3$ with MeOH gradient) when compounds **2**, loganin and **1** were eluted, in this order, with $CHCl_3$–MeOH (4:1) as eluent. After filtration on Sephadex LH20 (MeOH), 200 mg of pure loganin was obtained by semi-prep. HPLC (Lichrosorb RP18, MeOH–H_2O, 2:3). The final purification of **1** and **2** was performed by semi-prep. HPLC (Novapak C18 cartridge) with MeOH–H_2O (3:7 and 2:3) as mobile phase for **1** and **2**, respectively. This gave 20 mg **1** and 1.5 mg **2**. Fr. A_6 was chromatographed on Sephadex LH20 (MeOH) to give three new frs; from the first fr. 2″-glucosyl isovitexine (18 mg)

Table 1. ^{1}H NMR data of compounds **1**, **2** and **3** in CD_3OD*

H	1	2	3†
Iridoid part			
Aglycone			
1a	5.33 *d* (4.5)		5.34 *d* (4.5)
3a	7.47 *d* (1)		7.47 *d* (1)
5a	3.21 *m* (9–8.5–8–1)		3.20 *m* (9–8.5–8–1)
6a	2.48 *br dd* (14–8.5–0.5)		2.46 *m* (14–8.5–0.7)
	1.88 *m* (14–8–5.5)		1.89 *m* (14–8–5)
7a	5.48 *m* (5.5–4.5–0.5)		5.45 *br ddd* (5–4.5–0.7)
8a	2.24 *m* (8.7–6.7–4.5)		2.23 *m* (8.5–6.5–4.5)
9a	2.18 *ddd* (9–8.7–4.5)		2.18 *ddd* (9–8.5–4.5)
10a	1.16 *d* (6.7)		1.14 *d* (6.5)
Sugar			
1'a	4.68 *d* (8)		4.69 *d* (8)
2'a–5'a	*ca* 3.21–3.40 *m*		*ca* 3.21–3.40 *m*
6'a	*ca* 3.92–3.62 *m*		3.92[a] *dd* (12–2)
			3.68[b] *dd* (12–6.5)
Secoiridoid part			
Aglycone			
1b	5.53 *d* (6.5)	5.59 *d* (6.6)	5.54 *d* (6)
3b	7.44 *d* (0.5)	7.50 *d* (0.5)	7.44 *d* (0.5)
5b	2.89 *br td* (7–7–5.5)	2.98 *br td* (7.3–7–5.5–0.5)	2.89 *m* (7.5–7–5.5–0.5)
6b	2.00 *m* (13.5–7–6.5–6)	2.14 *m* (13–7–6.5–6)	2.01 *m* (13.5–7–6.5–6.2)
	1.68 *m* (13.5–7–7–5.5)	1.99 *m* (13–7.3–7–5.5)	1.79 *m* (13.5–7.5–7–5.5)
7b	4.18 *m* (12–6–5.5)	4.42 *m*	4.18 *ddd* (12–6.2–5.5)
	4.13 *m* (12–7–6.5)		4.11 *ddd* (12–7–6.5)
8b	5.74 *ddd* (17–11–8.5)	5.82 *ddd* (17–10–8.5)	5.75 *ddd* (17.5–11.5–8.5)
9b	2.61 *m* (8.5–6.5–5.5)	2.69 *ddd* (8.5–6.6–5.5)	2.63 *ddd* (8.5–6–5.5)
10b	5.26 *dd* (17–1)	5.32 *dd* (17.4–1.2)	5.27 *dd* (17.5–1.5)
	5.20 *dd* (11–1)	5.27 *dd* (10.6–1.2)	5.21 *dd* (11.5–1.5)
MeO	3.65 *s*	3.63 *s*	3.65 *s*
Sugar			
1'b	4.71 *d* (8)	4.70 *d* (8)	4.72 *d* (8)
2'b–5'b	*ca* 3.20–3.40 *m*	*ca* 3.20–3.40 *m*	*ca* 3.21–3.40 *m*
6'b	*ca* 3.92–3.62 *m*	3.89[a] *dd* (11.8–1.9)	3.89[a] *dd* (12–1.5)
		3.68[b] *dd* (11.8–6.5)	3.66[b] *dd* (12–6)
Benzoic acid part			
4''	7.43 *dd* (8–1)	7.41 *dd* (8.3–1.9)	7.03 *dd* (8–1.5)
5''	6.90 *t* (8)	6.88 *t* (8.3)	6.77 *t* (8)
6''	7.56 *dd* (8–1)	7.55 *dd* (8.3–1.9)	7.34 *dd* (8–1.5)
Sugar			
1'''	4.92 *d* (7)	4.91 *d* (7.5)	
2'''–5'''	*ca* 3.20–3.40 *m*	*ca* 3.20–3.40 *m*	
6'''	*ca* 3.92–3.62 *m*	3.89[a] *dd* (11.8–1.9)	
		3.64[b] *dd* (12–6.5)	

*At 400 MHz for **1**, and 500 MHz for **2** and **3** (CD_3OD, δ 3.27).

†Taken from ref. [1].

[a,b]Values with the same symbol in one column are interchangeable.

isoscoparine (6 mg) after semi-prep. HPLC (Lichrosorb RP 18, MeOH–H_2O, 3:6).

7-[7-(3-*β*-*Glucopyranosyloxy*-2-*hydroxy*-*benzoyl*)-*loganoyl*] *secologanol* (3''-*glucosyl depresteroside*) (**1**). UV λ_{Max}^{MeOH} nm: 220, 235 sh, 277 sh, 317; $AlCl_3$ 225, 270, 350; $AlCl_3$ + HCl 277, 270 sh, 317, 350 sh; NaOAc 225, 250 sh, 277 sh, 330; NaOAc + H_3BO_3 225, 250 sh, 280 sh, 315; ^{1}H and ^{13}C NMR: Tables 1 $H_2O + H]^+$, 673 $[M - 2Glc - H_2O - CH_3OH + H]^+$, 561 $[M - 3Glc + H]^+$, 543 $[M - 3Glc - H_2O + H]^+$, 495 $[M - secologanol + H]^+$, 477 $[M - secologanol - H_2O + H]^+$ 373 $[M - (2\text{-hydroxy 3-glucosyl benzoyl-7-loganoate}) + H]^+$, 333 $[M - secologanol - Glc + H]^+$, 315 $[M - secologanol - OGlc + H]^+$, 229, 211, 179; FAB$^-$MS [glycerine]: *m/z* 1045 $[M - H]^-$, 883 $[M - Glc - H]^-$, 721 $[M - 2Glc - H]^-$, 703 $[M -$

Table 2. ^{13}C NMR data of compounds **1**, **2** and **3** in CD_3OD*

C	1	2	3†
Iridoid part			
Aglycone			
1a	97.7		97.6
3a	152.7		152.5
4a	115.1		114.1
5a	32.7		32.7
6a	40.5		40.5
7a	80.3		80.1
8a	41.2		41.2
9a	47.2		47.1
10a	13.7		13.8
11a	169.2		168.9
Sugar			
1'a	100.3[a]		100.1
2'a	74.7[b]		74.8[a]
3'a	77.7		78.0[b]
4'a	71.6[c]		71.6
5'a	78.3[d]		78.4
6'a	62.8[e]		62.8
Secoiridoid part			
Aglycone			
1b	97.7	98.0	97.7
2b	153.6	153.9	153.7
4b	111.5	108.0	111.4
5b	31.3	30.0	31.2
6b	30.1	31.7	30.0
7b	63.5	66.4	63.6
8b	135.6	136.9	135.7
9b	45.3	46.5	45.2
10b	119.5	118.0	119.6
11b	168.8	169.2	169.2
OMe	51.8	50.2	51.9
Sugar			
1'b	100.2[a]	101.0	100.3
2'b	74.7[b]	75.1	74.7[a]
3'b	77.7	77.7[a]	77.9[b]
4'b	71.6[c]	71.9	71.6
5'b	78.3[d]	78.3[b]	78.4
6'b	62.8[e]	62.8[c]	62.8
Benzoic acid part			
1''	113.2	113.0	113.2
2''	153.0	152.7	151.5
3''	147.3	147.4	147.2
4''	124.6	125.0	121.8
5''	120.1	120.3	120.1
6''	124.0	124.2	121.1
7''	170.8	170.9	171.3
Sugar			
1'''	103.3	104.3	
2'''	74.8[b]	75.1	
3'''	77.7	77.8[a]	
4'''	71.4[c]	71.9	
5'''	77.9[d]	78.4[b]	
6'''	62.5[e]	62.7[c]	

*At 100 MHz for **1** and 125 MHz for **2** and **3** (CD_3OD, δ 49.0).

†Taken from ref. [1].

behaviour: R_t 17 min. (Novapak C18 cartridge MeCN–H_2O, 1:4, 1 ml × min^{-1}), R_f 0, 15 (silica gel $CHCl_3$–MeOH, 17:3).

Acid hydrolysis of **1**. Compound **1** (3 mg) was dissolved in 2M HCl and heated under reflux at 100° for 1 hr. After concentn, the residue was examined for sugars by TLC [22] and for 2,3-dihydroxybenzoic acid by cellulose TLC (AcOH 5% aq.) and HPLC (C_{18} Novapak MeCN–H_2O, 1:9) in comparison with authentic samples.

Alkaline hydrolysis of **1**. Compound **1**, 12 mg, was treated as described in ref. [1]. After 20 min, the soln was neutralized by a cation-exchange resin (DOWEX 50). Pure compound **4** (2 mg) was obtained by semi-prep. HPLC [1], and examined by ^1H and ^{13}C NMR. Total alkaline hydrolysis of **1**, carried out under the same conditions, was obtained after 75 min. This procedure yielded loganic acid and secologanol. These compounds were identified by comparison with authentic samples (TLC, UV, ^1H and ^{13}C NMR).

7-(3-*β*-*Glucopyranosyloxy*-2-*hydroxy*-*benzoyl*) *secologanol* (*depressine*) (**2**). UV λ_{Max}^{MeOH} nm: 220, 235, 317; $AlCl_3$ 225, 267, 350; ^1H NMR and ^{13}C NMR: Tables 1 and 2. R_t 12.5 min; (Novapak C18 cartridge, MeCN–H_2O, 1:4, 1 ml × min^{-1}), R_f 0.30 (silica gel, $CHCl_3$–MeOH, 17:3).

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