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SHAWURENSINE, A NEW C_{19}-DITERPENOID ALKALOID FROM *Delphinium shawurense*

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The new norditerpenoid alkaloid shawurensine in addition to β*-sitosterol and the known alkaloids elatin, delbrulin, and methyllycaconitine were isolated from* Delphinium shawurense. *A structure for shawurensine was proposed based on spectral data.*

Key words: *Delphinium shawurense*, Ranunculaceae, C_{19}-norditerpenoid alkaloid, shawurensine.

Plants of the genus *Delphinium* (Ranunculaceae) are sources of diterpenoid alkaloids. Therefore, many species of this genus are used in folk medicine [1]. However, data on the chemical composition and use of *D. shawurense* W. T. Wang in folk medicine have not been published. The plant was collected in Xinjiang Autonomous Republic of the Chinese People's Republic during flowering in July 2005.

We investigated the alkaloid composition of the aerial part of *D. shawurense*. Ethanol extraction produced the total alkaloids, the yield of which was 0.56% of the air-dried plant mass. The known C_{19}-norditerpenoid alkaloids elatine, delbruline, and methyllycaconitine and the new base **1**, which we called shawurensine, in addition to *β*-sitosterol were isolated from the total alkaloids. The known compounds were identified by comparing their spectral properties with those published [2-5].

Shawurensine (**1**) was isolated as an amorphous white powder with molecular formula $C_{37}H_{32}N_2O_{11}$ as determined by high-resolution mass spectrometry (HR-ESI) and confirmed by PMR and ^{13}C NMR spectra.

The PMR spectrum of **1** contained signals for protons of *N*-ethyl, four methoxyls, 18- and 19-methylenes, NH groups, and *o*-substituted benzene ring (Table 1). The presence of two N atoms in **1** and the spectral data given above enable **1** to be classified as a C_{19}-norditerpenoid alkaloid with a C-18 ester.

Chemical shifts (CS) and multiplicities of proton signals at 3.92 ppm (br) and 3.61 (3H, t, J = 4.8 Hz) in the PMR spectrum of **1** confirmed the presence of C-6 and C-14 methoxyls, respectively [6, 7]. The ^{13}C NMR spectrum contained signals for 37 C atoms and confirmed the conclusions made on the basis of the mass and PMR spectra of **1** (Table 2). Furthermore, the ^{13}C NMR spectrum had signals at 90.8, 88.3, and 77.4 ppm, confirming the presence of a C-6 methoxyl and C-7 and C-8 hydroxyls [7]. CS in the ^{13}C NMR spectrum of **1** at 83.9 and 55.8 ppm in addition to 82.5 and 56.3 ppm were consistent with C-1 and C-16 methoxyls.

The molecular formula of **1** includes 11 O atoms from 4 methoxyls, an ester, 2 hydroxyls, and 2 carbonyls. Therefore, the last O atom belongs to a carboxyl group, which was confirmed by comparing the ^{13}C NMR spectra of **1** and delavaine A (**2**) [8], which showed that the CS of the C atoms were similar and differed only by an additional signal for OCH_3 at 51.7 ppm in the spectrum of **2**. The compositions differed only by a CH_2 group. The PMR spectrum of **1** had signals at 3.12 ppm (1H, br.s, CH–CH_3), 2.96 (2H, br.s, CH_2COOH), and 1.31 (3H, d, J = 7.2 Hz, CH–CH_3), which were due to a [*N*-(2″-methylsuccinyl)anthranyl] moiety in **1**. Analogous proton signals were observed in spectra of elatine [2], geyerline [9], and grandiflorine [9], which were due to proton signals of the [*N*-(2″-methylsuccinimido)anthranyl] moiety in these alkaloids. The PMR spectrum of **1** differed from those of elatine, geyerline, and grandiflorine by an NH signal, which was consistent with the presence in **1** of an [*N*-(2″-methylsuccinyl)anthranyl] moiety. Therefore, **1** had the structure 1.

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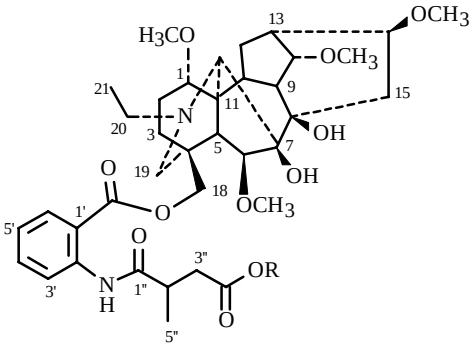
TABLE 1. Chemical Shifts in PMR Spectrum of Shawurensine (600 MHz, $CDCl_3$, 0 = TMS, δ, ppm, J/Hz)

Atom	**1**	Atom	**1**
1	3.00 (1H, m)	17	2.63 (1H, br.s)
2a	2.09 (1H, m)	2H-18	4.18 (2H, br.s)
2b	2.17 (1H, m)	19a	2.48 (1H, d, J = 11.4)
3a	1.53 (1H, m)	19b	2.73 (1H, d, J = 11.4)
3b	1.80 (1H, d, J = 15)	Ar-H-3′	7.95 (1H, d, J = 8.4)
5	1.73 (1H, br.s)	H-4′	7.09 (1H, t, J = 7.7)
6a	3.92 (1H, br.s)	H-5′	7.52 (1H, t, J = 7.5)
9	2.97 (1H, d, J = 7.2)	H-6′	8.68 (1H, d, J = 8.4)
10	1.95 (1H, q, J = 12.0, J = 6.0)	CO-NH	11.09 (1H, br.s)
12a	1.67 (1H, dd, J = 15.0 and 6.6)	2″	3.12 (1H, br.s)
12b	2.43 (1H, dd, J = 15.0 and 8.4)	3″	2.96 (2H, br.s)
13	2.35 (1H, dd, J = 6.6 and 4.8)	5″-CH_3	1.31 (3H, d, J = 7.2, N-CH_2CH_3)
14b	3.61 (1H, t, J = 4.5)	N-CH_2CH_3	2.84-2.81 (2H, m)
15a	1.85 (1H, dd, J = 14.4 and 7.8)	N-CH_2CH_3	(3H, t, J = 7.2)
15b	2.43 (1H, dd, J = 15.0 and 8.4)	4×OCH_3	3.40, 3.37, 3.34, 3.26 (3H each, s)
16a	3.08 (1H, t, J = 6.0)		

TABLE 2. Chemical Shifts in ^{13}C NMR Spectrum of Shawurensine (**1**) and Delavaine A (**2**) (600 MHz, $CDCl_3$, 0 = TMS, δ, ppm)

C atom	**1**	**2**	C atom	**1**	**2**
1	83.9	83.8	20	50.9	50.9
2	25.9	25.9	21	14.0	13.9
3	32.0	31.9	1′	114.9	114.6
4	37.5	37.5	2′	141.6	141.4
5	43.3	43.1	3′	120.7	120.5
6	90.8	90.8	4′	134.8	134.9
7	88.5	88.3	5′	122.6	122.5
8	77.5	77.4	6′	130.3	130.2
9	50.4	50.2	1″	174.8	172.4
10	38.4	38.0	2″	39.1	38.9
11	49.0	79.0	3″	37.0	37.5
12	28.6	28.6	4″	176.0	174.0
13	45.9	46.0	5″	17.9	17.9
14	83.9	83.8	1-OCH_3	55.8	55.8
15	33.6	33.5	6-OCH_3	57.8	57.8
16	82.5	82.5	14-OCH_3	58.1	58.0
17	64.5	64.4	16-OCH_3	56.3	56.2
18	69.8	69.7	ArC=O	167.9	167.9
19	52.3	52.4	$COOCH_3$	-	57.1

The mass spectrum of **1** had a peak for the molecular ion with *m*/*z* 701 $[M + 1]^+$ and a peak at *m*/*z* 587 that was probably formed by cleavage of a water molecule from the HN–CO–$CH(CH_3)$–CH_2–COOH moiety to form $C_5H_6NO_2$ whereas the peak with *m*/*z* 651 was apparently formed by simultaneous elimination of OCH_3 and H_2O from the molecular ion.



1, 2

1: R = H; **2:** R = CH_3

EXPERIMENTAL

General Comments. Melting points were determined on a MP-J3 apparatus (Yanaco). Mass spectra and elemental analyses were obtained in a FT ICR MS IonSpec 7.0T (USA) spectrometer. PMR and ^{13}C NMR spectra were recorded on Bruker Avance 600 NMR spectrometers relative to TMS (internal standard). ^{13}C NMR spectra were obtained with full C–H decoupling. TLC was performed on HSGF254 silica-gel plates (Qingdao Hai Yang Chemical Co., Ltd., Qingdao, China). Compounds on silica-gel plates were developed by iodine vapor, a fluorescent lamp, and spraying with Dragendorff's reagent. Column chromatography used silica gel (200-300 mesh, Qingdao, China).

Plant Material. The aerial part of *D. shawurense* W. T. Wang was identified by Prof. Shi Ming Duan of Xinjiang Institute of Ecology and Geography.

Extraction and Isolation of Alkaloids. Dried and ground raw material (2.9 kg) was extracted exhaustively with ethanol (80%) at room temperature. The extract was condensed in vacuo. The aqueous residue was diluted with H_2SO_4 solution (5%). The acidic solution was filtered and washed with $CHCl_3$.

The $CHCl_3$ extract was washed with saturated carbonate solution and water. The solvent was removed in vacuo to afford a mixture of alkaloids (6.3 g).

The acidic aqueous solution was made basic with carbonate solution until the pH was 8. Alkaloids were extracted with $CHCl_3$. Solvent was distilled off in vacuo to afford a mixture of alkaloids (10.1 g). The overall yield was 16.4 g or 0.56% of the dry starting material.

The alkaloid fraction (6.3 g) was worked up successively with boiling petroleum ether and acetone to afford fractions that were soluble and insoluble in acetone. The acetone-soluble fraction afforded elatine (1.0 g). The mother liquor from elatine was chromatographed over a column of silica gel with elution by petroleum ether:acetone (7:3 and 1:1). Preparative TLC of fractions 8-31 on silica-gel plates using hexane:$CHCl_3$:CH_3OH (7:3:1) produced methyllycaconitine (40 mg).

The acetone-insoluble part was fractionated over a column of LH-20 Sephadex with elution by CH_3OH. Fractions 4-9 were chromatographed over a column of silica gel with elution by petroleum ether:$CHCl_3$:CH_3OH (30:40:4 and 30:18:6) to afford amorphous **1** (20 mg).

The other alkaloid fraction (10.1 g) was separated by column chromatography over silica gel with gradient elution by petroleum ether:acetone (10:1→1:1). The first five fractions afforded β-sitosterol; fractions 33-37, delbruline (120 mg).

Shawurensine (1), $C_{37}H_{52}N_2O_{11}$, amorphous base. Mass spectrum (HRESI, *m*/*z*, %): 701 (100) $[M + 1]^+$, 651 (25), 587 (56.2), 273 (9.3), 219 (9.3).

Tables 1 and 2 give the PMR and ^{13}C NMR spectra of **1**.

Elatine (2), $C_{38}H_{50}N_2O_{10}$, mp 225-227°C (acetone). Mass spectrum (FAB—MS): 695 $[M + H]^+$.

PMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 8.05 (1H, d, J = 7.6, Ar–H-6′), 7.67 (1H, dt, J = 7.6, 1.6, Ar–H-5′), 7.53 (1H, dt, J = 7.6, 1.6, Ar–H-4′), 7.27 (1H, d, J = 7.6, Ar–H-3′), 5.07 (2H, s, OCH_2O), 4.12-4.02 (2H, m, H-18), 3.70 (1H, t, J = 5.6, H-14β), 3.64 (1H, s, H-6α), 3.26, 3.32, 3.34, 3.43 (3H each, s, 4 × OCH_3), 2.86-2.79 (1H, m, NCH_{2b}), 2.76 (1H, d, J = 11.6, H-19a), 2.73-2.63 (1H, m, NCH_{2a}), 2.58 (1H, dd, J = 14.0, 4.4, H-12a), 2.44 (1H, dd, J = 14.8, 8.4, H-15a), 2.40-2.30

(2H, m, H-19b, H-13), 1.87 (1H, dd, J = 14.8, 8.0, H-15b), 1.78-1.69 (1H, m, H-12b), 1.07 (3H, t, J = 7.2, NCH_2CH_3), 3.05 (1H, s, Suc-CH), 2.14 (m, Suc-CH_2), 1.47 (3H, d, J = 7.2, Suc-CH_3).

These spectral properties are identical to those published for elatine [2, 7].

Delbruline, $C_{26}H_{41}NO_7$, mp 142-143°C (acetone). Mass spectrum (ESI, *m*/*z*, %): 480.0 (100) $[M + 1]^+$, 292.0 (5.6), 240.1 (6.3), 229.1 (13.8), 207.2 (50).

The PMR and ^{13}C NMR spectra were identical to those published for delbruline [3].

Methyllycaconitine, $C_{37}H_{50}N_2O_{10}$, amorphous compound. Mass spectrum (ESI, *m*/*z*, %): 683.2 (88.2) $[M + 1]^+$, 665.2 (20.5) $[M - 17]^+$, 651.1 (100) $[M - 31]^+$, 633.1 (23.5) $[M - 49]^+$, 619.2 (69.4), 601.2 (23.5), 573.2 (17.6), 386.1 (11.8), 354.2 (10).

PMR spectrum (600 MHz, CD_3COCD_3, δ, ppm, J/Hz): 8.26 (1H, d, J = 7.8, Ar–H-6′), 7.86 (1H, t, J = 7.8, Ar–H-5′), 7.73 (1H, t, J = 7.8, Ar–H-4′), 7.49 (1H, d, J = 7.8, Ar–H-3′), 4.31-4.20 (2H, m, H-18), 4.05 (1H, s, H-6α), 3.69 (1H, t, J = 4.8, H-14β), 3.52, 3.41, 3.37, 3.36 (3H, s, 4 × OMe), 3.25 (1H, t, J = 7.8, H-16α), 2.86 (1H, d, J = 11.4, H-19a), 2.72-2.67 (2H, m, NCH_2CH_3), 1.15 (3H, t, J = 7.0, NCH_2CH_3), 3.05 (1H, s, Suc-CH), 2.38 (2H, m, Suc-CH_2), 1.50 (3H, d, J = 7.2, Suc-CH_3).

The spectral properties were identical to those published for methyllycaconitine [4, 7].

β-Sitosterol, mp 135-136°C (acetone).

The spectral properties were identical to those published for β-sitosterol [5].

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