NEW ALKALOIDS FROM *DELPHINIUM ANDERSONII* GRAY[+]

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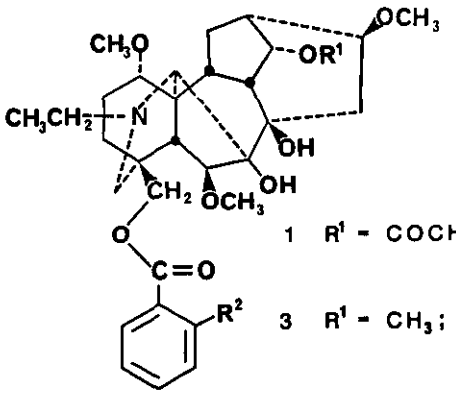
Abstract - Andersonine (1) and 14-deacetylnudicauline (2), two new C_{19}-diterpenoid alkaloids, have been isolated from *Delphinium andersonii* Gray. Structures were deduced by spectroscopic methods. The structures of andersonine and 14-deacetylnudicauline were confirmed by correlation with nudicauline (5). Six known alkaloids were also isolated: delavaine (3), delectinine (8) lycoctonine (7), methyllycaconitine (6), nudicauline (5), and takaosamine (4).

Extraction of the aerial parts of *D. andersonii* Gray with 95% ethanol, followed by gradient pH separation afforded a mixture of alkaloids in ca. 0.34% yield. Extensive chromatographic purification, including vacuum liquid chromatography (VLC)[1], and centrifugally accelerated, radial, thin-layer chromatography (Chromatotron)[2,3] of the pH 8 extract[4] furnished two new C_{19}-diterpenoid alkaloids designated as andersonine (1) and 14-deacetylnudicauline (2), and four known alkaloids, delavaine (3)[5], takaosamine (4)[6,7], nudicauline (5)[7,8] and methyllycaconitine (6).[9] Takaosamine isolated from *Aconitum japonicum*[6] was reported to have mp 174-175°C. Our sample (as well as a recent isolate[7]) was amorphous and was identified by ms, ^1H and ^{13}C nmr data. The sample also showed tlc behavior identical with that of an authentic sample of takaosamine. From the pH 12 extract two known C_{19}-diterpenoid alkaloids, lycoctonine (7)[10,11] and delectinine (8)[12,13] were also isolated by droplet counter current chromatography (dccc) and purification on a Chromatotron.

The ^1H and ^{13}C nmr spectral data of andersonine (1) showed similarities with those of delavaine (3)[6]. The only observable difference in their ^1H nmr spectra was that one of the methoxyl groups of delavaine (3) was replaced by an acetate group in andersonine. That the acetate function was located at C-14 was evident by the presence of a characteristic H-14β

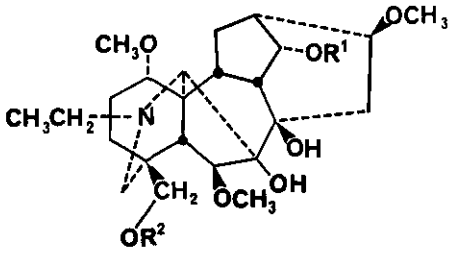
[+] Abstracted in part from the Ph.D. thesis of A. Mukendi Panu, University of Georgia, Athens (1986).

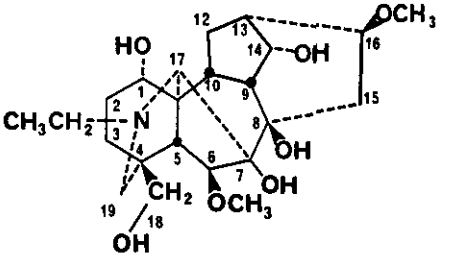
signal at δ4.76 (t, J = 4.5 Hz). Accordingly, the ^{13}C nmr spectrum showed an upfield shift for C-14 (Δ8.1 ppm). The carbon shifts of the andersonine nucleus were also in agreement with those of nudicauline[7], which differs only in the nature of the C-18 ester residue. Andersonine (1) may possibly be an artifact formed from nudicauline (5) during the isolation process, but this possibility is not certain. We have shown that when nudicauline (5) is stirred for two days in methanol in the presence of a large amount of alumina, some andersonine is produced.

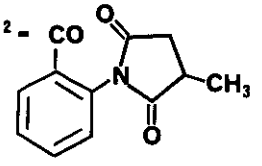


1 R^1 = $COCH_3$; R^2 = $HN-COCH_2CH(CH_3)-COOCH_3$ / $HN-COCH(CH_3)-CH_2COOCH_3$

3 R^1 = CH_3; R^2 = as above





2 R^1 = H; R^2 = 

4

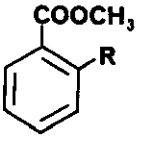
5 R^1 = $COCH_3$; R^2 = as above

6 R^1 = CH_3; R^2 = as above

7 R^1 = CH_3; R^2 = H

8 R^1 = R^2 = H

9 R^1 = H; R^2 = CH_3



10 R = $HN-COCH_2CH(CH_3)-COOCH_3$ / $HN-COCH(CH_3)-CH_2COOCH_3$

Structure 2 was deduced for the second new alkaloid, $[\alpha]_D$ +24° (c 0.55, $CHCl_3$), from its spectral data. The electron impact ms showed the molecular ion peak at m/z 668 ($C_{36}H_{48}N_2O_{10}$) and a base peak at 637 (M^+ -31) corresponding to the loss of the C-1 methoxyl group. The ^1H nmr spectrum was similar to that of methyllycaconitine (6), except that it lacked one methoxyl signal. In the ^{13}C nmr spectrum one of the oxygenated methine carbons showed an upfield shift (Δ8.6 ppm) when compared with that of 6, indicating that in 2 one of the methoxyl groups was replaced by a secondary hydroxyl group. The hydroxyl group must be placed at C-14 because the chemical shift (75.3 ppm) agrees with that found in similar compounds, such as browniine (9).[11] The structure of 14-deacetylnudicauline (2) was confirmed by conversion to its monoacetate which was identical in all respects with nudicauline (5).[7]

EXPERIMENTAL

Melting points are corrected and were determined on a Thomas-Kofler hot stage equipped with a microscope and polarizer. Infrared spectra were recorded on a Perkin-Elmer model 1420 spectrophotometer. ^1H and ^{13}C nmr spectra were obtained on JEOL FX-90 Q and FX-270 spectrometers in $CDCl_3$. Mass spectra were determined on a Finnigan Quadrupole 4023 and Ribermag R 10-10C QMS instruments. Chromatographic separations on a Chromatotron[2,3] were carried out on rotors coated with a 1 mm thick layer of neutral alumina (60 GF-254, type E, EM Art 1092). Vlc[1] was carried out with Merck alumina (60 HG-254, type E, Art 1094). Droplet counter current chromatography (dccc) was carried out on a model DCC-A instrument, manufactured by Tokyo Rikakikai Co. Ltd, Tokyo, Japan.

Plant Material - The aerial parts of *D. andersonii* Gray were collected 18 to 27 April, 1978 from 'Wildcat Hills' at an altitude of about 4800 feet, 18 miles southwest of Snowville, Utah. The stage of growth varied from plants having predominantly basal leaf clusters and flowering scapes 3 inches high to plants with flowering scapes 20 inches high. The major amount of plant material was collected from plants having a flowering scape of 12 inches in the bud stage. The plant was identified by Dr. Leila McReynolds Shultz, Curator of the Intermountain Herbarium, Utah State University, Logan, Utah. A voucher specimen (UTC accession no. 201385) has been deposited in the Intermountain Herbarium.

Extraction of Alkaloids - The dried, ground plant material (9444 g) was first defatted with hexane and then extracted with 95% EtOH at room temperature to give 1400 g of extract. A portion of the above extract (386.4 g) was fractionated by gradient pH extraction[4] to yield 8.38 g (pH 8) and 0.40 g (pH 12) of crude alkaloids.

Fractionation of the pH 8 Extract - A portion (7.7 g) of the pH 8 extract was chromatographed (vlc)[1] on alumina (450 g of a 1:1 mixture of tlc grade alumina, type E, Art 1094 and basic alumina, type E, EM Art 1085) to give fractions 1-9 (1% EtOH in hexane, 3.2 l, 0.08 g), 10-14 (2% EtOH in hexane, 1.8 l, 0.72 g), 15-19 (2% EtOH in hexane, 1.8 l, 0.17 g), 20-35 (2-10% EtOH in hexane, 5.6 l, 1.07 g), 36-39 (10% EtOH in hexane, 1.4 l, 0.36 g), 40 (MeOH, 7.5 l, 3.8 g), 41 (20% MeOH in $CHCl_3$, 3.8 l, 0.6 g) and 42(2.5% CH_3COOH in MeOH, 0.9 l, 0.5 g).

Fraction 40 (3.5 g) was further fractionated as described above to afford fractions 1-3 (2% EtOH in hexane, 0.35 l, 0.575 g), 4-6 (5% EtOH in hexane, 1.05 l, 0.91 g), 7(10% EtOH in hexane, 0.35 l, 0.453 g), 8(10% EtOH in hexane, 0.35 l, 0.113 g), 9(10% EtOH in hexane, 0.3 l, 0.1 g); 10 ($CHCl_3$, 0.325 l, 0.054 g), 11(50% MeOH in $CHCl_3$, 0.4 l, 0.418 g), 12(MeOH, 1 l, 0.192 g) and 13(2% CH_3COOH in MeOH, 0.5 l, 0.246 g).

Isolation of Andersonine (1), Delavaine (3) and Takaosamine (4) - Purification of fractions 1-3 (574 mg) obtained above on a vlc column (tlc grade alumina, 29 g) afforded fractions 1 (hexane, 0.125 l, 0.013 g), 2-3 (0.5% EtOH in hexane, 0.25 l, 0.004 g), 4-5 (1% EtOH in hexane, 0.2 l, 0.192 g), 6(1% EtOH in hexane, 0.1 l, 0.079 g), 7 (1% EtOH in hexane, 0.1 l, 0.063 g), 8(1% EtOH in hexane 0.13 l, 0.098 g), 9-10 (2% EtOH in hexane, 0.22 l, 0.041 g), 11(4% EtOH in hexane, 0.2 l, 0.012 g), 12(CH_2Cl_2, 0.2 l, 0.01 g) and 13(50% MeOH in $CHCl_3$, 0.1 l, 0.013 g). Fraction 6 (79 mg) was andersonine (1). Amorphous; $[\alpha]_D$ +34° (c 1.0, $CHCl_3$); ir(nujol): 3460, 3310, 1738, 1705, 1680, 1605, 1590 and 1250 cm^{-1}; eims: m/z(%) 462(M^+ -280, 35), 434(40), 418 (50), 388(40), 216(38) and 188(45); ^1H nmr: δ1.07(3H, t, J = 7.1 Hz, N-CH_2-CH_3), 1.28 and 1.35 (3H, each d, J = 5.8 Hz, CH-CH_3), 2.07 (3H, s, OAc), 3.27(3H, s) and 3.34(6H, s) (OCH_3), 3.68 and 3.71 (3H, each s, $COOCH_3$), 4.76(1H, t, J = 4.5 Hz, H-14β), 7.12(1H, td, J = 9, 1.5 Hz), 7.56(1H, td, J = 9, 1.5 Hz), 7.96(1H, dd, J = 9, 1.5 Hz), 8.72(1H, dd J = 9, 1.5 Hz) (aromatic protons). For ^{13}C nmr data see Table 1. Fraction 8 (98 mg) contained mainly one component and was repurified on alumina (vlc) to yield a homogeneous substance (29 mg) which was identical with delavaine by ^1H and ^{13}C nmr spectra.[5] Fraction 11 (375 mg) obtained from vlc of fractions 1-3 (574 mg, see above) was repurified on a Chromatotron to afford 78 mg of takaosamine (4), identified by ^1H and ^{13}C nmr spectra.[7]

Isolation of Nudicauline (5), Methyllycaconitine (6) and 14-Deacetylnudicauline (2) - Another sample of pH 8 alkaloidal extract (8.0 g) was chromatographed on a gravity column packed with neutral alumina (activity III, 300 g). Fractions were collected as follows: 1-23 (0-0.75% EtOH in hexane, 4 l, 0.14 g), 24-26 (0.75% EtOH in hexane, 0.6 l, 0.06 g), 27-37 (0.75% EtOH in hexane, 2 l, 0.13 g), 38-51 (0.75-1% EtOH in hexane, 2.6 l, 0.11 g), 52-90 (2-5% EtOH in

hexane, 8 l, 1.2 g), 91-109 (5-10% EtOH in hexane, 4 l, 2.73 g), 110-112 (20% EtOH in hexane, 0.6 l, 1.48 g) and 113-115 (2% CH_3COOH in MeOH, 1.5 l, 1.71 g). A portion of fractions 91-109 (1.02 g) was rechromatographed (vlc) on alumina. Elution was carried out with hexane, hexane-acetone, acetone and $CHCl_3$-MeOH. The fraction eluted with 30-50% acetone in hexane (218 mg) was further chromatographed (vlc) on alumina to give 84.5 mg of pure nudicauline (5). Nudicauline was identified by $[\alpha]_D$, ^1H and ^{13}C nmr spectra.[7] The fraction eluted with 50% acetone in hexane (160 mg) was identified as methyllycaconitine (6) by comparison (^1H and ^{13}C nmr spectra) with an authentic sample.[9] The fraction eluted with acetone (223 mg) was further purified (vlc) on alumina. Elution with 29% MeOH in acetone gave a fraction (81 mg) which upon repurification on a Chromatotron furnished 36 mg of 14-deacetylnudicauline (2). Amorphous; ir (nujol): 3460, 1715, 1465, 1380 cm^{-1}; eims: m/z (%) 668 (M^+, $C_{36}H_{48}N_2O_{10}$,3), 653 (M^+ -15, 17), 637 (M^+ -31, 100), 216(75), 188(25) and 146(25); cims (methane): m/z (%) 697 (M +29), 669 (M +1), 651(95), 637(18), 619(20), 216(100), 190(20) and 149(20); ^1H nmr: δ 1.06 (3H, t, J = N-CH_2-CH_3), 1.44 (3H, d, J = 6.3 Hz, CH-CH_3), 3.25 (3H, s, OMe) and 3.36 (6H, s, OMe), 4.09 (1H, s, OH), 7.28 (1H, dd, J = 8.5, 1.5 Hz), 7.62 (2H, m) and 8.04 (1H, dd, J = 8.5, 1.5 Hz) (aromatic protons). For ^{13}C nmr data see Table 1.

Isolation of Lycoctonine (7) and Delectinine (8) - A total of 1.58 g of the pH 12 alkaloid extract was initially fractionated by dccc. Elution with benzene-$CHCl_3$-MeOH-H_2O, 5:5:7:2 gave a mixture of two compounds (260 mg). Purification of this mixture on a Chromatotron gave lycoctonine (28 mg), mp 83.5-86.5°C (CH_2Cl_2), identified by ^{13}C nmr spectrum, and an impure fraction. The latter was repurified as above to give 17 mg of delectinine identified by ^1H and ^{13}C nmr data.

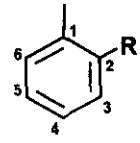
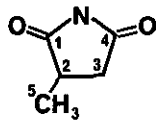
Correlation of Nudicauline (5) with Delectinine (8) - Nudicauline (22 mg) was stirred with 5 ml of 5% methanolic KOH at room temperature for two days. 50 ml of ice-cold water was added and the resulting solution was extracted with $CHCl_3$ (4 x 50 ml). Evaporation of solvent gave 18 mg of delectinine (8). Although delectinine thus obtained was amorphous (lit.[12] mp 167-169°C) it had identical ^1H and ^{13}C nmr data with those reported.[13]

Correlation of Andersonine (1) with Nudicauline (5) - A solution of nudicauline (12 mg) in 3 ml of MeOH was stirred with 400 mg of a 1:1 mixture of the tlc grade alumina (basic, type E, Art 1094) and alumina (basic, type E, Art 1085) at room temperature for two days. The mixture was filtered through a sintered funnel and the alumina was washed with 10 ml of MeOH. The combined filtrate was purified on a silica rotor (HF-254 + 366, 1 mm, Art 7741) of a Chromatotron to yield 1 mg of ester 10 and 7 mg of andersonine (1), identified by ^1H and ^{13}C

nmr spectral data.

Correlation of 14-Deacetylnudicauline (2) with Nudicauline (5) - A mixture of 14-deacetylnudicauline (39 mg), 1.5 ml of pyridine and 1.5 ml of AC_2O was kept at room temperature for three days. Usual workup and subsequent purification on a Chromatotron gave nudicauline (5) (29 mg), which exhibited identical tlc behavior, and ^1H and ^{13}C nmr data with those of an authentic sample.[7]

Table 1. ^{13}C Nmr data (22.49 MHz) of andersonine (1) and 14-deacetylnudicauline (2)

Carbon	1	2	Carbon		1	2
1	83.8	84.9	C=O		168.1	164.2
2	26.1	25.5		1	114.7,114.8	127.1
3	32.2	32.2		2	141.7,141.9	133.1
4	37.6	37.9		3	120.7	129.4
5	42.5	45.2		4	135.0	133.7
6	90.7	90.4		5	122.7	131.1
7	88.4	89.2		6	130.3	130.1
8	77.4	76.3	HN-C=O		172.2	-
9	50.1	50.3	CH-CH_3		39.1,18.0	-
10	45.2	46.1	CH_2		39.1	-
11	49.0	48.4	C=O		174.1	-
12	28.2	27.5	OCH_3		51.7	-
13	38.2	36.5	HN-C=O		169.9	-
14	75.9	75.3	CH_2		41.5	-
15	33.7	33.2	CH-CH_3		35.9,17.1	-
16	82.4	81.8	C=O		175.9	-
17	64.5	65.1	OCH_3		51.9	-
18	69.6	69.5		1	-	179.8
19	52.3	52.4		2	-	35.3
N-CH_2	51.0	51.2		3	-	37.1
CH_3	14.1	14.2		4	-	175.9
1′	55.8	56.0		5	-	16.5
6′	58.1	58.3	C=O		171.9	-
16′	56.3	56.5	CH_3		21.6	-

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