

LIGNAN BIS-GLUCOSIDES FROM *GALIUM SINAICUM*

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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

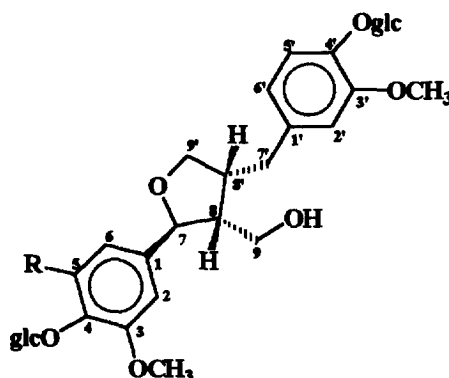
INTRODUCTION

In previous studies [1–3], a total of 30 anthraquinones were isolated from the *n*-hexane, Et₂O, CH₂Cl₂ 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

**R = H** (1)**R = OCH₃** (2)

glucosyl moieties. The ¹H NMR data of **1** and its aglycone **1A** (Table 1) disclosed a substituted diaryl epoxy lignan skeleton [5–8] characterized by the presence of only one downfield-shifted benzylic methine proton (H-7), one benzylic methylene proton (H₂-7′) and a primary carbinol methylene (H₂-9). 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

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

Proton	1	1A †	δ_{H} (ppm, J in Hz)*	3	3A †
H-2	6.88 <i>d</i> (1.6)	6.70 <i>d</i> (1.8)	6.59 <i>s</i>	6.65 <i>s</i>	6.58 <i>s</i>
H-5	7.02 <i>d</i> (8.3)	6.87 <i>d</i> (8.3)	—	—	—
H-6	6.78 <i>dd</i> (8.3, 1.6)	6.83 <i>dd</i> (8.3, 1.8)	6.59 <i>s</i>	6.65 <i>s</i>	6.58 <i>s</i>
H-7	4.72 <i>d</i> (6.0)	4.79 <i>d</i> (6.8)	4.72 <i>d</i> (5.7)	4.66 <i>d</i> (3.8)	4.73 <i>d</i> (4.4)
H-8	2.20 <i>dddd</i> (6.0)	2.42 <i>dddd</i> (7.0)	2.23 <i>dddd</i> (5.9)	3.09 <i>m</i>	3.09 <i>m</i>
H _a -9	3.44 <i>m</i>	3.75 <i>dd</i> (11.0, 6.2)	3.51 <i>m</i>	3.82 <i>dd</i> (9.1, 3.3)	3.89†
H _b -9	3.67 <i>m</i>	3.92 <i>dd</i> (11, 7.0)	3.66 <i>m</i>	4.20 <i>dd</i> (9.1, 6.6)	4.28 <i>dd</i> (9.1, 6.9)
H-2'	6.82 <i>d</i> (1.6)	6.68 <i>d</i> (1.8)	6.82 <i>d</i> (1.8)	6.65 <i>s</i>	6.58 <i>s</i>
H-5'	6.98 <i>d</i> (8.3)	6.85 <i>d</i> (8.3)	6.97 <i>d</i> (8.4)	—	—
H-6'	6.68 <i>dd</i> (8.3, 1.6)	6.81 <i>dd</i> (8.3, 1.8)	6.69 <i>dd</i> (8.4, 1.8)	6.65 <i>s</i>	6.58 <i>s</i>
H _a -7'	2.47 <i>dd</i> (13.5, 11.0)	2.56 <i>dd</i> (13.5, 10.7)	2.47 <i>dd</i> (13.5, 11.0)	—	—
H _b -7'	2.86 <i>dd</i> (13.5, 4.4)	2.92 <i>dd</i> (13.5, 5.2)	2.83 <i>dd</i> (13.5, 4.5)	4.66 <i>d</i> (3.8)	4.73 <i>d</i> (4.4)
H-8'	2.61 <i>m</i>	2.73 <i>m</i>	2.60 <i>m</i>	3.09 <i>m</i>	3.09 <i>m</i>
H _a -9'	3.58 <i>t</i> (7.3)	3.79 <i>dd</i> (8.6, 6.1)	3.58 <i>t</i> (7.3)	3.82 <i>dd</i> (9.1, 3.3)	3.89†
H _b -9'	3.90 <i>t</i> (7.3)	4.06 <i>dd</i> (8.6, 6.6)	3.90 <i>t</i> (7.3)	4.20 <i>dd</i> (9.1, 6.6)	4.28 <i>dd</i> (9.1, 6.9)
H-1''	4.83 <i>d</i> (7.5)	—	4.84 <i>d</i> (7.6)	4.87 <i>d</i> (7.2)	—
H-1'''	4.86 <i>d</i> (7.5)	—	4.86 <i>d</i> (7.6)	4.87 <i>d</i> (7.2)	—
OMe-3	3.75 <i>s</i>	3.89 <i>s</i>	3.74 <i>s</i>	3.75 <i>s</i>	3.90 <i>s</i>
OMe-5	—	—	3.74 <i>s</i>	3.75 <i>s</i>	3.90 <i>s</i>
OMe-3'	3.75 <i>s</i>	3.89 <i>s</i>	3.74 <i>s</i>	3.75 <i>s</i>	3.90 <i>s</i>
OMe-5'	—	—	—	3.75 <i>s</i>	3.90 <i>s</i>

* 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, $\text{DMSO}-d_6$) spectral data of compounds 1–3

Carbon	1	δ_0 (ppm)* 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.

$[\text{M} + \text{Na}]^+$ 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 $42.0 \mu\text{g ml}^{-1}$, respectively.

It is of interest to note that very few lignans have been reported from the Rubiaceae. (+)-Syringaresinol, ($\pm$)-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 Araliaceae plants [21] and, recently, from *Albizia julibrissin* (Leguminosae) [4].

EXPERIMENTAL

General. Mps: uncorr. $[\alpha]_{\text{D}}^{26.5}$ pyridine. ^1H and ^{13}C NMR: $\text{DMSO}-d_6$ 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 F₂₅₄ plates; detection UV at 254 nm and spraying with 10% H_2SO_4 in MeOH. Cytotoxicity bioassay: Tosoh MPR A4i 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-(–)-*Lariciresinol-4,4'-bis-O- β -D-glucopyranoside* (**1**). White amorphous powder (15 mg, MeOH), mp, $200\text{--}203^\circ$. $[\alpha]_D^{26.5} -50.0^\circ$ (pyridine; *c* 0.16). R_f 0.28 in CHCl_3 –MeOH– H_2O (70:30:3). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 228, 276. IR ν^{KBr} cm^{-1} : 3402 (OH), 1597, 1516 (C = C), 1226, 1077. FAB-MS m/z (rel. int): 707 $[\text{M} + \text{Na}]^+$ (51) calcd for $\text{C}_{32}\text{H}_{44}\text{O}_{16}$, 545 $[\text{M} - \text{glc} + \text{Na}]^+$ (12), 523 $[\text{M} - \text{glc} + \text{H}]^+$ (9), 522 $[\text{M} - \text{glc}]^+$ (9), 399 $[\text{M} - 2\text{glc} + \text{K}]^+$ (47), 361 $[\text{M} - 2\text{glc} + \text{H}]^+$ (18), 360 $[\text{M} - 2\text{glc}]^+$ (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-Methoxylariciresinol-4,4'-bis-O- β -D-glucopyranoside (**2**). White amorphous powder (6 mg, MeOH), mp, $139\text{--}140^\circ$. $[\alpha]_D^{26.5} -40.0^\circ$ (pyridine; *c* 0.29). R_f 0.24 in CHCl_3 –MeOH– H_2O (70:30:3). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 230, 280. IR ν^{KBr} cm^{-1} : 3400 (OH), 1595, 1511 (C = C), 1228, 1125, 1072. FAB-MS m/z (rel. int.): 737 $[\text{M} + \text{Na}]^+$ (47) calcd for $\text{C}_{33}\text{H}_{46}\text{O}$, 575 $[\text{M} - \text{glc} + \text{Na}]^+$ (12), 413 $[\text{M} - 2\text{glc} + \text{Na}]^+$ (26), 391 $[\text{M} - 2\text{glc} + \text{H}]^+$ (54), 390 $[\text{M} - 2\text{glc}]^+$ (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, $245\text{--}248^\circ$. $[\alpha]_D^{26.5} -10.7^\circ$ (pyridine, *c* 0.13). R_f 0.52 in CHCl_3 –MeOH– H_2O (70:30:3). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 240, 270. IR ν^{KBr} cm^{-1} : 3400 (OH), 1595, 1515 (C = C), 1240, 1137, 1090. FAB-MS m/z (rel. int.): 765 $[\text{M} + \text{Na}]^+$ (35) calcd for $\text{C}_{34}\text{H}_{46}\text{O}_{18}$, 626 $[\text{M} - \text{glc} + 2\text{Na}]^+$ (12), 619 $[\text{M} - \text{glc} + \text{K}]^+$ (8), 603

$(\text{M} - \text{glc} + \text{Na})^+$ (7), 480 $[\text{M} - 2\text{glc} + \text{Na} + \text{K}]^+$ (10), 441 $[\text{M} - 2\text{glc} + \text{Na}]^+$ (9), 418 $[\text{M} - 2\text{glc}]^+$ (20). ^1H NMR (400 MHz, DMSO- d_6): Table 1. ^{13}C NMR (100 MHz, DMSO- d_6): Table 2.

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