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A New Prenylated Isoflavonoid From *Limonium leptophyllum*

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Abstract

Phytochemical investigation of methanolic extract of *Limonium leptophyllum* (Plumbaginaceae), led to the isolation of 1 new isoflavonoid with a rare 5-membered dihydrofuran ring (**1**, leptoisoflavone A) and 8 known compounds. The known isolated compounds were identified as euchrenone b_9 (**2**), auriculasin (**3**), kaempferol (**4**), avicularoside (**5**), myricetin-3-arabinoside (**6**), *trans-N*-feruloyltyramine (**7**), *trans-N*-caffeoyltyramine (**8**), and β-sitosterol (**9**). The crude methanolic extract exhibited moderate activity toward endocannabinoid receptors. Auriculasin (**3**) showed activity toward cannabinoid receptor type 1 (86.7% displacement with IC_{50} 8.92 μM).

Keywords

Limonium leptophyllum, isoflavonoids, leptoisoflavone A, isolation, cannabinoid receptors

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The genus *Limonium* (Plumbaginaceae family) is known as halophytes and consists of 300 species worldwide, of which 18 are found in Kazakhstan.[1] Species in this genus are also known as statice, caspia, sea-lavender, or marsh-rosemary. The genus is widely distributed in Asia, Africa, Australia, Europe, and North America. More recently, several species of the *Limonium* genus are used to treat arthritis and fever.[2] This genus possesses anti-inflammatory, antibacterial,[3,4] antiviral,[5] and cytotoxic properties.[3] Previous phytochemical and biological studies on the *Limonium* genus have shown the presence of different classes of compounds with various biological activities.[6–14] In continuation of our previous work and search for novel plant-derived biological agents,[6,7,15–17] further investigations on the phytochemical constituents of *Limonium leptophyllum* (Schrenk) O. Kuntze were carried out. Herein, we are reporting the isolation and characterization of 1 new and 8 known compounds from *L. leptophyllum*.

Compound **1** was obtained as a yellow solid. The high-resolution mass spectrometric (MS) data showed a peak corresponding to the molecular formula $C_{25}H_{22}O_5$, based on the $[M + H]^+$ ion signal at *m/z* 403.1532 (calc. 403.1545). The ^{13}C nuclear magnetic resonance (NMR) data of **1** (Table 1) showed resonances of 25 carbon atoms, which were classified by distortionless enhancement by polarization transfer 135 and heteronuclear single quantum coherence (HSQC) experiments as 3 methyls, 2 methylenes, 8 methines, and 12 quaternary carbons. The ^1H and ^{13}C NMR spectra (Table 1) showed resonances of 3 methyls [δ_H/δ_C 1.48/28.3, 1.52/28.5, and 1.92/19.9 (C-5‴, 4‴, and 5″)], methylene [δ_H/δ_C 3.16 (dd, J = 9.0, 14.0 Hz), 3.03 (dd, J = 4.5, 14.0 Hz)/23.4 (C-1″)], an oxymethine proton [δ_H/δ_C 4.45 (dd, J = 4.5, 9.0 Hz)/86.6 (C-2″)], olefinic methylene [δ_H/δ_C 5.01 (s)/113.1 (C-4″)], a pair of olefinic protons [δ_H/δ_C 5.61 (d, J = 10.0 Hz)/127.3, 6.71 (d, J = 10.0 Hz)/115.0, (C-2‴, 1‴)], a set of A_2B_2/ortho coupled protons of 1,4-disubstituted aromatic ring [δ_H/δ_C 6.90 (d, J = 8.5 Hz)/115.7 (C-3′, 5′), and 7.39 (d, J = 8.5 Hz)/130.5 (C-2′, 6′)], and an oxygenated aromatic proton [δ_H/δ_C 7.90 (s)/152.7 (C-2)]. Furthermore, the ^{13}C NMR showed

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Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1** and **2** in $CDCl_3$ ($\delta_C$and δ_H in parts per minute; *J* in Hertz).

Position	Leptoisoflavone A (1)		Euchrenone b_9 (2)	
	^{1}H NMR[a]	^{13}C NMR[b]	^{1}H NMR[a]	^{13}C NMR[b]
2	7.90 (s)	152.7 (D)	7.87 (s)	152.58 (D)
3	-	127.3 (S)	-	123.05 (S)
4	-	181.2 (S)	-	181.10 (S)
4a	-	105.6 (S)	-	105.7 (S)
5	-	159.9 (S)	-	160.09 (S)
6	-	109.6 (S)	-	110.03 (S)
7	-	157.5 (S)	-	157.44 (S)
8	-	101.1 (S)	-	100.99 (S)
8a	-	151.1 (S)	-	150.8 (S)
1′	-	123.7 (S)	-	123.63 (S)
2′	7.39 (d, 8.5)	130.5 (D)	7.37 (d, 8.0)	130.39 (D)
3′	6.90 (d, 8.5)	115.7 (D)	6.85 (d, 8.0)	115.70 (D)
4′	-	156.1 (S)	-	156.16 (S)
5′	6.90 (d, 8.5)	115.7 (D)	6.85 (d, 8.0)	115.70 (D)
6′	7.39 (d, 8.5)	130.5 (D)	7.37 (d, 8.0)	130.39 (D)
1″	3.16 (dd, 9.0, 14.0)	23.4 (T)	2.90 (dd, 9.2, 14.0)	29.20 (T)
	3.03 (dd, 4.5, 14.0)		3.03 (dd, 3.6, 14.0)	
2″	4.45 (dd, 4.5, 9.0)	86.6 (D)	4.30 (dd, 3.6, 9.2)	76.14 (D)
3″	-	144.4 (S)	-	147.57 (S)
4″	5.01 (s)	113.1 (T)	4.85 (s), 5.03 (s)	110.40 (T)
5″	1.92 (s)	19.9 (Q)	1.88 (s)	18.39 (Q)
1‴	6.71 (d, 10.0)	115.0 (D)	5.60 (d, 10.0)	127.22 (D)
2‴	5.61 (d, 10.0)	127.3 (D)	6.71 (d, 10.0)	114.99 (D)
3‴	-	78.8 (S)	-	78.60 (S)
4‴	1.52 (s)	28.5 (Q)	1.51 (s)	28.57 (Q)
5‴	1.48 (s)	28.3 (Q)	1.45 (s)	28.37 (Q)

NMR, nuclearmagnetic resonance.
Recorded at [a]500 MHz, [b]125MHz.

characteristic resonances attributed to an isoflavonoid skeleton of the conjugated oxo group [δ_C 181.2 (C-4)], 5 nonprotonated aromatic carbons [δ_C 101.1, 105.6, 109.6, 123.7, and 127.3 (C-8, 4a, 6, 1′, and 3)], 4 oxygenated aromatic carbons [δ_C 159.9, 157.5, 156.1, and 151.1 (C-5, 7, 4′, and 8a)], nonprotonated olefinic carbon [δ_C 144.4 (C-3″)], and nonprotonated oxygenated carbon [δ_C 78.8 (C-3‴)]. The assignments of ^{1}H and ^{13}C NMR spectroscopic data of **1** (Table 1) were based on HSQC, heteronuclear multiple bond correlation (HMBC) correlations, and ^{1}H-^{1}H correlated spectroscopy (COSY) couplings (Figure 1). The HMBC spectrum of compound **1** showed the following key ^{2}J and ^{3}J correlations (Figure 1): H-4‴ and H-5‴ exhibited correlation with C-2‴, C-3‴, and with each other, which confirmed the attachment of methyls to C-3‴. The methyl (H-5″) in the isoprenyl moiety showed correlations with C-2″, 3″, and 4″. Compound **1** showed similarities with the known compound **2** with observed variation at C-2″ (δ_C 86.6 for compound **1** whereas δ_C 76.1 for compound **2**), which was similar to δ_C 89.5, observed in lachnoisoflavones A,[18] which suggested the cyclization in compound **1**. It was also confirmed by comparing the oxo carbon (C-4) value of compound **1** (δ_C 181.2) with lachnoisoflavone A (182.9 ppm). Correlated spectroscopy spectrum showed the following correlations (Figure 1): δ_H 7.39 (H-2′, 6′) with δ_H 6.90 (H-3′, 5′), δ_H 6.71 (H-1‴) with δ_H 5.61 (H-2‴), 4.45 (H-2″) with δ_H 3.16 (H-1″a) and δ_H 3.03(H-1″b). Based on spectral data, compound **1** is identified to be a rare new natural pentacyclic isoflavonoid including a rare 5-membered dihydrofuran ring between C-5 and C-6. Finally, compound **1** was elucidated as 10-(4-hydroxyphenyl)-5,5-dimethyl-2-(prop-1-en-2-yl)-2,3-dihydro-5H,11H-furo[2,3 f]pyrano[2,3 h]chromen-11-one and named as leptoisoflavone A.

The known isolated compounds (Figure 2) were identified as euchrenone b_9 (**2**),[19] auriculasin (**3**),[20] kaempferol (**4**), avicularoside (**5**),[21] myricetin-3-arabinoside (**6**),[22]

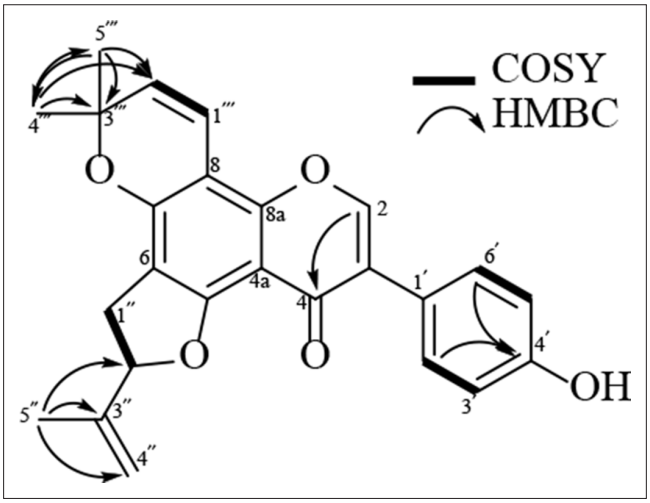

Figure 1. Key HMBC and COSY correlations of compound **1**.

trans-N-feruloyltyramine (**7**), *trans-N*-caffeoyltyramine (**8**),[23] and β-sitosterol (**9**).

Crude methanolic extract of aerial parts of *L. leptophyllum* exhibited moderate activity toward endocannabinoid receptors (cannabinoid receptor type 1 [CB1] and CB2). Auriculasin (**3**) showed activity toward CB1 receptor (86.7% displacement with IC_{50} 8.92 µM).[15]

Experimental

General

Bruker model AMX 500 NMR and 400 NMR spectrometers operating on a standard pulse system were used to acquire ^{1}H and ^{13}C NMR and 2D spectra. The instruments ran at 500 and 400 MHz for ^{1}H whereas they ran at 125 and 100 MHz for ^{13}C. $CDCl_3$, dimethyl sulfoxide-d_6, and acetone-d_6 were used as NMR solvents, and trimethyl silane was used as an internal standard. Electrospray ionization-mass spectrometric (ESI-MS) data were recorded on Thermo Orbitrap Fusion (Thermo Scientific). Samples were analyzed in the positive mode of ionization and were directly infused at 3 µL/min. Mass was analyzed in Orbitrap (mass error on the instrument <2 ppm). Electrospray ionization-MS data were obtained on a Micromass Q-Tof micromass spectrometer. Fourier transform mass spectrometry-ESI was analyzed on Thermo Orbitrap Fusion (Thermo Scientific). The sample was analyzed in the positive mode of ionization. Thin-layer chromatography was performed on precoated silica gel GF254 plates and column chromatography was performed on silica gel (200-300 mesh) and Sorbadex-LH20 (Sorbent Technologies, Atlanta, GA, USA) with detection provided by UV light (254 and 366 nm) and by spraying with 1% vanillin-H_2SO_4 reagent followed by heating for 5 to 10 minutes (105°C). Quantitative high-performance liquid chromatography (HPLC) was conducted using an Agilent 1100 HPLC system equipped with a degasser (G1379A), quaternary pump (G13311A), auto sampler (G1313A), column oven (G1316A), and UV-diode array detector (G1315B) controlled by Chemstation software. The analysis was carried out on RP-C18 columns (150 × 4.6 mm; particle size 5 µm; Luna) and (250 × 10.0 mm; particle size 10 µm; Luna) with column oven temperature set at 25°C and using the isocratic system of eluent water (A) and acetonitrile (B) for the separation of the target compounds. Acetonitrile and water solvents were of HPLC grade, where acetic acid was added as a modifier to achieve a final concentration of 0.1% in each solvent. The flow rates of the solvent were 1.0 mL/min for the analytical injections whereas 5 mL/min for the semipreparative ones and the injection volumes were 5.0 and 50 µL for the analytical and semipreparatives, respectively. All the analysis was carried out at wavelengths of 254 and 280 nm with a run time of 40 minutes.

Plant Material

Limonium leptophyllum (Schrenk) O. Kuntze aerial parts were collected in August 2016, from Kyzylorda region, Kazakhstan. The plant material was authenticated by Akhtaeva Nursulu and the specimen voucher (No.0916.) was stored at the department of biology and biotechnology of al-Farabi Kazakh National University.

Extraction and Isolation

Limonium leptophyllum aerial parts (830 g) were extracted using MeOH (3 L × 3 times); the extract was thereafter filtered and concentrated on a rotary evaporator at 40°C yielding 90.3 g of crude methanolic extract. This extract (50 g) was fractionated over HP 20 gel (200 g) column using H_2O-methanol (1:9), methanol, acetone, and dichloromethane (DCM) (each 750 mL). Each fraction was evaporated to dryness to give 16.47 g fraction A (H_2O:methanol 1:9), 7.87 g fraction B (methanol), 11.25 g fraction C (acetone), and 1.17 g fraction D (DCM).

Fractions C and D were combined and the mixture (E, 12 g) was loaded onto a normal phase silica gel vacuum liquid column (210 g) and eluted using hexane-acetone gradient to yield 12 fractions (E1-E12) and continued with methanol-DCM gradient to yield 10 more fractions (E13-E22). Fraction E5 was identified as β-sitosterol (**9**, 20 mg). Fraction E13 was identified as kaempferol (**4**, 10 mg). Fractions E5-E8 were combined together and purified on HPLC using water-acetonitrile gradient 60:40 to 20:80 over a period of 20 minutes, to yield euchrenone b_9 (**2**, 2.5 mg, RT 13.1 min) and compound **1** (2.1 mg, RT 14.6 min). Fractions E11 and 12 were combined (497 mg) and

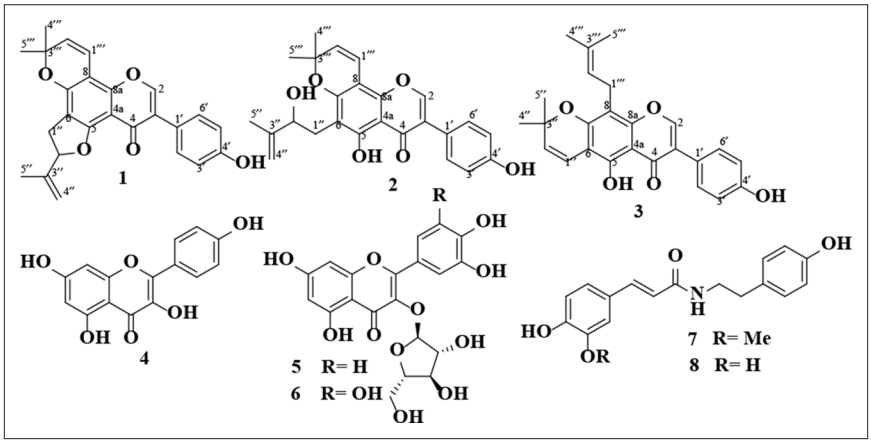

Figure 2. Compounds isolated from *L. leptophyllum*.

purified over silica gel column to afford auriculasin (**3**, 25 mg). Combined fractions E20-22 (5 g) were loaded on silica gel and purified using methanol-DCM gradient system to afford avicularoside (**5**, 10 mg), myricetin-3-arabinoside (**6**, 15 mg), *trans*-*N*-feruloyltyramine (**7**, 5 mg), and *trans*-*N*-caffeoyltyramine (**8**, 8 mg).

Leptoisoflavone a (1)

Yellow solid.
$[\alpha]_D^{22.5}$: –3.125 (*c* 0.064, MeOH).
^{1}H and ^{13}C NMR: Table 1.
High-resolution ESI-MS: *m/z* 403.1532 $[M + H]^+$ (calc. $C_{25}H_{22}O_5$ + H, 403.1545).

Cannabinoid and Opioid Receptor Assays

The affinity of the total extracts, fractions, and isolated compounds toward cannabinoid and opioid receptors was analyzed according to the published method.[24] The crude methanolic extract exhibited moderate activity toward endocannabinoid receptors. Compound **3** showed activity toward CB1 receptor.

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Declaration of Conflicting Interests

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Supplemental Material

Supplemental Material:Supplemental material for this article is available online.

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