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LIGNAN BIS-GLUCOSIDES FROM *GALIUM SINAICUM*

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Key Word Index—*Galium sinaicum*; Rubiaceae; roots; lignan glycosides; cytotoxic activity.

Abstract—The new lariciresinol-based lignan bis-glucosides, 7*S*, 8*R*, 8′*R*-(−)-lariciresinol-4,4′-bis-*O*-*β*-D-glucopyranoside and 7*S*, 8*R*, 8′*R*-(−)-5-methoxylariciresinol-4,4′-bis-*O*-*β*-D-glucopyranoside, together with (−)-syringaresinol-4,4′-bis-*O*-*β*-D-glucopyranoside were isolated from the *n*-butanol extract of *Galium sinaicum* roots and their structures were established by various spectroscopic techniques. The isolated compounds represent the first report of lignan glycosides from the Rubiaceae. The two lariciresinol-type glucosides exhibited weak cytotoxic activity against the P388 cell line. ©1997 Elsevier Science Ltd. All rights reserved

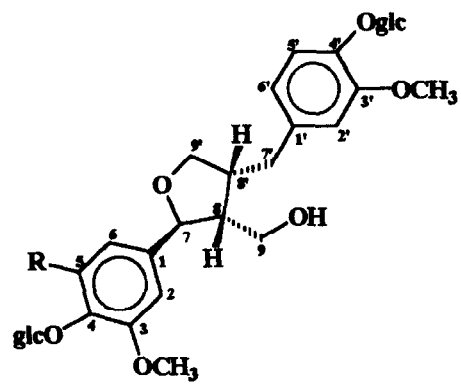
INTRODUCTION

In previous studies [1–3], a total of 30 anthraquinones were isolated from the *n*-hexane, Et_2O, CH_2Cl_2 and *n*-BuOH extracts of *Galium sinaicum* roots, of which 20 new ones were reported. In the present study, the early fractions of the *n*-BuOH extract were investigated for their non-anthraquinone content and the structural elucidation of the new isolated compounds is discussed.

RESULTS AND DISCUSSION

From the early fractions of the *n*-BuOH extract, two new lariciresinol-based lignan bis-glucosides were isolated and identified as 7*S*, 8*R*, 8′*R*-(−)-lariciresinol-4,4′-bis-*O*-*β*-D-glucopyranoside (**1**) and 7*S*, 8*R*, 8′*R*-(−)-5-methoxylariciresinol-4,4′-bis-*O*-*β*-D-glucopyranoside (**2**). (−)-Syringaresinol-4,4′-bis-*O*-*β*-D-glucopyranoside (**3**) [4] was also characterized. The three compounds were generally characterized by strong and broad IR bands at *ca.* 3400 and 1226–1240 cm^{-1} for free OH and C-O stretches, together with the aromatic skeletal bands (C=C) at 1595 and 1515 cm^{-1}. The corresponding sugar and aglycone residues were readily liberated by enzymatic hydrolysis with *β*-glucosidase enzyme suggesting that all were *O*-*β*-glucosides.

The FAB-mass spectrum of **1** displayed a $[M+Na]^+$ at *m/z* 707 and the fragment ions at *m/z* 545 $[M-glc+Na]^+$ and 361 $[M-2glc+H]^+$ corresponding with the successive loss of one and two glucosyl moieties. The ^1H NMR data of **1** and its aglycone **1A** (Table 1) disclosed a substituted diaryl epoxylignan skeleton [5–8] characterized by the presence of only one downfield-shifted benzylic methine proton (H-7), one benzylic methylene proton (H_2-7′) and a primary carbinol methylene (H_2-9). The diaryl substituents showed the 1,3,4-trisubstitution pattern by two sets of resonances, each of three coupled aromatic protons. Such an NMR pattern, together with two OMe resonances and two anomeric protons (H-1″, H-1‴) with diaxial coupling, suggested that the diaryl substituents are two identical guaiacyl-4-*O*-*β*-D-glucopyranosides. It could, therefore, be concluded that the aglycone moiety of (**1**) is lariciresinol [5, 7, 9–11]. The 2D NOESY established the *cis*-configuration of H-8 and H-8′ and the *trans*-orientation of H-7 and



R = H **(1)**
R = OCH_3 **(2)**

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Table 1. ^{1}H NMR (400 MHz, DMSO-d_6) spectral data of compounds **1–3** and the aglycones **1A** and **3A**

Proton	δ_H (ppm, J in Hz)*				
	1	**1A**†	**2**	**3**	**3A**†
H-2	6.88	6.70	6.59 *s*	6.65 *s*	6.58 *s*
	d (1.6)	*d* (1.8)	—	—	—
H-5	7.02	6.87	—	—	—
	d (8.3)	*d* (8.3)	—	—	—
H-6	6.78	6.83	6.59 *s*	6.65 *s*	6.58 *s*
	dd (8.3, 1.6)	*dd* (8.3, 1.8)	—	—	—
H-7	4.72	4.79	4.72	4.66	4.73
	d (6.0)	*d* (6.8)	*d* (5.7)	*d* (3.8)	*d* (4.4)
H-8	2.20	2.42	2.23	3.09 *m*	3.09 *m*
	dddd (6.0)	*dddd* (7.0)	*dddd* (5.9)	—	—
H_a-9	3.44 *m*	3.75	3.51 *m*	3.82	3.89‡
	—	*dd* (11.0, 6.2)	—	*dd* (9.1, 3.3)	—
H_b-9	3.67 *m*	3.92	3.66 *m*	4.20	4.28
	—	*dd* (11, 7.0)	—	*dd* (9.1, 6.6)	*dd* (9.1, 6.9)
H-2′	6.82	6.68	6.82	6.65 *s*	6.58 *s*
	d (1.6)	*d* (1.8)	*d* (1.8)	—	—
H-5′	6.98	6.85	6.97	—	—
	d (8.3)	*d* (8.3)	*d* (8.4)	—	—
H-6′	6.68	6.81	6.69	6.65 *s*	6.58 *s*
	dd (8.3, 1.6)	*dd* (8.3, 1.8)	*dd* (8.4, 1.8)	—	—
H_a-7′	2.47	2.56	2.47	—	—
	dd (13.5, 11.0)	*dd* (13.5, 10.7)	*dd* (13.5, 11.0)	—	—
H_b-7′	2.86	2.92	2.83	4.66	4.73
	dd (13.5, 4.4)	*dd* (13.5, 5.2)	*dd* (13.5, 4.5)	*d* (3.8)	*d* (4.4)
H-8′	2.61 *m*	2.73 *m*	2.60 *m*	3.09 *m*	3.09 *m*
H_a-9′	3.58	3.79	3.58	3.82	3.89†
	t (7.3)	*dd* (8.6, 6.1)	*t* (7.3)	*dd* (9.1, 3.3)	—
H_b-9′	3.90	4.06	3.90	4.20	4.28
	t (7.3)	*dd* (8.6, 6.6)	*t* (7.3)	*dd* (9.1, 6.6)	*dd* (9.1, 6.9)
H-1″	4.83	—	4.84	4.87	—
	d (7.5)	—	*d* (7.6)	*d* (7.2)	—
H-1‴	4.86	—	4.86	4.87	—
	d (7.5)	—	*d* (7.6)	*d* (7.2)	—
OMe-3	3.75 *s*	3.89 *s*	3.74 *s*	3.75 *s*	3.90 *s*
OMe-5	—	—	3.74 *s*	3.75 *s*	3.90 *s*
OMe-3′	3.75 *s*	3.89 *s*	3.74 *s*	3.75 *s*	3.90 *s*
OMe-5′	—	—	—	3.75 *s*	3.90 *s*

* Coupling constants (J) in Hz are given in parentheses.
† Measured in $CDCl_3$.
‡ J values could not be calculated due to overlapping with OMe resonance.

H-8. The disposition of the two OMe groups at C-3 and C-3′, as well as the glycosylation sites at C-4 and C-4′ were indicated by cross-peaks between the two OMe groups and their vicinal protons H-2 and H-2′, as well as between the two anomeric protons and the two aromatic ones H-5 and H-5′, respectively. The ^{13}C NMR data (Table 2) and DEPT experiments revealed the basic C_{18}-lignan skeleton of the 7,9′-monoepoxy type [12] characterized by three signals ascribed for C-7, C-7′ and C-9. These signals, together with those of the guaiacyl groups and the two anomeric carbons C-1″ and C-1‴, are in accordance with a lariciresinol diglucoside structure. The HMQC and HMBC illustrated the key connectivities between the two diaryl substituents and the central tetrahydrofuran ring through two- and three-bond correlations from H-7 to C-1, C-2 and C-6 and from H_a-7 to C-1′, C-2′, C-6′ and C-8′. Significant correlations were also observed from H-7 to C-9, from H-1″ to C-4′ and from H-1‴ to C-4.

Since no NOESY correlations were observed between H-7 and H-8 and the NMR signals due to the aliphatic part of **1** closely resembled those of lariciresinol-4-*O*-β-D-glucoside, lariciresinol-4′-*O*-β-D-glucoside [10], lariciresinol-4,4′-bis-*O*-α-L-rhamnoside and 5,5′-dimethoxylariciresinol-4,4′-bis-*O*-α-L-rhamnoside [7], the aryl substituent at C-7 must therefore be axially oriented. Moreover, the fact that all five compounds are laevorotatory, having identical relative configurations, indicated their common absolute configuration. Therefore, compound **1** is identified as 7*S*, 8*R*, 8′*R*-(−)-lariciresinol-4,4′-bis-*O*-β-D-glucopyranoside.

The FAB-mass spectrum of **2** displayed a

Table 2. ^{13}C NMR (100 MHz, DMSO-d_6) spectral data of compounds 1–3

Carbon	δ_0 (ppm)* 1	2	3
C-1	137.6 *s*	133.5 *s*	133.9 *s*
C-2	110.1 *d*	103.9 *d*	104.3 *d*
C-3	148.8 *s*	152.5 *s*	152.7 *s*
C-4	145.5 *s*	139.8 *s*	137.2 *s*
C-5	115.1 *d*	152.5 *s*	152.7 *s*
C-6	117.7 *d*	103.9 *d*	104.3 *d*
C-7	81.6 *d*	81.8 *d*	85.1 *d*
C-8	52.4 *d*	52.2 *d*	53.7 *d*
C-9	58.6 *t*	58.7 *t*	71.4 *t*
C-1′	134.6 *s*	134.7 *s*	133.9 *s*
C-2′	113.1 *d*	113.1 *d*	104.3 *d*
C-3′	148.8 *s*	148.8 *s*	152.7 *s*
C-4′	144.8 *s*	144.8 *s*	137.2 *s*
C-5′	115.3 *d*	115.3 *d*	152.7 *s*
C-6′	120.3 *d*	120.4 *d*	104.3 *d*
C-7′	32.1 *t*	32.1 *t*	85.1 *d*
C-8′	41.8 *d*	41.8 *d*	53.7 *d*
C-9′	71.9 *t*	72.0 *t*	71.4 *t*
OMe-3	55.7 *q*	56.4 *q*	56.5 *q*
OMe-5	—	56.4 *q*	56.5 *q*
OMe-3′	55.7 *q*	55.7 *q*	56.5 *q*
OMe-5′	—	—	56.5 *q*
C-1″	100.3 *d*	100.2 *d*	102.8 *d*
C-2″	73.2 *d*	73.2 *d*	74.2 *d*
C-3″	76.8 *d*	76.5 *d*	76.5 *d*
C-4″	66.9 *d*	69.7 *d*	70.0 *d*
C-5″	77.0 *d*	76.4 *d*	77.2 *d*
C-6″	60.6 *t*	60.7 *t*	61.0 *t*
C-1‴	100.2 *d*	102.8 *d*	102.8 *d*
C-2‴	73.2 *d*	74.2 *d*	74.2 *d*
C-3‴	76.8 *d*	76.8 *d*	76.5 *d*
C-4‴	69.7 *d*	69.9 *d*	70.0 *d*
C-5‴	77.0 *d*	77.1 *d*	77.2 *d*
C-6‴	60.7 *t*	60.9 *t*	61.0 *t*

* ^{13}C Multiplicities determined by DEPT pulse sequence.

$[M+Na]^+$ at *m/z* 737, 30 mu more than that of **1**. The ^1H NMR data (Table 1) showed, like **1**, an identical splitting pattern for a 7,9′-monoepoxy lignan-type, but the diaryl substituents showed the 1,3,4- and 1,3,4,5-substitution patterns by two sets of resonances ascribed for three coupled protons (H-2′, H-5′, H-6′) and two proton singlet (H-2, H-6), respectively. Such a splitting pattern together with the three OMe resonance and two anomeric protons (H-1″, H-1‴) with diaxial coupling suggested that compound **2** is the methoxyl derivative of **1**. The ^{13}C NMR data (Table 2) showed, for compounds **2** and **1**, identical carbon resonances for both the guaiacyl derivative and the central epoxy structure, while those of the second aryl substituent of **2** displayed a strong downfield-shift for C-5 (+37.4) and upfield-shifts for the *o*- and *p*-carbons (C-6, −13.8; C-4, −5.7; C-2, −6.2). The 2D NOESY established the same relative configuration as in **1** and located the third OMe at C-5 through a cross-peak with H-6. As the chemical shift of C-1 is sensitive both to change in substituents on the aromatic ring and to their stereochemistry [13–15], comparison between the chemical shift of C-1 (δ 133.9) of the axially-oriented syringyl grouping of (−)-syringaresinol-4,4′-bis-*O*-β-D-glucopyranoside (**3**) with that of **2** (δ 133.5) (Table 2), alongside with the above NOESY observations, confirmed the location of the syringyl grouping in compound **2** at C-7 and it must therefore be axially-oriented. The HMQC and HMBC confirmed the proposed structure of **2** through two and three bond correlations from H-7 to C-2 and C-6, from OMe groups to C-3, C-5 and C-3′ and from H-2 (6) to C-7, C-6 (2), C-1 and C-3 (5). Based upon the above spectral findings, compound **2** is the 5-methoxyl derivative of **1** and because both compounds are laevorotatory and having identical relative configurations, compound **2** is identified as 7*S*,8*R*,8′*R*-(−)-5-methoxylariciresinol-4,4′-bis-*O*-β-D-glucopyranoside.

The two lignan glucosides **1** and **2** were subjected to cytotoxicity bioassay against the P388 leukemia cell line [16, 17]. The tested compounds exhibited weak, *in vitro*, cytotoxic activity with IC_{50} values of 100 and 42.0 μg ml^{-1}, respectively.

It is of interest to note that very few lignans have been reported from the Rubiaceae. (+)-Syringaresinol, (±)-dilignans and sesquilignans were isolated from the leaves of *Hedyotis lawsoniae* [18] and (+)-isolariciresinol was reported from the whole plant of *Rubia akane* [19]. The present isolation of (−)-lariciresinol-4-4′-bis-*O*-glucoside (**1**), (−)-5-methoxylariciresinol-4-4′-bis-*O*-glucoside (**2**) and (−)-syringaresinol-4,4′-bis-*O*-glucoside (**3**) represents the first report of lignan glycosides from the Rubiaceae. Whereas the 4-, 4′- and 9-monoglucosides of lariciresinol [5, 10], as well as the 4,4′-dirhamnosides of lariciresinol and 5,5′-dimethoxylariciresinol [7] are known natural products, (−)-lariciresinol-4,4′-bis-*O*-glucoside (**1**) and (−)-5-methoxylariciresinol-4,4′-bis-*O*-glucoside (**2**), reported in the present study, are hitherto unreported lignan glycoside. However, (+)-lariciresinol-4,4′bis-*O*-glucoside (clemastanin B) was recently reported, during the preparations of this paper, from the roots of *Clematis stans* (Ranunculaceae) [20]. (−)-Syringaresinol-4,4′-bis-*O*-glucoside **3** was previously isolated from some Araliaceous plants [21] and, recently, from *Albizzia julibrissin* (Leguminosae) [4].

EXPERIMENTAL

General. Mps: uncorr. $[\alpha]_D^{26.5}$ pyridine. ^1H and ^{13}C NMR: DMSO-d_6 or $CDCl_3$ with TMS as int. standard, Bruker AM400. HMQC, HMBC, NOESY, Bruker AM500. FAB-MS: 8kV, glycerol. IR: KBr. UV: MeOH. CC: Diaion HP-20 and Kieselgel 60. TLC: silica gel 60 F_{254} plates; detection UV at 254 nm and spraying with 10% H_2SO_4 in MeOH. Cytotoxicity bioassay: Tosoh MPR A4*i* microplate reader.

Plant material. Refer to ref. [2]. Herbarium voucher, 101 St C./1991

Extraction and isolation. Successive *n*-BuOH extraction of 11 g of *G. sinaicum* roots, remaining after extraction with *n*-hexane and CH_2Cl_2 [2, 3], was coarsely fractionated over a Diaion HP-20 column eluted with a H_2O–MeOH gradient to give 3 frs, A (0.5 g), B (0.6 g) and C (1.4 g). CC of A on Kieselgel 60 (50 g, 2.5 cm i.d.) eluted with $CHCl_3$–MeOH–H_2O (70:30:3) afforded **1** (15 mg) and **2** (6 mg). CC of B on Kieselgel 60 (60 g, 2.5 cm i.d.) eluted with $CHCl_3$–MeOH–H_2O (85:15:1.5) afforded **3** (10 mg). Fr. C was saved for further investigation.

Enzymatic hydrolysis. Aq. solns of **1** and **3** (5 mg in 3 ml H_2O) were incubated overnight with β-glucosidase (10 mg) at 37°. Hydrolysates were dild with H_2O and extracted with *n*-BuOH. The obtained extracts were concd to small vol. and then purified on silica columns eluted with $CHCl_3$–MeOH–H_2O (14:2:3) [7, 10]. The aglycones-containing frs were evapd to dryness and dissolved in MeOH to afford white amorphous powders of the corresponding aglycones **1A** and **3A**. The remaining aq. mother liquors were evapd to dryness, dissolved in pyridine and the identity of the liberated sugar with D-glucose was confirmed by TLC on silica gel against a ref. sample using *n*-BuOH–pyridine–HOAc–EtOAc–H_2O (5:2:1:2.5:5:2, double run).

7S,8R,8′R-(−)-*Laricinesinol*-4,4′-*bis*-O-β-D-*glucopyranoside* (**1**). White amorphous powder (15 mg, MeOH), mp, 200–203°. $[\alpha]_D^{26.5}$ −50.0° (pyridine; *c* 0.16). R_f 0.28 in $CHCl_3$–MeOH–H_2O (70:30:3). UV λ_{max}^{MeOH} nm: 228, 276. IR ν^{KBr} cm^{-1}: 3402 (OH), 1597, 1516 (C = C), 1226, 1077. FAB-MS *m/z* (rel. int): 707 $[M+Na]^+$ (51) calcd for $C_{32}H_{44}O_{16}$, 545 $[M-glc+Na]^+$ (12), 523 $[M-glc+H]^+$ (9), 522 $[M-glc]^+$ (9), 399 $[M-2glc+K]^+$ (47), 361 $[M-2glc+H]^+$ (18), 360 $[M-2glc]^+$ (52). ^1H NMR (400 MHz, DMSO-d_6): Table 1. ^{13}C NMR (100 MHz, DMSO-d_6): Table 2.

7S,8R,8′R-(−)-5-*Methoxylaricinesinol*-4,4′-*bis*-O-β-D-*glucopyranoside* (**2**). White amorphous powder (6 mg, MeOH), mp, 139–140°. $[\alpha]_D^{26.5}$ −40.0° (pyridine; *c* 0.29). R_f 0.24 in $CHCl_3$–MeOH–H_2O (70:30:3). UV λ_{max}^{MeOH} nm: 230, 280. IR ν^{KBr} cm^{-1}: 3400 (OH), 1595, 1511 (C = C), 1228, 1125, 1072. FAB-MS *m/z* (rel. int.): 737 $[M+Na]^+$ (47) calcd for $C_{33}H_{46}O$, 575 $[M-glc+Na]^+$ (12), 413 $[M-2glc+Na]^+$ (26), 391 $[M-2glc+H]^+$ (54), 390 $[M-2glc]^+$ (38). ^1H NMR (400 MHz, DMSO-d_6): Table 1. ^{13}C NMR (100 MHz, DMSO-d_6): Table 2.

(−)-*Syringaresinol*-4,4′-*bis*-O-β-D-*glucopyranoside* (**3**). White amorphous powder (10 mg, MeOH), mp, 245–248°. $[\alpha]_D^{26.5}$ −10.7° (pyridine, *c* 0.13). R_f 0.52 in $CHCl_3$–MeOH–H_2O (70:30:3). UV λ_{max}^{MeOH} nm: 240, 270. IR ν^{KBr} cm^{-1}: 3400 (OH), 1595, 1515 (C = C), 1240, 1137, 1090. FAB-MS *m/z* (rel. int.): 765 $[M+Na]^+$ (35) calcd for $C_{34}H_{46}O_{18}$, 626 $[M-glc+2Na]^+$ (12), 619 $[M-glc+K]^+$ (8), 603 $[M-glc+Na]^+$ (7), 480 $[M-2glc+Na+K]^+$ (10), 441 $[M-2glc+Na]^+$ (9), 418 $[M-2glc]^+$ (20). ^1H NMR (400 MHz, DMSO-d_6): Table 1. ^{13}C NMR (100 MHz, DMSO-d_6): Table 2.

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