Stilbene and 2-Arylbenzofuran Glucosides from the Rhizomes of *Schoenocaulon officinale*

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Two stilbene glucosides, oxyresveratrol 2-*O*-β-glucopyranoside and resveratrol 3,4′-*O*,*O*′-di-β-D-glucopyranoside, and a 2-arylbenzofuran glucoside, schoenoside, were isolated from the rhizomes of *Schoenocaulon officinale*, along with five known compounds, oxyresveratrol 3′-*O*-β-D-glucopyranoside, oxyresveratrol, resveratrol 3-*O*-β-D-glucopyranoside, mulberroside A and moracin M 3′-*O*-β-D-glucopyranoside. The structural elucidations were based on analyses of both physical and spectroscopic data.

Key words *Schoenocaulon officinale*; Liliaceae; stilbene glucoside; 2-arylbenzofuran glucoside; schoenoside

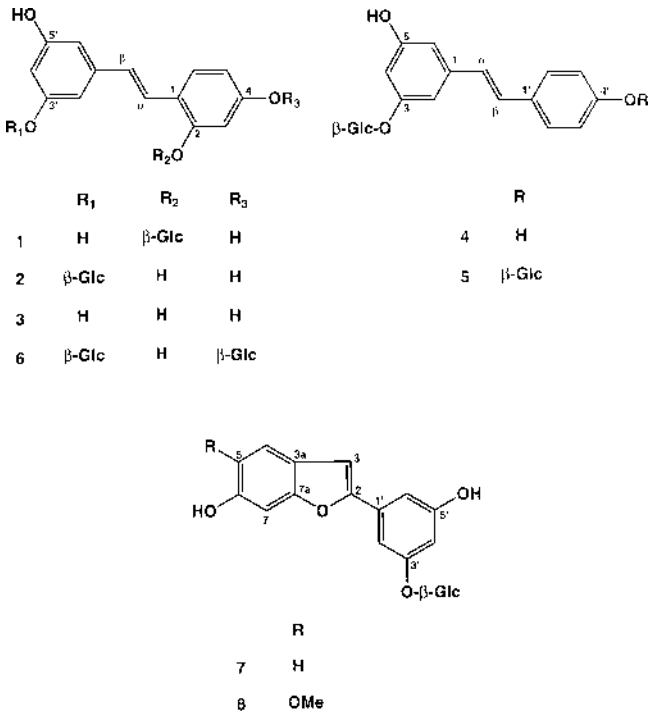
Schoenocaulon officinale A. GRAY ex BENTH. (Liliaceae) is an ornamental plant growing in the Sinai region of Egypt. The literature shows that *Veratrum* alkaloids have been reported from the seeds of *S. officinale* and showed potent insecticide properties as well as antihypertensive activities.[1,2] The present study deals with the isolation and structural determination of two new stilbene glucosides (**1**, **5**), and one new 2-arylbenzofuran glucoside (**8**), along with five known compounds (**2**—**4**, **6**, **7**) from the rhizomes of this plant.

Results and Discussion

The methanolic extract of the rhizomes of *S. officinale* was suspended in H_2O and defatted with Et_2O. The aqueous layer was subjected to a column of highly porous copolymer resin of styrene and divinylbenzene, using H_2O, MeOH and Me_2CO, successively. The fraction eluted with MeOH was repeatedly chromatographed on columns of silica gel, RP-18, or prep. HPLC to afford eight compounds. Five were identified as known compounds, oxyresveratrol 3′-*O*-β-D-glucopyranoside (**2**), oxyresveratrol (**3**),[3] resveratrol 3-*O*-β-D-glucopyranoside (**4**),[4] mulberroside A (**6**)[5] and moracin M 3′-*O*-β-D-glucopyranoside (**7**)[6] by both physical and spectroscopic evidence.

The molecular formula of compound **1** was determined as $C_{20}H_{22}O_9$ by high resolution (HR)-FAB mass spectrometry. The ^{13}C-NMR spectrum indicated the presence of one β-D-glucopyranosyl unit together with 14 carbon signals for the aglycone moiety. Acid hydrolysis gave D-glucose which was identified by TLC and comparison of the optical rotation with authentic sample. The ^{1}H-NMR showed the signals of an ABX aromatic ring system at δ 7.37 (1H, d, J=9.3 Hz), δ 6.56 (1H, d, J=2.4 Hz) and δ 6.54 (1H, dd, J=9.3, 2.4 Hz), and a 1,3,5-trisubstituted symmetrical aromatic ring at δ 6.42 (2H, d, J=2.0 Hz) and δ 6.12 (1H, t, J=2.0 Hz), as well as two *trans*-oleofinic protons at δ 7.23 (1H, d, J=16.4 Hz) and δ 6.84 (1H, d, J=16.4 Hz). These spectral data of **1** were very similar to those of oxyreaveratrol (**3**), except for the additional signals of sugar moiety as shown in Table 1. The glucopyranosyl unit was determined to be located at C-2 by irradiation of the anomeric signal at δ 4.83 (1H, d, J=7.3 Hz) which caused an increase to the nuclear Overhauser effect (NOE) enhancement only at δ 6.56 (H-3). On the basis of these spectral data, the structure of compound **1** was determined to be oxyresveratrol 2-*O*-β-D-glucopyranoside.

The molecular formula of compound **5** was determined as $C_{26}H_{32}O_{13}$ by HR-FAB mass spectrometry. The ^{1}H-NMR spectrum showed the presence of two anomeric protons at δ 4.87 (1H, d, J=7.3 Hz), and δ 4.79 (1H, d, J=7.6 Hz), in addition to the signals of a 1,3,5-trisubstituted aromatic ring at δ 6.76 (1H, br s), δ 6.59 (1H, br s) and δ 6.35 (1H, br s), and *para*-disubstituted aromatic ring at δ 7.50 (2H, d, J=8.6 Hz) and δ 7.01 (2H, d, J=8.6 Hz), and two *trans*-olefinic protons at δ 7.08 (1H, d, J=16.4 Hz) and δ 6.96 (1H, d, J=16.4 Hz). Acid hydrolysis provided D-glucose, identical by TLC and comparison of the optical rotation with authentic sample.



	R_1	R_2	R_3
1	H	β-Glc	H
2	β-Glc	H	H
3	H	H	H
6	β-Glc	H	β-Glc

	R
4	H
5	β-Glc

	R
7	H
8	OMe

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Table 1. ^{13}C-NMR Spectral Data of Compounds **1**—**6** (400 MHz)

C	**1**[a)]	**2**[a)]	**3**[a)]	**4**[b)]	**5**[b)]	**6**[b)]
1	120.3	117.6	117.8	139.5	139.2	117.9
2	157.0	157.2	157.3	102.9	103.0	155.9
3	104.9	103.5	103.5	159.0	159.0	103.9
4	159.4	159.1	159.2	104.9	105.0	158.0
5	109.3	108.0	108.4	158.4	158.4	106.6
6	128.2	128.5	128.4	107.3	107.3	123.5
1′	141.8	142.1	142.2	128.1	130.8	139.9
2′	105.8	107.0	105.6	128.1	127.7	102.7
3′	159.2	160.2	159.6	115.7	116.5	159.0
4′	102.5	103.6	102.3	157.4	157.1	105.4
5′	159.2	159.3	159.6	115.7	116.5	158.8
6′	105.8	108.4	105.6	128.1	127.7	107.5
α	124.3	125.2	124.9	125.3	126.8	126.1
β	127.9	126.1	126.5	128.7	128.1	127.3
G-1	102.0	102.1		100.8	100.3, 100.8	100.4, 100.8
G-2	74.8	74.8		73.4	73.3, 73.3	73.3, 73.4
G-3	77.9	77.9		76.8	76.7, 76.8	76.7, 76.7
G-4	71.2	71.2		69.9	69.8, 69.8	69.6, 69.7
G-5	78.0	77.9		77.2	77.1, 77.2	77.1, 77.1
G-6	62.4	62.3		60.8	60.8, 60.8	60.7, 60.7

a) Measured in CD_3OD. *b*) Measured in DMSO-d_6.

Table 2. ^{13}C-NMR Spectral Data of Compounds **7**—**8** (400 MHz, DMSO-d_6)

C	**7**	**8**
2	153.5	153.5
3	102.1	102.4
3a	120.7	119.8
4	121.2	102.9
5	112.5	145.7
6	155.8	145.7
7	97.5	98.1
7a	155.8	149.1
1′	131.7	131.8
2′	103.6	103.4
3′	159.0	159.1
4′	103.3	103.3
5′	158.7	158.7
6′	104.7	104.6
MeO	—	56.1
G-1	100.7	100.7
G-2	73.3	73.3
G-3	76.6	76.6
G-4	69.7	69.7
G-5	77.1	77.1
G-6	60.7	60.7

The ^{1}H- and ^{13}C-NMR spectral data were almost the same as those of resveratrol 3-*O*-β-D-glucopyranoside (**4**), except for the signals for one more glucopyranosyl moiety as shown in Table 1. On irradiation of the anomeric signal at δ 4.87 (1H, d, J=7.3 Hz), the intensity of H-3′, 5′ at δ 7.01 was enhanced, indicating the position of an additional glucopyranosyl moiety on C-4′. Consequently, the structure of compound **5** was elucidated as resveratrol 3,4′-*O*,*O*′-di-β-D-glucopyranoside.

The molecular formula of compound **8** was deduced as $C_{21}H_{22}O_{10}$ by HR-FAB mass spectrometry. The ^{13}C-NMR spectral data provided the presence of one β-D-glucopyranosyl unit, one methoxyl signal, as well as 14 signals for the aglycone moiety. The ^{1}H- and ^{13}C-NMR spectral data of **8** were very similar to those of moracin M 3′-*O*-β-D-glucopyranoside (**7**) (Table 2). However, the presence of two singlet signals from ^{1}H-NMR spectrum at δ 7.08 (1H, s) and δ 6.99 (1H, s), along with the downfield shift of C-5 from δ 112.5 to δ 145.7, and the upfield of C-4 from δ 121.2 to δ 102.9 and C-6 from δ 155.8 to δ 145.7 indicated that ring A is a 1,2,4,5-tetrasubstituted with an oxygenated group on C-5. The methoxyl group was determined to locate at C-5 by irradiation of this methoxy signal at δ 3.78, which caused increase to the NOE enhancement at H-4 (δ 7.08). The structure of **8** was supported by heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond connectivity (HMBC) as shown in Fig. 1. Therefore, the structure of compound **8** was concluded to be 5-methoxy-6,3′,5′-trihydroxy-2-arylbenzofuran 3′-*O*-β-D-glucopyranoside, and called schoenoside.

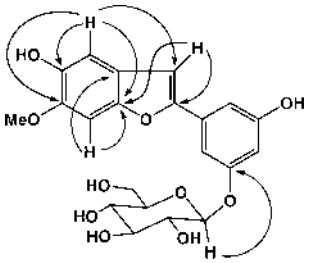

Fig. 1. The Significant HMBC Correlation of Schoenoside (**8**)

Experimental

General Procedures NMR spectra were recorded in CD_3OD or DMSO-d_6 using a JEOL JNM A-400 spectrometer (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR) with tetramethylsilane (TMS) as internal standard. MS were recorded on a JEOL JMS-SX 102 spectrometer. Preparative HPLC was carried out on a column of ODS (150×20 mm i.d., YMC) with a Tosoh refraction index (RI-8) detector. The flow rate was 6 ml/min for ODS. For CC, silica gel G 60 (Merck), RP-18 (50 mm, YMC) and a highly porous copolymer of styrene and divinylbenzene (Mitsubishi Chem. Ind. Co., Ltd.) were used. The solvent systems were: (I) EtOAc–MeOH (9 : 1), (II) EtOAc–MeOH–H_2O (4 : 1 : 0.1), (III) EtOAc–MeOH–H_2O (7 : 3 : 0.3), (IV) EtOAc–MeOH–H_2O (6 : 4 : 1), (V) 20—50% MeOH, (VI) 15% MeCN, (VII) 20% MeCN, (VIII) 25% MeCN, (IX) 10—70% MeOH and (X) 10% MeCN. The spray reagent used was 10% H_2SO_4 in ethanol.

Plant Material Rhizomes of *Schoenocaulon officinale* were collected from Sinai, Egypt, in April 1997. The plant was identified by Professor A. Fayed, Department of Botany, Faculty of Sciences, Assuit University, Egypt. A voucher sample is deposited in the Herbarium of the Department of Pharmacognosy, Assuit University.

Extraction and Isolation Air dried powdered rhizomes (900 g) of *S. officinale* were extracted with hot MeOH. After removal of the solvent by evaporation, the residue (114.0 g) was defatted with Et_2O. The aqueous layer was subjected to a column of highly porous copolymer of styrene and divinylbenzene, using H_2O, MeOH and Me_2CO, successively. The fraction eluted with MeOH (14.1 g) was subjected to a column of silica gel (systems I, II, III and IV, respectively) affording four fractions. Fraction 2 (1.5 g) was repeatedly chromatographed on a column of RP-18 using system V to provide nine fractions. Fractions 2-2 and 2-3 were purified by prep. HPLC-ODS (system VI) to give compounds **1** (55 mg) and **2** (155 mg). Fraction 2-4 was purified by prep. HPLC-ODS (system VII) to afford compound **4** (72 mg). Fraction 2-5 was similarly separated by prep. HPLC-ODS (system VIII) to provide compounds **3** (5 mg) and **7** (5 mg). Fraction 2-6 was purified by prep. HPLC-ODS (system VII) to provide compound **8** (7 mg). Fraction **3** (2.1 g) was subjected to a column of RP-18 (system IX), and finally purified by prep. HPLC-ODS (system X) to provide compounds **5** (309 mg) and **6** (105 mg).

Oxyresveratrol 2-*O*-β-D-glucopyranoside (**1**): Amorphous powder. $[\alpha]_D^{21}$ −59.1° (c=2.13, MeOH); ^{1}H-NMR (CD_3OD) δ: 7.37 (1H, d, J=9.3 Hz, H-6), 7.23 (1H, d, J=16.4 Hz, H-α), 6.84 (1H, d, J=16.4 Hz, H-β), 6.56 (1H, d,

J=2.4 Hz, H-3), 6.54 (1H, dd, J=9.3, 2.4 Hz, H-5), 6.42 (2H, d, J=2.0 Hz, H-2′, 6′), 6.12 (1H, t, J=2.0 Hz, H-4′), 4.83 (1H, d, J=7.3 Hz, H-1 Glc), 3.87 (1H, dd, J=12.3, 1.7 Hz, H-6 Glc), 3.68 (1H, dd, J=12.3, 4.9 Hz, H-6 Glc); ^{13}C-NMR: Table 1; negative HR-FAB-MS, *m/z*: 405.1192 $[M-H]^-$ ($C_{20}H_{21}O_9$ reduires 405.1185).

Resveratrol 3,4′-*O*,*O*′-di-β-D-glucopyranoside (**5**): Amorphous powder. $[\alpha]_D^{21}$ −74.7° (c=3.53, MeOH); ^{1}H-NMR (DMSO-d_6) δ: 7.50 (2H, d, J=8.6 Hz, H-2′, 6′), 7.08 (1H, d, J=16.4 Hz, H-β), 7.01 (2H, d, J=8.6 Hz, H-3′, 5′), 6.96 (1H, d, J=16.4 Hz, H-α), 6.76 (1H, br s, H-2), 6.59 (1H, br s, H-6), 6.35 (1H, br s, H-4), 4.87 (1H, d, J=7.3 Hz, H-1 Glc), 4.79 (1H, d, J=7.6 Hz, H-1′ Glc); ^{13}C-NMR: Table 1; negative HR-FAB-MS, *m/z*: 551.1759 $[M-H]^-$ ($C_{26}H_{31}O_{13}$ requires 551.1764).

Acid Hydrolysis of Compounds 1 and 5 Compound **1** (25 mg) was dissolved in 5% HCl and heated at 90 °C for 2 h. After cooling, the reaction mixture was extracted with Et_2O. The aqueous layer was neutralized with saturated $NaHCO_3$ and concentrated to dryness. The residue was then applied to a silica gel column (system III), affording D-glucose (7 mg, $[\alpha]_D^{21}$ +49.2°) in comparison with authentic sample. By the same method, compound **5** provided D-glucose (11 mg, $[\alpha]_D^{21}$ +50.3°).

Schoenoside (**8**): Amorphous powder. $[\alpha]_D^{21}$ −84.2° (c=0.48, MeOH); ^{1}H-NMR (DMSO-d_6) δ: 7.13 (1H, s, H-3), 7.08 (1H, s, H-4), 6.99 (1H, s, H-7), 6.94 (1H, br s, H-2′), 6.86 (1H, br s, H-6′), 6.42 (1H, br s, H-4′), 4.83 (1H, d, J=7.3 Hz, H-1 Glc); ^{13}C-NMR: Table 2; negative HR-FAB-MS, *m/z*: 433.1140 $[M-H]^-$ ($C_{21}H_{21}O_{10}$ reduires 433.1134).

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References

1) Kupchan S. M., By A. W., "The Alkaloids," Vol. 10, ed. by Manske R. H. F., Academic Press, New York, 1968, pp. 193—285.
2) Ujvary I., Casida J. E., *Phytochemistry*, **44**, 1257—1260 (1997).
3) Qiu F., Komatsu K., Kawasaki K., Saito K., Yao X., Kano Y., *Planta Med*., **62**, 559—561 (1996).
4) Nyemba A. M., Mpondo T. N., Kumbu S. F., Connolly J. D., *Phytochemistry*, **39**, 892—898 (1995).
5) Hirakura K., Fujimoto Y., Fukai T., Nomura T., *J. Nat. Prod*., **49**, 218—224 (1986).
6) Basnet P., Kadota S., Terashima S., Shimizu M., Namba T., *Chem. Pharm. Bull*., **41**, 1238—1243 (1993).