Journal of Natural Products
Vol. 58, No. 2, pp. 291-295, February 1995

TWO NEW NORDITERPENOID ALKALOIDS FROM *DELPHINIUM ELATUM* VAR. "BLACK NIGHT"

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ABSTRACT.—Two new norditerpenoid alkaloids, blacknine [**1**] and blacknidine [**2**], have been isolated from the whole plants of *Delphinium elatum* var. "black night" along with six known alkaloids. The structures of the new alkaloids **1** and **2** were derived from their spectroscopic data (^{1}H, ^{13}C, DEPT, COSY, HETCOR, and selective INEPT nmr experiments). The phytochemistry of this plant variety has not been examined previously.

Delphinium elatum L. (Ranunculaceae) has proven to be a rich source of norditerpenoid and diterpenoid alkaloids. Delpheline (1), deltaline (2–4), deltamine (5), elatine (2–4), and methyllycaconitine (1, 6) have been isolated from the whole plant. The following norditerpenoid alkaloids have been found in the seeds: 14-deacetylnudicauline (7), andersonidine (10), delcorine (10), delectinine (8), delelatine (9), delpheline (7, 11), deltaline (7), eladine (7, 10), elanine (8), elasine (7), elatine (7), isodelpheline (7), lycoctonine (7), methyllycaconitine (7), nudicauline (7), pacidine (10), pacifidine (10), pacifiline (10), pacifinine (10), paciline (11), pacinine (8,11), and yunadelphinine (10); two diterpenoid alkaloids have also been found: ajaconine (8) and delatisine (12).

We now report the isolation and characterization of two new norditerpenoid alkaloids, blacknine [**1**] and blacknidine [**2**], along with six known alkaloids, 14-deacetylnudicauline [**3**], delectinine [**7**], delelatine [**4**], delpheline [**5**], methyllycaconitine [**6**], and ajaconine [**8**] from the whole plants of *Delphinium elatum* var. "black night." Structures **1** and **2** were derived from spectroscopic data (ir, mass, ^{1}H, ^{13}C, DEPT, COSY, HETCOR, and selective INEPT nmr experiments). The known alkaloids were identified by comparison of their tlc behavior and ir, ^{1}H-, and ^{13}C-nmr data with those of authentic samples.

Blacknine [**1**] is a homogeneous amorphous solid. Its molecular formula $C_{23}H_{35}NO_6$ was derived from fabms and ^{13}C-nmr spectral studies. The ir spectrum of **1** showed the presence of -OH (3420 cm^{-1}) and ether groups (1122, 1070 cm^{-1}). The ^{1}H-nmr spectrum of compound **1** indicated the presence of a tertiary-Me (δ 1.05), an *N*-Et (δ 1.12), one methoxyl (δ 3.45), a carbinol proton (δ 4.20), and methylenedioxy protons (δ 5.09 and 5.15). The ^{13}C-nmr spectrum showed resonances for the 23 carbon atoms. The DEPT spectra indicated the presence of four quaternary, nine methine, seven methylene, and three methyl carbons, among the 23 carbons, as well as 32 hydrogen atoms in the molecule. Seven of these ^{13}C-nmr signals (δ 93.2 t, 92.3 s, 84.2 s, 81.3 d, 79.0 d, 74.5 d, and 72.1 d) could be attributed to oxygenated carbons. The presence of a methylenedioxy group accounts for three of these carbons (δ 93.2 t, 92.3 s, and 84.2 s). The multiplicity of the remaining oxygenated carbons was used to establish secondary functionality.

The methylenedioxy group (sharing two oxygens) and one methoxyl group accounted for three of the six oxygens of blacknine [**1**]. Therefore, the three remaining oxygens were present as hydroxyl groups. The three hydroxyl hy-

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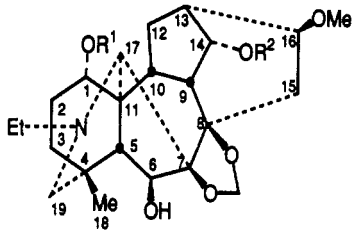
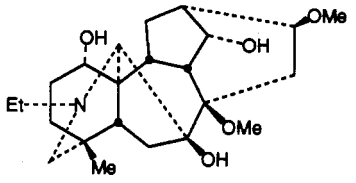


1 $R^1=R^2=H$
4 $R^1=Me, R^2=H$
5 $R^1=R^2=Me$



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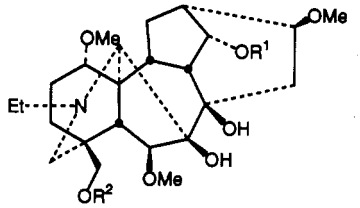
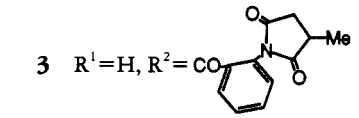
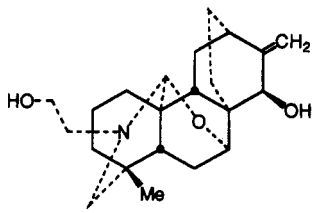




3 $R^1=H, R^2=$

6 $R^1=Me$, $R^2=$same as in **3**
7 $R^1=R^2=H$



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drogens bring the total number of hydrogens present in the molecule to 35, in accordance with the molecular formula of compound **1**.

The ^1H- and ^{13}C-nmr spectral characteristics of compound **1** are indicative of norditerpenoid alkaloids previously characterized from *D. elatum*. However, comparison of the spectral data for compound **1** with spectral data for previously characterized norditerpenoid alkaloids with the same molecular formula showed no correlation. Compound **1** was therefore established as a new norditerpenoid alkaloid.

Structure **1** for this new alkaloid was determined through nmr spectral experiments. The protons were identified by COSY experiments. Assignments of the carbons and placement of the functional groups were achieved by studying the HETCOR and the selective INEPT experiments. The methylenedioxy group was assigned to C-7 and C-8 by analogy with the naturally occurring alkaloids that have this group on these carbons. This assignment was confirmed by selectively pulsing protons at δ 5.15 and 4.27 (H-6), whereby the first experiment showed a response to a signal at δ 84.2 s (C-8) and the second one showed responses to the signals at δ 92.3 s (C-7), 50.9 s (C-11), and 32.8 s (C-4), When H-14 (δ 4.20) was selectively pulsed, responses of the signals at δ 84.2 s (C-8) and 81.3 d which should be for C-16 (3 bonds away from H-14) were observed; this result indicates that the only methoxyl group present in the molecule is at C-16. A β-OH group was assigned to C-6 because when H-5 (δ 1.47) was selectively pulsed, the responses observed were for the carbon signals at δ 79.0 d (C-6), 50.9 s (C-11), 92.3 s (C-7), 65.0 d (C-17), and 32.8 s (C-4). When H_3-18 (δ 1.05) was selectively pulsed, the responses observed were at δ 61.3 t (C-19), 52.4 d (C-5), 32.8 s (C-4), and 31.8 t (C-3). One of the two remaining -OH groups was assigned to C-1 (δ 72.1 d) because the ^{13}C-nmr chemical shift for a C-1α-OH is about 72 ppm, and the second -OH group (δ 74.5 d) was assigned to C-14 on account of a broad triplet for H-14. Thus, all the ^1H- and

^{13}C-nmr chemical shift assignments (see Table 1) for blacknine [**1**] agree with structure **1**.

The structure of blacknidine [**2**], the second new alkaloid, was derived through the application of similar methodology. The compound ($C_{23}H_{37}NO_5$) was a homogeneous amorphous solid which displayed hydroxyl (3400 cm^{-1}) and ether (1090 cm^{-1}) ir stretching bands. Its ^{1}H-nmr spectrum showed the presence of a tertiary-Me (δ 0.92), an *N*-Et (δ 1.12), two methoxyl groups (δ 3.40 and 3.45), and two carbinol protons (δ 3.65 and δ 4.03). The ^{13}C-nmr spectrum showed twenty-one signals of which two signals (27.6 t and 44.6 d) were coincident for two carbons. The DEPT spectra established four quaternary, eight methine, seven methylene, and four methyl carbons in the molecule. Five carbons were attached to oxygen.

The presence of four quaternary carbons (including two oxygenated) in the ^{13}C-nmr spectrum coupled with the appearance of two carbinol protons and two methoxyls in the ^{1}H-nmr spectrum was consistent with compound **2** being a norditerpenoid of the C-1, C-6, C-7, C-8, C-14, C-16 oxygenated lycoctonine-type with one less oxygenated carbon. DEPT experiments confirmed the presence of an additional methylene carbon in the molecule compared with the lycoctonine-type alkaloids, and this additional methylene can be assigned to C-

TABLE 1. ^{13}C- and ^{1}H-Nmr Chemical Shift Assignments for Compounds **1** and **2**.[a]

Position	Compound			
	1		**2**	
	δ_C	δ_H	δ_C	δ_H
1	72.1 d	3.75 1H, br s	72.5 d	3.65 1H, br m
2	29.5 t	1.29, 1.65 m	29.2 t	1.59, 1.76 m
3	31.8 t	1.52, 1.79 m	31.3 t	1.52, 1.67 m
4	32.8 s	—	33.2 s	—
5	52.4 d	1.47 br s	46.3 d	1.54 br s
6	79.0 d	4.27 br s	27.6 t	1.65 m
7	92.3 s	—	87.5 s	—
8	84.2 s	—	80.8 s	—
9	42.1 d	3.61 br s	44.6 d	2.35 m
10	45.4 d	2.17 m	44.6 d	1.85 m
11	50.9 s	—	50.1 s	—
12	28.6 t		27.6 t	1.90, 2.03 m
13	38.6 d	1.21, 2.18 m	39.6 d	2.33 m
14	74.5 d	4.20 br t	74.8 d	4.03 br t
15	34.0 t	1.82, 1.90 m	34.6 t	1.90, 2.87 m
16	81.3 d	3.40 m	82.0 d	3.45 m
17	65.0 d	3.07 br s	64.1 d	2.76 br s
18	26.5 q	1.05 3H, s	27.3 q	0.92 3H, s
19	61.3 t	2.32, 2.55 AB, J=11.6 Hz	59.5 t	2.39, 2.64 AB, J=11.5 Hz
20	49.9 t	2.75 m	50.6 t	2.94 m
21	13.5 q	1.12 3H, t, J=7.2 Hz	13.9 q	1.12 3H, t, J=7.1 Hz
8-OMe	—	—	52.6 q	3.45 3H, s
16-OMe	56.3 q	3.45 3H, s	56.5 q	3.40 3H, s
1-OH	—		—	5.98 br s
6-OH	—	2.85 br s	—	—
O-CH_2-O	93.2 t	5.09, 5.15 each s	—	—
7-OH	—	—	—	1.25 br s
14-OH	—	1.65 br s	—	1.25 br s

[a]Spectra were recorded in $CDCl_3$. Chemical shifts are in ppm downfield from TMS.

6 on the basis of selective INEPT nmr experiments. The presence of a C-8 methoxyl is revealed by the presence of a methyl carbon at δ 52.6 q as in ambiguine (13) and was confirmed when one of the methoxyl signals (δ 3.45) was selectively pulsed to show a response by a signal at δ 80.8 s (C-8). The second methoxyl group was assigned to C-16 on the basis of responses observed for the carbon at δ 82.0 d when the protons at δ 3.40 (OMe) and δ 4.03 (H-14), three bonds removed from the carbon, were selectively pulsed.

A β-OH group was assigned to C-7 because H-14β (δ 4.03) showed a broad triplet resulting from the coupling with H-9 and H-13 and hence this -OH group could not be assigned to C-9 or C-13. The other possibility, C-10, was ruled out as C-11 resonated at δ 50.1 which would have otherwise shown a downfield shift of around 5–6 ppm (a β-effect). It is interesting to note that although C-11 is in a β-position to C-1 (bearing an α-OH group) it did not experience a β-effect. This may be because the C-1 α-OH is strongly hydrogen bonded to the *N* atom with ring A in a boat conformation (13,14). The question of compound **2** being an artifact is less likely since no lycoctonine-type alkaloids having a C-8 acetyl group, capable of undergoing methanolysis, have been found in nature. These data are in accordance with the designation of structure **2** for blacknidine.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The optical rotations were measured on a Perkin-Elmer model 141 polarimeter in $CHCl_3$. Ir spectra were determined in Nujol, on a Perkin-Elmer model 1420 spectrophotometer. The fabms were determined on a JEOL model JMS-SX/SX 102A spectrometer and eims were determined on a Finnegan Quadrupole 4023 spectrometer. Nmr spectra were recorded on a Bruker AC 300 spectrometer operating at 300.13 MHz for ^1H and at 75.47 MHz for ^{13}C. The pulse sequences employed in the 1D, DEPT, and 2D nmr experiments were those of the standard Bruker software. The pulse sequence for the selective INEPT experiments was obtained by modifying the Bruker standard INEPT sequence and the critical parameters used were as described previously (15). Chromatographic separations on a Chromatotron (16) were carried out on rotors coated with 1-mm thick layers of Merck Al_2O_3 60 PF 254, 365 (EM 1104) or SiO_2 60H PF 254 (EM 7749); vacuum-liquid chromatography (vlc) (17) was carried out with Merck Al_2O_3 (EM 1085) and SiO_2 60H (EM 7736). All the known compounds isolated were identified by comparing their spectral data and tlc behavior with those of authentic samples available in our laboratory.

PLANT MATERIAL.—The plants of *Delphinium elatum* var. "black night" were grown by Goodness Grows, Inc., Lexington, Georgia, from seeds supplied by Park Seeds Wholesale, Greenwood, SC. A herbarium specimen of the plant has been deposited in the herbarium of the Botany Department at the University of Georgia. The whole plants bearing large violet flowers grown as annuals were collected in August 1993, and were air-dried in the shade and ground to a 30 mesh size.

EXTRACTION OF ALKALOIDS.—The finely powdered plant material (1,887 g) was first defatted (in a percolator) with hexane (3×3 liters) and then exhaustively extracted with aqueous 70% EtOH (7×2.5 liters) at room temperature. The EtOH extract was concentrated *in vacuo* to a syrupy mass (340.3 g). The concentrate was treated with $CHCl_3$ (500 ml) and then extracted with 2% H_2SO_4 (v/v) (5×200 ml). After the ice cooled, the combined H_2SO_4 extract was subjected to a gradient pH separation as usual (18) to give crude alkaloid mixtures at pH 4.5 (2.21 g), pH 10 (3.2 g), and pH 14 (1.16 g). The crude alkaloid mixtures obtained at pH 4.5 and pH 10 were each subjected to an acid/base extraction to give the purified alkaloid mixtures A (1.46 g, pH 4.5) and B (1.094 g, pH 10).

Isolation of delpheline [**5**], *methyllycaconitine* [**6**], *14-deacetylnudicauline* [**3**], *delelatine* [**4**], *and blacknine* [**1**].—The alkaloid mixture A (1.43 g) dissolved in $CHCl_3$ (10 ml) was passed over a small column of Al_2O_3 (neutral, activity III). The column was eluted with $CHCl_3$ (100 ml). Evaporation of the eluate *in vacuo* gave a clean residue (1.186 g) from which a 300-mg aliquot was fractionated on an Al_2O_3 rotor of a Chromatotron. Elution of the alkaloids was carried out with a solvent gradient of hexane, $CHCl_3$, and MeOH. The fractions were collected according to the uv-visible bands (254 or 365 nm) eluted from the rotor. Nineteen fractions were collected, concentrated, and then analyzed by tlc. The results of the tlc indicated the presence of five homogeneous alkaloid fractions. The residues from each fraction were analyzed by nmr spectra (^1H and ^{13}C). Fraction 2 (eluted with hexane/20% $CHCl_3$) gave delpheline [**5**] (38.5 mg), fraction 8 (hexane/40%

$CHCl_3$) gave methyllycaconitine [**6**] (26.0 mg), fraction 12 (hexane/55% $CHCl_3$) gave 14-deacetylnudicauline [**3**] (34.5 mg), and fraction 13 (hexane/60% $CHCl_3$) and fraction 14 (hexane/70% $CHCl_3$) gave delelatine [**4**] (7.1 mg and 24.1 mg). Fraction 15 ($CHCl_3$) gave the new alkaloid blacknine [**1**] (12.5 mg).

Blacknine [**1**], amorphous solid; $[\alpha]_D$ −5.13° (c=0.887); fabms *m/z* 422.27 $[M+1]^+$ ($C_{23}H_{35}NO_6$ requires *m/z* 421.2464); eims *m/z* 390 $[M-31]^+$ (18.9), 377 (28.3), 361 (8.4), 359 (7.1), 105 (11.4), 100 (14.8), 95 (11.7), 43 (100); ir ν max 3420, 1122, 1070, 750 cm^{-1}. For ^1H- and ^{13}C-nmr assignments, see Table 1.

Isolation of blacknidine [**2**], *delectinine* [**7**], *and ajaconine* [**8**].—Fraction B (pH 10) (1.094 g) was purified on a small column of Al_2O_3 (neutral, activity III) as described above, to give a clean alkaloid mixture (0.61 g). An aliquot containing 300 mg was loaded on an Al_2O_3 rotor of a Chromatotron. The elution was carried out with a gradient of hexane, $CHCl_3$, and MeOH. Twenty fractions were collected according to the uv-visible bands (254 or 365 nm) eluted from the rotor. Fraction 4 [eluted with hexane-$CHCl_3$ (1:1)] gave the new alkaloid blacknidine [**2**] (8.1 mg); fraction 15 (hexane/80% $CHCl_3$) gave delectinine [**7**] (16.5 mg), and fraction 17 ($CHCl_3$) gave ajaconine [**8**] (15.3 mg).

Blacknidine [**2**], amorphous solid; $[\alpha]_D$ +8.05° (c=0.642); fabms *m/z* 408.25 $[M+1]^+$ ($C_{23}H_{37}NO_5$ requires *m/z* 407.2671); eims *m/z* 376 $[M-31]^+$ (11.8), 361 (62.5), 81 (18.2), 77 (16.3), 72 (12.9), 69 (24.6), and 43 (100); ir ν max 3400, 1260, 1090, 800 cm^{-1}. For ^1H- and ^{13}C-nmr assignments, see Table 1.

ACKNOWLEDGMENTS

This work was partially supported by Grant HL 32562 from the National Institutes of Health. We thank Dr. Balawant S. Joshi for reading the manuscript and making helpful suggestions. We thank Dr. R.C. Orlando of the Complex Carbohydrate Center, University of Georgia, for the fabms and Mr. Courtney Pape for the eims. One of the authors (J.C.P.) wishes to thank the Yonam Foundation, Republic of Korea, for providing financial support.

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Received 8 August 1994