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ALKALOIDS FROM *Delphinium oreophilum*. THE NEW DITERPENE ALKALOID 15-EPINAVICULINE B

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4-{[2-(Methoxycarbonyl)phenyl]amino}-2-methyl-4-oxobutanoic acid amide, the diterpenoid alkaloid delavaine B, and the new talassamine-type alkaloid 15-epinaviculine B were isolated for the first time from the aerial part of Delphinium oreophilum *Huth. The structures of the compounds were elucidated using 1D and 2D NMR spectroscopy, mass spectrometry, and an X-ray crystal structure analysis for the new alkaloid. The stereochemistry of the talassamine framework and H-bonds in the crystal of 15-epinaviculine B were analyzed.*

Keywords: *Delphinium oreophilum*, 15-epinaviculine B, 4-{[2-(methoxycarbonyl)phenyl]amino}-2-methyl-4-oxobutanoic acid, NMR spectroscopy, MS, XSA.

Essential oil, macroscopic and trace elements [1, 2], and the alkaloid composition of *Delphinium oreophilum* Huth were previously studied [3]. In continuation of research on the alkaloid composition of the plant, a new C_{20}-diterpene alkaloid with the talassamine skeleton (**1**) was isolated from the aerial part of *D. oreophilum*. In addition, 4{[2-(methoxycarbonyl)-phenyl]amino}-2-methyl-4-oxobutanoic acid amide (**2**) [4] and the diterpenoid alkaloid delavaine B (**3**) [5] were observed for the first time in this plant. The structures of the compounds were elucidated using 1D and 2D NMR spectroscopy and mass spectrometry. Single crystals of the new alkaloid **1** were studied using an X-ray crystal structure analysis (XSA).

The chemical structure of new compound **1** was established by analyzing 1D (^{1}H and ^{13}C) and 2D NMR spectra (HSQC, HMBC, COSY, and NOESY). Proton and ^{13}C resonances in ^{1}H and ^{13}C NMR spectra were assigned using HSQC and COSY experiments. The aliphatic part of the ^{1}H NMR spectrum of **1** showed a singlet for a methyl at δ_H 1.05 (H-18) and several overlapping multiplets in the range 1.15–1.95 ppm. Next, the spectrum exhibited 1H resonances at δ_H 2.05 (dt, J = 10.4, 2.8 Hz, H-14), 2.14 (td, J = 13.9, 6.6 Hz, H-7a), 2.32 (m, H-12). Resonances at δ_H 3.52 (br.t, J = 2.8 Hz) and 3.99 (t, J = 2.4 Hz) were assigned to protons of C atoms with heteroatoms, H-20 and H-15, respectively. The middle of the spectrum contained two 1H resonances characteristic of exomethylene protons (H-17) at δ_H 4.84 (m) and 4.94 (dd, J = 2.3, 1.7 Hz). The aromatic region of the spectrum had only one 1H doublet at δ_H 7.39 (d, J = 2.4 Hz, H-19) that corresponded to the proton on the double bond.

Analyses of the ^{13}C NMR and HSQC spectra of **1** showed resonances for 20 C atoms representing one methyl; eight methylene, including one exomethylene; six methine, and five quaternary C atoms. Three quaternary C atoms resonated in the weak-field and aromatic regions of the ^{13}C NMR spectrum at δ_C 173.11 (C-19), 158.27 (C-16), and 104.44 (C-17). Three resonances were observed at stronger field that were characteristic of C atoms bound to heteroatoms at δ_C 80.86 (C-20), 72.91 (C-15), and 72.58 (C-5). The other 14 C resonances appeared in the strong-field part of the spectrum in the range 19–49 ppm (Table 1). The positions of the functional groups were established based on data from an HMBC experiment.

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TABLE 1. Chemical Shifts in ^1H and ^{13}C NMR Spectra of **1** ($CDCl_3$, δ, ppm, J/Hz) and Data from HMBC Experiment

C atom	δ_H	δ_C	HMBC (H→C)
1	1.64 (m)	27.33	2, 3, 5
	1.68 (m)		
2	1.20 (m)	21.98	
	1.55 (m)		
3	1.26 (m)	31.74	1, 2, 5, 19
	1.79 (td, J = 13.6, 4.6)		2, 4
4	–	46.55	
5	–	72.58	
6	1.42 (td, J = 14.3, 6.7)	32.24	4, 7
	1.72 (m)		5, 7, 8, 10
7	2.14 (td, J = 13.9, 6.6)	29.31	6, 9, 14
	1.23 (m)		6, 9, 14
8	–	48.78	
9	2.05 (dt, J = 10.4, 2.8)	40.03	
10	–	46.80	
11	1.67 (m)	30.37	8, 12, 13, 16
	1.88 (m)		8, 20
12	2.32 (m)	36.48	9
13	1.90 (m)	38.29	11, 12, 16
14	1.75 (m)	46.47	9, 20
15	3.99 (t, J = 2.4)	72.91	9, 14, 16, 17
16	–	158.27	
17	4.84 (m)	104.44	12, 15
	4.94 (dd, J = 2.3, 1.7)		12, 15, 16
18	1.05 (s)	19.24	3, 4, 5, 19
19	7.39 (d, J = 2.4)	173.11	4, 5, 18, 20
20	3.52 (br.t, J = 2.8)	80.86	1, 5, 8, 9, 11, 19

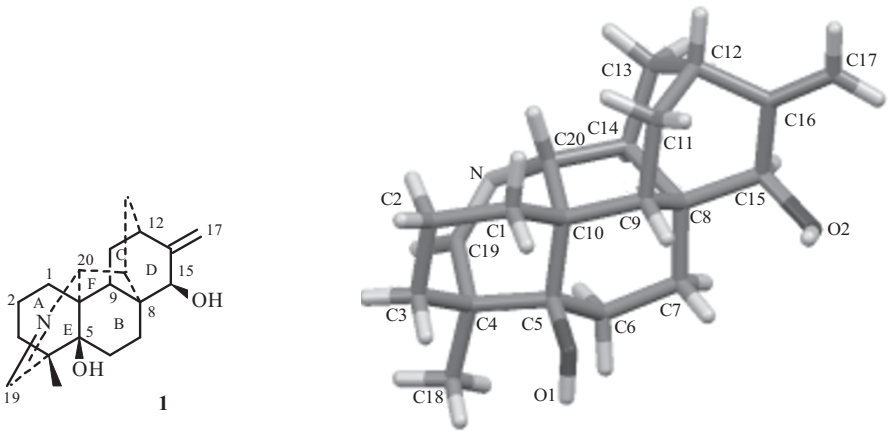

Fig. 1. Molecular structure of **1** (one of two molecules shown).

The HMBC spectrum showed cross-peaks for H-18/C-3, C-4, C-5, C-19; H-17/C-12, C-15, C-16; H-15/C-9, C-14, C-16, C-17, which indicated that the methyl was situated on C-4; the exomethylene, C-16, and the hydroxyl, C-15.

An analysis of the ^1H and ^{13}C NMR spectroscopic data found that **1** was a diterpene alkaloid. A comparison of the spectral data for **1** with those of the diterpene alkaloid naviculine B [6] revealed these two compounds had similar structures. A significant difference in the ^{13}C NMR spectrum of **1** was shielding of C-15 (δ_C 72.91) and C-17 (δ_C 104.44) by $\Delta\delta_C$ = –1.0 and –2.56 ppm, respectively, and deshielding of C-14 (δ_C 46.47), C-16 (δ_C 158.27) by $\Delta\delta_C$ = +3.57, 0.77, respectively, as compared to naviculine B. The relative configuration of **1** was established based on data from the NOESY spectrum. NOE correlations between H-15/H-13, H-14, and H-7ax indicated these protons were coplanar with the α-orientation. Based on the results, diterpene alkaloid **1** was an epimer at C-15 of naviculine B. Table 1 presents detailed data of the ^1H and ^{13}C spectra and HMBC correlations for **1**. Thus, the chemical structure the new alkaloid **1**, which is shown in Fig. 1, was elucidated based on the data.

TABLE 2. Main Crystallographic Parameters and Characteristics of X-ray Structure Analysis

Structure	**1**	Structure	**1**
Molecular formula	$C_{40}H_{54}N_2O_4$	ρ, g/cm^3	1.274
MM, g/mol	626.85	Crystal size, mm	0.30 × 0.15 × 0.05
Space group	$P2_12_12_1$, Z = 4	Scan range	3.0 ≤ θ ≤ 71.5
a, Å	7.23435(7)	Number of reflections	6329
b, Å	19.2435(2)	Number of reflections with I > 2σ (I)	5734
c, Å	23.4676(3)	R_1 (I > 2σ(I) and total)	0.037 (0.041)
α, deg	90	WR_2	0.110 (0.114)
β, deg	90	GOOF	0.882
γ, deg	90	Difference electron density peaks, eÅ^{-3}	0.19 and –0.13
V, Å^3	3267.03(6)	CCDC	2260523

The asymmetric part of the unit cell in crystals of alkaloid **1** contained two structurally identical molecules. Figure 1 shows the molecular structure of one of them from the XSA. The absolute configuration of the molecule was experimentally established from the Flack parameter [0.03(12)]. The chiral centers in both molecules of **1** had the configurations 4*S*,5*R*,8*S*,9*R*,10*S*,12*R*,14*S*,15*R*,20*R*. Figure 1 shows that **1** had *β*-oriented C5 and C15 hydroxyls. Naviculine B with an *α*-oriented C15 OH is known in the literature [6].

The molecule of **1** formed a framework of four cyclohexane rings A (C1–5 and C10), B (C5–10), C (C8, C9, C11–14), and D (C8, C9, C11, C12, C15, C16); heterocycle E (C4, C5, C10, C20, C18, N); and five-membered ring F (C10, C9, C8, C14, C20). Six-membered rings A and B in the two independent molecules of **1** in the crystal adopted chair conformations; C and D, boats but with slight deviations from the ideal shape. Heterocycle E adopted a conformation close to a half-chair with atom C5 deviating from the plane of the other five atoms because of the C19=N double bond [the bond lengths for the two independent molecules were 1.269(3) and 1.271(3) E, respectively]. Five-membered ring F had a twist conformation.

According to the CCDC XSA database, the molecular structures of related alkaloids such as talassamine [7], talassimidine (7-acetoxytalassamine) [8], and 15-epitalassamine [7] are known. They differ from **1** by the positions of the C7 and C15 hydroxyls. A comparison using the Mercury program [9] of the molecular structures of the talassamine skeleton of **1** with those observed in the three known natural derivatives did not reveal conformational changes of the talassamine skeleton, which indicated that the framework in them was stable.

Intermolecular H-bonds between the two independent (asymmetric) molecules were observed in the crystal of **1**. The C5 hydroxyl H atom in one molecule approached the unshared electron pair of the N atom of the other molecule (O1–H…N′). The parameters of this bond were distances O1…N′ 2.899(3) and H…N′ 2.05(2) Å and angle 168(2)°. The crystal also had H-bonds between the C15 hydroxyl and the N atom of identical molecules (O2–H…N) translated along the *a* axis with parameters 2.822(3), 1.91(2), and 169(2). The next intermolecular H-bond was O–H…O between the C15 hydroxyl H atom and the same O2 atom translated by 2_1 symmetry along the *c* axis (O2′–H…O2). The parameters of this bond were 2.926(3), 1.90(2), and 155(2). These H-bonds formed a two-dimensional network in the *a0c* plane. The above intermolecular H-bonds differed from those observed in crystals of the related talassamine alkaloids [7, 8].

In conclusion, it is noteworthy that the presence or absence of hydroxyls and inter- and intramolecular H-bonds in the talassamine-type diterpenoid alkaloids did not substantially change the conformations.

EXPERIMENTAL

General. Mass spectra were measured on an LC/MS-IT-TOF mass spectrometer (Shimadzu, Kyoto, Japan). NMR spectra were recorded from $CDCl_3$ solutions on JNM-ECZ600R (JEOL, Japan) and Avance III-600 spectrometers (Bruker, Germany) at operating frequency 600 MHz for ^{1}H. The internal standard for PMR spectra was TMS (0 ppm); for ^{13}C NMR spectra, the solvent chemical shift ($CDCl_3$, 77.16 ppm vs. TMS). TLC used GF254 silica gel plates (Yantai Jiangyou Silicon Development Co., Yantai, China). Column chromatography (CC) used silica gel (200–300 mesh, Linyi Haixiang Co., Ltd., Linyi, China) and Sephadex LH-20 (Amersham Bioscience, Sweden).

Extraction and Isolation. The aerial part of air-dried *D. oreophilum* (2.8 kg) was extracted (3 ×) with EtOH (70%). The extracts were filtered and evaporated under vacuum. The solid was dissolved in H_2O (1 L) and extracted with EtOAc.

Dilute NaOH solution was added to the mother liquor to adjust the pH to 9–10. The solution was extracted (3 ×) with $CHCl_3$. The solvent was evaporated to produce a solid (22 g) that was chromatographed over a column of silica gel with elution by a $CHCl_3$–MeOH gradient (19:1, 9:1, 7:1, 4:1) to give fractions A–F. Fraction E (3.34 g) was rechromatographed over a column of silica gel with elution by petroleum ether–Me_2CO–Et_2NH (18:2:1–12:8:1) to produce fractions E-1–E-6. Fraction E-2 (844 mg) was rechromatographed over a column of silica gel with elution by petroleum ether–Me_2CO–Et_2NH (9:1:0.5–7:3:0.5) to produce fractions E-2-1–E-2-4. Chromatography of fraction E-2-4 (195 mg) over a column of silica gel with elution by $CHCl_3$–MeOH (19:1, 9:1) isolated **1** (15 mg). Fraction B (1.154 g) was rechromatographed over a column of silica gel with elution by $CHCl_3$–MeOH–Et_2NH (49:1:0.5–18:2:0.2) to produce fractions B-1–B-3. Fraction B-2 (168 mg) was chromatographed over a column of Sephadex LH-20 with elution by MeOH to afford **2** (18 mg). Fraction E-4 (1.221 g) was chromatographed over silica gel with elution by petroleum ether–Me_2CO–Et_2NH (8:2:0.5–12:8:1) to produce fractions E-4-1–E-4-3. Chromatography of fraction E-4-3 (75 mg) over a column of silica gel with elution by petroleum ether–Me_2CO–Et_2NH (95:5:5–80:20:5) isolated **3** (15 mg).

15-Epinaviculine B (1), colorless needle-like crystals, $C_{20}H_{27}NO_2$, mp 254–256°C. MS *m/z* 314.0580 $[M + H]^+$ (calcd for $C_{20}H_{28}NO_2$ 314.2120). Table 1 presents the ^1H and ^{13}C NMR spectra.

4-{[2-(Methoxycarbonyl)phenyl]amino}-2-methyl-4-oxobutanoic acid (2), colorless needle-like crystals, $C_{13}H_{15}NO_5$. ^1H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 11.18 (1H, s, NH), 8.67 (1H, dd, J = 8.5, 1.2, H-3), 8.03 (1H, dd, J = 8.1, 1.7, H-6), 7.53 (1H, ddd, J = 8.6, 7.2, 1.7, H-4), 7.09 (1H, ddd, J = 8.2, 7.2, 1.2, H-5), 3.93 (3H, s, 7-OCH_3), 3.09 (1H, m, H-12), 2.90 (1H, dd, J = 15.7, 8.1, H-11b), 2.57 (1H, dd, J = 15.7, 5.6, H-11a), 1.31 (3H, d, J = 7.1, H-14). ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm): 115.12 (C-1), 141.34 (C-2), 120.60 (C-3), 134.80 (C-4), 122.81 (C-5), 130.96 (C-6), 168.85 (C-7), 52.54 (C-8), 170.46 (C-10), 41.33 (C-11), 36.13 (C-12), 180.21 (C-13), 17.17 (C-14). MS *m/z* 264.0877 $[M - H]^-$ (calcd for $C_{13}H_{14}NO_5$, 264.0843).

Delavaine B (3), white compound. ^1H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.08 (3H, t, J = 7.1, N-CH_2 $\underline{CH_3}$), 1.28 (3H, d, J =7.2, H-5″), 1.69 (1H, dd, J =15.3, 6.9, H-3a), 1.74 (1H, br.s, H-5), 1.86 (1H, m, H-12a), 1.98 (1H, dt, J = 12.1, 6.4, H-10), 2.10 (1H, m, H-2a), 2.20 (1H, m, H-2b), 3.27 (3H, s, 1-OCH_3), 3.35 (3H, s, 16-OCH_3), 3.39 (3H, s, 14-OCH_3), 3.42 (3H, s, 6-OCH_3), 3.68 (3H, s, 4″-OCH_3), 3.92 (1H, br.s, H-6), 7.11 (1H, t, J = 7.1, H-5′), 7.56 (1H, m, H-4′), 7.98 (1H, m, H-6′), 8.71 (1H, dd, J = 8.6, 1.0, H-3′). ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm): 84.02 (C-1), 26.23 (C-2), 32.34 (C-3), 37.69 (C-4), 43.38 (C-5), 91.07 (C-6), 88.67 (C-7), 77.64 (C-8), 50.51 (C-9), 46.23 (C-10), 49.19 (C-11), 28.80 (C-12), 38.27 (C-13), 84.02 (C-14), 33.79 (C-15), 82.66 (C-16), 64.64 (C-17), 69.88 (C-18), 52.47 (C-19), 51.14 (N-CH_2), 14.21 (CH_3), 55.97 (1-OCH_3), 57.99 (6-OCH_3), 58.25 (14-OCH_3), 56.45 (16-OCH_3) 168.18 (C=O), 114.83 (C-1′), 142.00 (C-2′), 120.79 (C-3′), 135.06 (C-4′), 122.68 (C-5′), 130.42 (C-6′), 170.10 (C-1″), 41.56 (C-2″), 35.96 (C-3″), 176.14 (C-4″), 17.25 (C-5″), 51.90 (4″-OCH_3). Based on MS, ^1H and ^{13}C NMR spectra, **3** was identified as delavaine B [5].

XSA. Single crystals of **1** were grown by slow evaporation from MeOH at ambient temperature. The crystals were transparent and prismatic in shape. The unit-cell parameters of a crystal were determined and refined on an HPC XtaLAB Synergy diffractometer (Rigaku, Japan) using Cu Kα-radiation (T = 293 K, graphite monochromator). A three-dimensional dataset of reflections was obtained on the same diffractometer. Absorption corrections were applied semi-empirically using the SADABS program [10]. Table 2 presents the main crystallographic parameters and characteristics of the XSA and structure refinement of 1.

The structure was solved by direct methods using the SHELXS-97 program suite [11] and was refined using the SHELXL-97 program [12]. All nonhydrogen atoms were refined by anisotropic full-matrix least-squares methods (over F^2). Positions of H atoms were found geometrically and refined with fixed isotropic shift parameters $U_{iso} = nU_{eq}$, where n = 1.5 for methyls and 1.2 for others and U_{eq} is the equivalent isotropic shift parameter of the corresponding C atom. Hydroxyl H atoms of **1** were found in difference electron density syntheses and refined isotropically.

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