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LIGNAN GLYCOSIDES FROM STEMS OF *SALVADORA PERSICA*

M. S. KAMEL, K. OHTANI,* M. H. ASSAF, R. KASAI,* M. A. EL-SHANAWANI, K. YAMASAKI,* A. A. ALI and O. TANAKA*

Pharmacognosy Department, Assiut University, Faculty of Pharmacy, Assiut, Egypt; *Institute of Pharmaceutical Sciences, Hiroshima University, School of Medicine, Kasumi, Minami-Ku, Hiroshima 734, Japan

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Key Word Index—*Salvadora persica*; Salvadoraceae; salvadoside; salvadoraside; lignan glycosides.

Abstract—From stems of *Salvadora persica*, five glycosides were isolated. Two are new and identified as sodium 1-*O*-benzyl-β-D-glucopyranoside-2-sulphate (salvadoside) and 5,5'-dimethoxylariciresinol 4,4'bis-*O*-β-D-glucopyranoside (salvadoraside), in addition to syringin, liriodendrin and sitosterol 3-*O*-glucopyranoside. This represents the first report of syringin and lignan glycosides from the family Salvadoraceae.

INTRODUCTION

Salvadora persica L. (Salvadoraceae) is a plant native to Egypt, Saudi Arabia and India [1], of medicinal interest [2]. This paper deals with isolation and characterization of the major glycosides of the stem.

RESULTS AND DISCUSSION

The ethanolic extract of the stems was defatted and subjected to column chromatography on a highly porous synthetic polymer (Diaion HP-20) followed by column chromatography on silica gel and LiChroprep RP-8 affording five compounds (**1**–**5**). Compounds **1** and **2** were identified as syringin and sitosterol 3-*O*-β-D-glucopyranoside on the bases of physical, chemical and spectral data (^{1}H, ^{13}C NMR and FAB-MS) [3–5].

Compound **3** showed an $[M+Na]^+$ peak at *m/z* 395 in the FAB mass spectrum. High resolution FAB-MS $[M+Na]^+$ revealed the molecular formula as $C_{13}H_{17}O_9SNa$. ^{1}H NMR spectral data of **3** (Table 1) showed one anomeric proton for β-glucose at δ4.97 [6] as well as the downfield shift of H-2 glucose (at δ5.0) which was observed clearly from ^{13}C NMR analysis (Table 2), in which C-2 glucose appeared at δ80.3, indicating the attachment of an $NaSO_3$ group to this position. In addition, the chemical shifts of the other carbon signals were very similar to those reported for benzyl glucoside [7].

Solvolysis of **3** [8] afforded **3a**, which was identified as benzyl glucoside on the bases of co-chromatography with an authentic sample [R_f 0.55, system I) indicating the attachment of sulphate to the oxygen of C-2 glucose, which was identified by GLC after acid hydrolysis of **3a** with 2 N HCl. Consequently, **3** is sodium 1-*O*-benzyl-β-D-glucopyranoside-2-sulphate and provisionally named salvadoside.

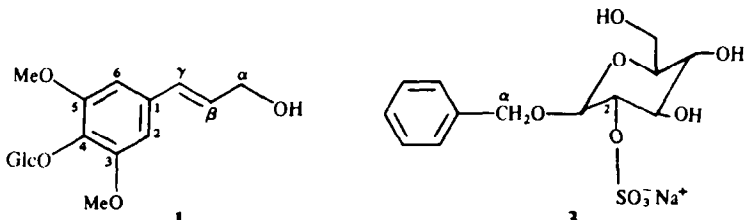
Compound **4** was investigated as liriodendrin. Upon hydrolysis **4a** was produced and identified as (+)-syringaresinol. The structure determination of the aforementioned lignans was based on comparison of their physical, chemical and spectral data (^{1}H, ^{13}C NMR and FAB-MS) with those reported for liriodendrin and (+)-syringaresinol [9].

Compound **5** showed an $[M-H]^-$ peak at *m/z* 743.0527 in the FAB-MS, corresponding to a molecular formula of $C_{34}H_{48}O_{18}$. The following significant FAB-MS fragment peaks were observed: $[M-H-Glc]^-$ at *m/z* 581 and $[M-H-2Glc]^-$ at *m/z* 419. Acid hydrolysis with 2 N HCl afforded D-glucose. ^{1}H NMR and ^{1}H–^{1}H 2D-COSY spectral data (Table 1) revealed the presence of four methoxyl groups from signals at δ3.72 and 3.71, as well as two anomeric protons for β-glucose from the signals at 5.73 and 5.69 ($J=6.6$ and 6.8 Hz, respectively). The phenolic proton signals—two singlet signals for two protons each—were observed at δ7.0 and 6.68, suggesting the presence of two 3,5-dimethoxy-4-hydroxyphenyl residues. In the 2D-NOESY spectrum, the cross peaks were observed between H-2', H-6' and H-7', OMe; H-2, H-6 and OMe; H-1 glucose and OMe as well as between H-7' and H-8'.

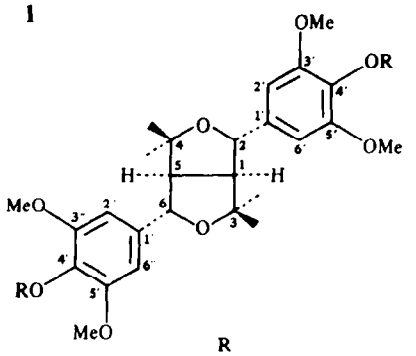
^{13}C NMR and DEPT ^{13}C NMR spectra of **5** (Table 2) showed two anomeric carbons for β-glucose at δ105.1 and other sugar signals identical to those reported for β-glucopyranose [10]. Four methoxylated carbon signals were observed at δ153.7 along with methoxyl signals at δ56.5. The other carbon signals were similar to those reported for 5,5'-dimethoxy-lariciresinol-4,4'-bis-*O*-α-L-rhamnoside [11]. From the aforementioned data, **5** was identified as 5,5-dimethyoxylariciresinol-4,4'-bis-*O*-β-D-glucopyranoside and termed salvadoraside.

EXPERIMENTAL

Nuclear magnetic resonance (NMR) spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR using tetramethylsilane (TMS) as int. standard. GLC conditions: Shimadzu GC-8A apparatus equipped with a dual flame ionization detector, carrier gas He 3.1 ml min^{-1}, Neutra Bond-1 column (0.25 mm i.d. × 25 m), column temp. 150° and injection temp. 220°. R_t D-glucose was 22.1 min. TLC was carried out on precoated silica gel plates (Kieselgel 60 F_{254}, Merck). For CC, silica gel G (E. Merck), LiChroprep RP-8 (40–63 μm, Merck) and Diaion HP-20 (Mitsubishi Chem. Ind. Co., Ltd.) were used. The solvent systems



1 3



R
4 Glc
4a H

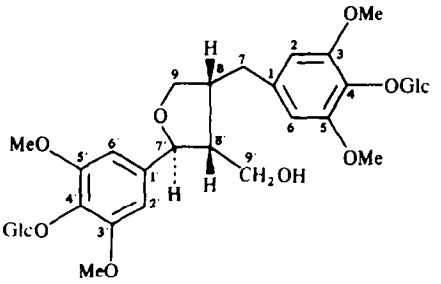


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Table 1. ^{1}H NMR spectral data of 3 and 5 (400 MHz, pyridine-d_5)

H	3	H	5
2, 3, 4, 5, 6	7.21-7.31 *m*	2′, 6′	7.00 *s*
α′	5.11 *d*, *J* = 12.46 Hz	2, 6	6.68 *s*
Glc-2	5.00 *t*, *J* = 8.4 Hz	Glc-1	5.73 *d*, *J* = 6.6 Hz
Glc-1	4.97 *d*, *J* = 7.7 Hz	Glc-1	5.69 *d*, *J* = 6.8 Hz
α	4.85 *d*, *J* = 12.46 Hz	7′	5.33 *d*, *J* = 5.7 Hz
Glc-6a	4.40 *dd*, *J* = 2.02 and 12.0 Hz	9	4.21 *m*
Glc-4	4.32 *t*, *J* = 8.8 Hz	9	4.00 *t*, *J* = 6.94 Hz
Glc-6b	4.23 *dd*, *J* = 5.32 and 12.0 Hz	9′	4.11 *dd*, *J* = 10.8 and 7.2 Hz
Glc-3	4.17 *t*, *J* = 9.2 Hz	2-OMe	3.72 *s*
Glc-5	3.8 *m*	2-OMe	3.71 *s*
		7	3.19 *dd*, *J* = 13.89 and 3.6 Hz
		7	2.78 *dd*, *J* = 13.89 and 10.8 Hz
		8	2.95 *m*
		8′	2.69 *m*

were: (I) EtOAc–MeOH–H_2O (75:25:3); (II) EtOAc–MeOH–H_2O (85:15:5); (III) 20% MeOH; (IV) 22% MeOH and (V) $CHCl_3$–MeOH–H_2O (60:40:10). Spray reagents: 10% H_2SO_4 and triphenyltertrazolium chloride (TTC), unless otherwise stated. Plant material was collected in March 1988 at the flowering stage from Wady Ghadier, Eastern desert of Egypt. The identity of the plant was confirmed by Prof. N. El-Hadidy, Cairo University. A voucher sample is kept in the Herbarium of the Faculty of Pharmacy, Assiut University.

Extraction and isolation. Dried stems of *Salvadora perica* L. (2 kg) were extracted with EtOH. After removal of the solvent by evapn, the residue (150 g) was defatted with diethyl ether. The mother liquor was chromatographed on Diaion HP-20 and eluted with H_2O, MeOH and Me_2CO successively. The MeOH

Table 2. ^{13}C NMR spectral data of 3 and 5 (100 MHz, pyridine-d_5)

C	3	C	5
1	138.8	1	*
3, 5	128.6	2, 6	107.3
2, 6	127.9[a]	3,3', 5,5'	153.7
4	127.6[a]	4	137.0
α	70.3	7	34.0
Glc-1	101.1	8	42.9
2	80.3	9	73.0
3	77.8	1'	134.3
4	71.3	2', 6'	104.5
5	78.0	4'	140.9
6	62.2	7'	83.2
		8'	53.5
		9'	60.1
		4-OMe	56.5
		Glc-1	105.1, 105.1
		2	76.0, 76.0
		3	78.3, 78.3[b]
		4	71.5, 71.5
		5	78.6, 78.5[b]
		6	62.5, 62.5

*Obscured by solvent.

[a,b]Values may be interchangeable in each column.

eluate was concd to produce a residue (43 g) and subjected to CC on silica gel (system II) affording four fractions. Fraction 2 was chromatographed on LiChroprep RP-8 (system III) to give two compounds, **1** and **2**. Fraction 3 was subjected to CC on RP-8 using system IV to afford one compound (**3**). Fraction 4 was evapd and the residue dissolved in $CHCl_3$–MeOH (4:1) for crystallization. The crystals were separated by filtration and recrystallized from the same solvent to give compound **4** as fine needles. The supernatant was dried to produce compound **5** as a powder.

Syringin (**1**). Powder (7 mg). $[\alpha]_D^{23}$ −32° (MeOH; *c* 0.025) and R_f 0.66 (system II).

Sitosterol 3-β-D-glucopyranoside (**2**). Powder (20 mg). $[\alpha]_D^{23}$ −49° (DMSO; *c* 0.025) and R_f 0.41 (system II).

Salvadoside (**3**). Colourless needles from MeOH (15 mg). $[\alpha]_D^{23}$ −36° (MeOH; *c* 0.025); mp 162–164° and R_f 0.24 (system II). High resolution FAB-MS $[M+Na]^+$ Calc. for $C_{13}H_{17}O_9SNa_2$: 395.0381; Found: 395.0385.

Solvolysis of **3**. A soln of **3** (10 mg) in dioxane (0.5 ml)–pyridine (2 ml) was heated under reflux for 2 hr. The solvent was evapd under red. pres. to dryness and the residue was treated with NaOMe–MeOH (1.5 ml) and heated under reflux for 2 hr. After dilution with MeOH (5 ml), the total soln was neutralized with Dowex 50 W-X 8, filtered and evapd under red. pres. The residue was then partitioned into *n*-BuOH–H_2O. The *n*-BuOH layer was taken and evapd under red. pres. to give **3a** (5 mg).

Liriodendrin (**4**). Needles (60 mg) from $CHCl_3$–MeOH (4:1). $[\alpha]_D^{17}$ −18.5° (pyridine; *c* 0.2); mp 272–274° and R_f 0.16 (system II).

Salvadoraside (**5**). Powder (10 mg). $[\alpha]_D^{23}$ −82° (DMSO; *c* 0.025) and R_f 0.10 (system II). High resolution FAB-MS $[M-H]^-$, Calc. for $C_{34}H_{48}O_{18}$: 743.0524; Found: 743.0527.

Acid hydrolysis of isolated glycosides. A sample (5 mg) was heated with 2 N HCl in H_2O–dioxane (1:1) in a sealed microtube at 80° for 3 hr. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The aq. layer was neutralized with Ag_2CO_3 and subjected to TLC on silica gel with solvent V (detection: TTC reagent). R_f value of D-glucose was 0.17. The aq. layer was concd, trimethylsilylated and subjected to GLC analysis. The identification of the aglycones isolated from the $CHCl_3$ layer was based on co-chromatography alongisde authentic samples or ^{13}C NMR spectral analysis and comparison with reported data.

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