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Lignans and neolignans from *Stelleropsis antoninae*

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ABSTRACT

Background and the purpose of the study: *Stelleropsis antoninae* Pobed. (Family: Thymelaeaceae) grows wildly as an herbaceous plant in Iran. Most of the Thymelaeaceous plants contain lignans and neolignans, which have important pharmacologically properties. In the present study, the isolation and identification of the main lignans and neolignans of *S. antoninae,* which has not been previously reported is described and compared to other species.

Methods: Column (CC) and High Performance Liquid Chromatographic (HPLC) methods were used for the isolation and purification, and ^{1}H-NMR, ^{13}C-NMR, HMBC, HMQC, H-H COSY and MS were employed for the identification of the compounds isolated from the methanol extract.

Results: From the methanol extract of the aerial parts of *S. antoninae* four lignans, syringaresinol (**1**), syringaresinol 4-*O*-*β*-D-glucopyranoside (**4**), syringaresinol 4-*O*- *β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (**5**), liriodendrin (**6**), and two neolignans, 5-methoxylariciresinol 4'-*O*-*β*-D-glucopyranoside (**3**) and dehydrodiconiferyl alcohol 4-*O*-*β* -D-glucopyranoside (**2**) were isolated and identified.

Major conclusion: The results of this study show that siringaresinol, a well-known bioavtive compound, and its glucosides are the main lignans, and lariciresinol and coniferyl alcohol derivatives are the main neolignans of *S. antoninae.*

Keywords: Syringaresinol, Liriodendrin, Lariciresinol derivatives, Coniferyl alcohol derivatives.

INTRODUCTION

The genus *Stelleropsis* Pobed. belongs to the plant family Thymelaeaceae (1), which is middle size family of the dicotyledons and found throughout the tropical areas of the world (2). *Stelleropsis*, has two species in Iran, *S. irania*n (restrictively growing in central and North- East of Iran) and *S. antoninae* (3) which grows wildly in Afghanistan, Iran and Turkmenistan (3, 4).

The Thymelaeaceae plants contain coumarins, flavonoids, chromones, lignans, and neolignans (5-7). Phytochemical studies on the Thymelaeaceous plants due to their widespread uses in medicine have been reported (2) and there are reports on the toxicity of these plants (2). Lignans and neolignans consist of two phenyl propane monomers linked through C–C or C–O bonds and play an important role in resistance against opportunistic pathogens in vascular plants and also, display pharmacological activities in mammalian cells (8).

Recently, the phytochemical and chemotaxonomic investigation of *S. irania*n resulting in the identification of syringin, yuankanin, syringaresinol, syringinoside, β-sitosterol and gengkwanin was reported (9). In this article, separation and identification of some lignans and neolignans from the aerial parts of *S. antoninae,* which has not previously been reported, is described.

MATERIAL AND METHODS

Plant material

Aerial parts of *Stelleropsis antoninae* Pobed., at flowering stage, were collected from Firuzkuh (Tehran Province, the North-East of Iran), in July 2007. Plant specimen was identified by Dr. Gholamreza Amin (Faculty of Pharmacy, Tehran University of Medical Sciences), and a voucher specimen (6681- TEH) was deposited at the Herbarium of Faculty of Pharmacy.

Experimental

^{1}H- and ^{13}C-NMR spectra were measured on a Brucker Avance 500 DRX (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer with tetramethylsilane as an internal standard and chemical shifts are given in δ (ppm). EI-MS data were recorded on Agilent Technology (HP) instrument with 5973 Network Mass Selective Detector (MS model). HRFAB-MS were measured on a JEOL JMS-HX/HX110A

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spectrometer. Silica gel $60F_{254}$ pre-coated plates (Merck) were used for TLC. The spots were detected by spraying anisaldehyde-H_2SO_4 reagent followed by heating.

Isolation process

The flowering aerial parts of *S. antoninae* (700 g) were dried at room temperature, cut into small pieces and extracted with MeOH by percolation. The MeOH extract (43 g) was subjected to silica gel column chromatography with $CHCl_3$:AcOEt (1:1) and AcOEt:MeOH (9:1, 1:1, 0:1) as eluents to give eight fractions (A-H). Fraction B (750 mg) was fractionated on a silica gel CC with hexane:acetone (9:1, 8:2, 0:1) to yield eight parts (B_a-B_h). Compound **1** (14 mg) was obtained from fraction B_h (77 mg) via CC with $CHCl_3$:AcOEt (8:2).

Fraction E (960 mg) was subjected to reverse phase (RP) silica gel CC with aqueous MeOH (20%, 40% and 80%) to give seven fractions (E_a-E_g). Fraction E_d (87 mg) was subjected to RP-HPLC with aqueous methanol to afford compounds **2** (8 mg), **3** (5 mg) and **4** (20 mg). HPLC conditions: Vertex column C18 (250 x 20 mm I.D.), Kenauer. Isocratic elution: 40% MeOH, Flow-rate: 3 ml/min^{-1}. Injection volume and detector were 2 ml and PDA (UV spectra were collected across the range of 200-500 nm).

Chromatography of fractions E_e (66 mg) twice on Sephadex LH_{20} (MeOH) resulted in compound **5** (42 mg). Fraction G was subjected to RP silica gel CC using aqueous methanol (20%, 40% and 80%) to give eight fractions (G_a-G_h). Purification of G_e (95 mg) on Sephadex LH_{20} (MeOH) resulted in compound **6** (60 mg).

RESULTS AND DISCUSSION

Isolated compounds (Fig. 1) from the MeOH extract of *S. antoninae* were identified as syringaresinol **1** (10), dehydrodiconiferyl alcohol 4-*O*-*β*-D-glucopyranoside **2** (11), 5-methoxylariciresinol 4'-*O*-*β*-D-glucopyranoside **3** (12), syringaresinol 4-*O*-*β*-D-glucopyranoside **4** (13), syringaresinol 4-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside **5** (14) and syringaresinol 4, 4′-di-*O*-*β*-D-glucopyranoside (liriodendrin) **6** (15) by comparison of their NMR (^{1}H-NMR, ^{13}C-NMR, HMBC, HMQC and ^{1}H-^{1}H COSY) and MS (EI-Mass, HRFAB-Mass) spectral data with those reported in the literature. ^{13}C-NMR data of the constituents are given in table 1. Four isolated compounds of *S. antoninae* are syringaresinol and its glucosides, therefore, the important HMBC correlations of syringaresinol are indicated in figure 2. ^{1}H-NMR data of the isolated compounds are:

***Syringaresinol* 1**: ^{1}H-NMR (500 MHz, $CDCl_3$): *δ* 6.59 (4H, *s*, aromatic protons), 5.57 (2H, *s,* OH-4, 4'), 4.74 (2H, *d, J* = 4.3 Hz, H-8, 8'), 4.29 (2H, *dd*, *J* = 9.2, 6.7 Hz, H-9b, 9'b), 3.90 (12H, *s*, four OMe), 3.90 (2H, *m*, H-9a 9'a), 3.10 (2H, *m*, H-7,7').

***Dehydrodiconiferyl alcohol 4-O-β-D-glucopyranoside* 2**: ^{1}H-NMR (500 MHz, methanol-*d4*) δ (ppm): 7.14 (1H, *d*, *J* = 8.4 Hz, H-5), 7.02 (1H, *d, J* = 1.8 Hz, H-2), 6.95 (2H, *d, J* = 1.8 Hz, H-2', 6'), 6.93 (1H, *dd, J* = 8.2, 1.8 Hz, H-6), 6.53 (1H, d, J = 15.9 Hz, H-7'), 6.22 (1H, *dt, J* = 15.8, 5.9 Hz, H-8'), 5.58 (1H, *d, J* = 5.9 Hz, H-7), 4.19 (2H, *dd*, *J* = 5.9, 1.1 Hz, H-9'), 3.87 (3H, *s*, 3'- OMe), 3.84 (1H, *m,* H-9a), 3.82 (3H, *s*, 3- OMe), 3.78 (1H, *m*, H-9b), 3.46 (1H, *m*, H-8).

***5-methoxylariciresinol 4-O-β-D-glucopyranoside* 3**: ^{1}H-NMR (500 MHz, DMSO-*d6*) δ (ppm): 7.00 (1H, *dd, J* = 8.2, 1.5 Hz, H-5), 6.82 (1H, *d, J* = 1.5 Hz, H-2), 6.68 (1H, *dd, J* = 8.2, 1.5 Hz, H- 6), 6.55 (2H, *s,* H-2', 6'), 4.84 (1H, *d, J* = 7.5 Hz, Glc H-1"), 4.68 (1H, *d, J* = 6.5 Hz, H-7'), 3.90 (1H, *m,* H-9b), 3.76 (3H, *s*, 3- OMe), 3.75 (6H, *s*, 3', 5'- OMe), 3.66 (1H, *m,* H-9'b), 3.55 (1H, *m,* H-9a), 3.49 (1H, *m,* H-9'a), 2.85 (1H, *m,* H-7b), 2.59 (1H, *m*, H-8), 2.45 (1H, *m*, H-7a), 2.20 (1H*, m*, H-8').

***Syringaresinol 4-O-β-D-glucopyranoside* 4**: ^{1}H-NMR (500 MHz, methanol-d4) δ (ppm): 6.71 (2H, *s*, H-2, 6), 6.65 (2H, *s*, H-2', 6'), 4.86 (1H, *d*, *J* = 7.5 Hz, Glc H-1"), 4.76 (1H, *d*, *J* = 4.0 Hz, H-7), 4.71 (1H, *d*, *J* = 4.2 Hz, H-7'), 4.27 (2H, *m*, H-9b, 9'b), 3.90 (2H, *m*, H-9a, 9'a), 3.85 (6H, *s*, two OMe), 3.83 (6H, *s*, two OMe), 3.75 (1H, *m*, H-6"b), 3.65 (1H, *m*, H-6"a), 3.46 (1H, *m*, H-2"), 3.41 (2H, *m*, H-3", 4"), 3.19 (1H, *m*, H-5"), 3.12 (2H, *m*, H-8, 8').

***Syringaresinol 4-O- β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside* 5**: ^{1}H-NMR (500 MHz, methanol-d4) δ (ppm): 6.71 (2H, *s*, H-2, 6), 6.65 (2H, *s*, H-2', 6'), 4.88 (1H, *d, J* = 7.5 Hz, Glc H-1"),4.76 (1H, *d, J* = 4.0 Hz, H-7),), 4.71 (1H, *d*, *J* = 4.2 Hz, H-7'), 4.27 (2H, *m*, H-9b, 9'b), 3.84 (6H, *s*, two OMe), 3.83 (6H, *s*, two OMe), 3.12 (2H, *m*, H-8, 8').

***Liriodendrin* 6**: ^{1}H-NMR (500 MHz, DMSO-d_6) *δ* (ppm): 6.65 (4H, *s*, H-2, 2′, 6, 6′), 4.88 (2H, *m*, H-1", 1"′), 4.68 (2H, *d*, *J* = 3.5 Hz, H-7, 7′), 4.19 (2H, *dd*, *J* = 8, 6 Hz, H-9eq, 9′eq), 3.82 (2H, *d*, *J* = 8.5, 3.0 Hz, H-9ax, H-9′ax), 3.76 (12H, *s*, four-OMe), 3.11 (2H, *m*, H-8, 8′).

This is the first report on the presence of compounds **1-6** in *S. antoninae.* Syringaresinol **1**, a furofuran-type lignan has shown antifungal, anti-inflammatory, anti-malarial activities, inhibition of cAMP phosphodiesterase, antioxidant and cytotoxic properties (16). Isolation of this lignan has previously been reported from *Dirca occidentalis* (5), *D. genkwa* (17) and *Stelleropsis iranica* (9). Dehydrodiconiferyl alcohol 4-*O*-*β*-D-glucopyranoside **2** has previously been isolated from *Pedicularis torta* and *Bellardia trixago* (both belong to the Scrophulariaceae) (10, 18), and are highly accumulated in free and immobilized *Linum usitatissimum* cell cultures (8). 5-Methoxylariciresinol 4'-*O*-*β*-D-glucopyranoside (**3**) has been reported as one of the antipsoriasis constituents of *Oplopanax elatus* (19). This compound

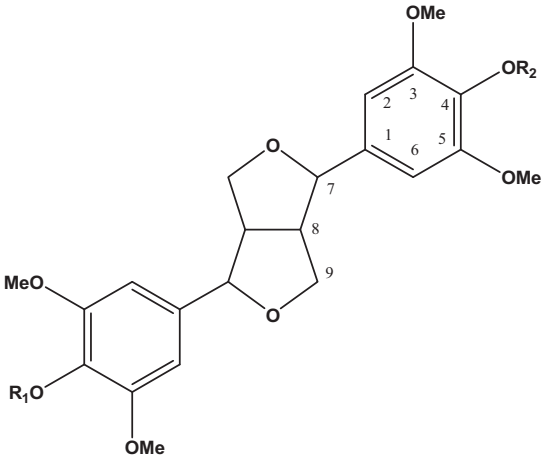
Table 1. ^{13}C-NMR of compounds **1-6.**

No.	1[a]	2[b]	3[b]	4[b]	5[b]	6[c]
1	132.0	138.2	135.4	139.6	139.6	137.2
2	102.6	111.2	104.6	104.9	104.9	104.3
3	147.1	151.1	154.4	154.5	154.5	152.7
4	134.2	147.8	133.5	135.7	135.7	133.8
5	147.1	118.1	154.4	154.5	154.5	152.7
6	102.6	119.5	104.6	104.9	104.9	104.3
7	86.0	88.8	84.0	87.2	87.2	85.1
8	54.2	55.4	54.0	55.7	55.7	53.6
9	71.7	65.0	60.6	72.9	72.9	71.4
1’	132.0	130.1	133.5	133.1	133.2	137.2
2’	102.6	112.2	113.4	104.5	104.6	104.3
3’	147.1	145.7	146.0	149.5	149.5	152.7
4’	134.2	149.3	149.1	136.3	136.3	133.8
5’	147.1	132.8	116.2	149.5	149.5	152.7
6’	102.6	116.6	122.1	104.5	104.6	104.3
7’	86.0	132.0	33.6	87.6	87.2	85.1
8’	54.2	127.7	43.8	55.5	55.5	53.6
9’	71.7	63.9	73.8	72.9	72.9	71.4
3-OCH_3	56.3	56.7	56.4	56.8	56.7	56.4
5-OCH_3	56.3		56.4	56.8	56.7	56.4
3’-OCH_3	56.3	56.8	57.0	57.0	56.8	56.4
5’-OCH_3	56.3			57.0	56.8	56.4
1”		102.8	105.5	105.4	105.4	102.7
2”		74.9	75.8	75.7	75.7	74.2
3”		78.2	77.9	77.8	77.8	76.5
4”		71.4	71.4	71.3	71.3	70.0
5”		77.9	78.4	78.4	78.3	77.2
6”		62.5	62.6	62.6	71.3	60.9
1”’					104.3	102.7
2”’					74.9	74.2
3”’					77.8	76.5
4”’					71.3	70.0
5”’					78.2	77.2
6”’					62.6	60.9

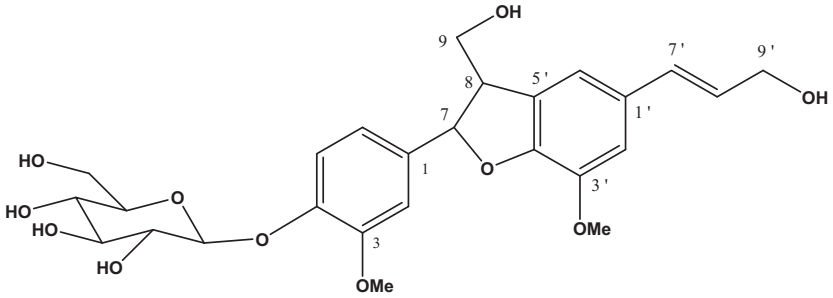
[a] $CDCl_3$
[b] Methanol-d_4
[c] DMSO-d_6

is a methoxy glucosylated form of lariciresinol which is a dietary neolignan and noteworthy, significant phytoestrogenic activity (20). Recent epidemiological studies suggest that high dietary intake of lignans and lariciresinol is associated with reduced risk of breast cancer. Pharmacological studies show that administration of lariciresinol enhanced tumor cell apoptosis and increased estrogen receptor beta expression (20).

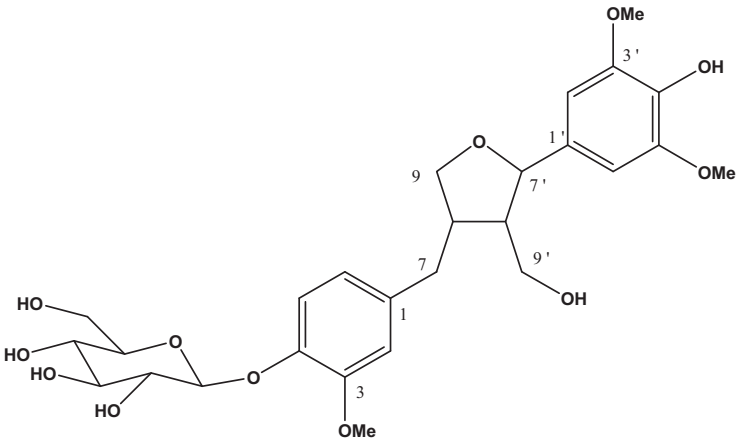
Syringaresinol 4-*O*-*β*-D-glucopyranoside (**4**) is previously isolated from *Cressa cretica* (Convulvulaceae) (13). Liriodendrin (**6**) has shown



1

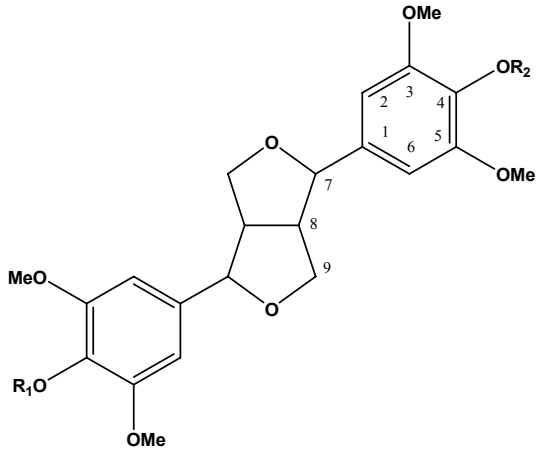


2

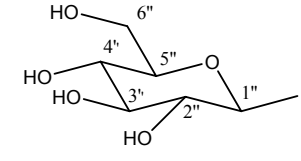


3

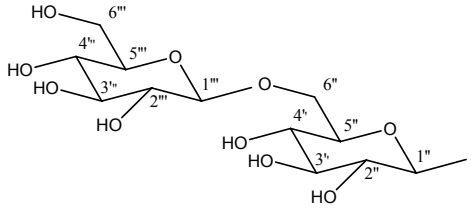
Figure 1. structure of compound 1-3 isolated from S. *antoninae*



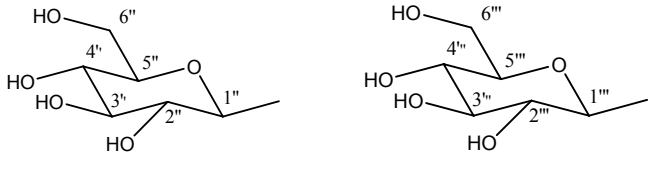
	R_1	R_2
1	H	H



4 H



5 H



6

Figure 1. (continued) structure of compound 4-6 isolated from S. *antoninae.*

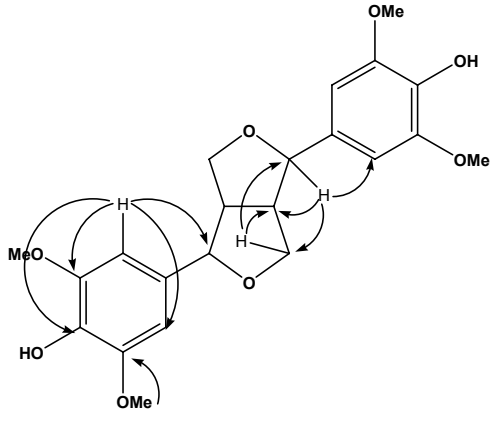


Figure 2. HMBC correlations of the compound **1**, isolated from *S. antoninae***.**

anti-inflammatory and antinociceptive effects following oral administration and has been isolated from *Acanthopanax senticosus* (21).

In conclusion, the results of this study show that the main lignans and neolignans of *S. antoninae* are different from those of *S. iranica* which contain siringin and its glucoside as the main lignans. Only siringaresinol was found similar in both species (9).

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