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NORDITERPENOID ALKALOIDS FROM THE STEMS AND LEAVES OF *DELPHINIUM AJACIS*

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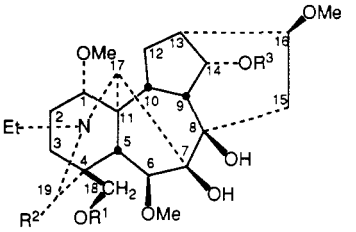
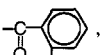
ABSTRACT.—Two new and eleven known norditerpenoid alkaloids from the stems and six known norditerpenoid alkaloids from the leaves have been isolated from *Delphinium ajacis*. Six alkaloids from the stems and three from the leaves isolated in the present work are new to this plant species. The new alkaloids, 19-oxoanthranoyllycoctonine and 19-oxodelphatine, were assigned the structures **7** and **8**, respectively, on the basis of ^{1}H- and ^{13}C-nmr spectral data and synthesis. Oxidation of ajacine [**9**] with OsO_4 afforded 19-oxoajacine [**10**], which was hydrolyzed to 19-oxoanthranoyllycoctonine [**7**]. Oxidation of anthranoyllycoctonine [**11**] with OsO_4 also yielded **7**. 19-Oxoajacine is a new synthetic alkaloid.

The diterpenoid alkaloids from *Aconitum* and *Delphinium* species are isolated mainly from the seeds, roots, or aerial parts of the plant. *Delphinium ajacis* L. (Ranunculaceae) is an ornamental plant with the common name of garden larkspur. From the seeds of *D. ajacis* [syn. = *Consolida ambigua* (L.) P.W. Ball & Heyw.] several lycoctonine-type diterpenoid alkaloids have been isolated (1,2). We now report the isolation and identification of thirteen norditerpenoid alkaloids from the stems and six from the leaves of *D. ajacis* cultivated in Assiut, Egypt.

RESULTS AND DISCUSSION

Of the thirteen alkaloids isolated from the stems, two are new diterpenoid alkaloids, five were previously reported from the seeds of *D. ajacis* (2), and six known alkaloids are being reported for the first time. The five previously reported (1) alkaloids are anthranoyllycoctonine, browniine, delcosine, delphatine, and delsoline. The six



1 R^1=  , R^2=H_2, R^3=H

9 R^1=  , R^2=H_2, R^3=Me

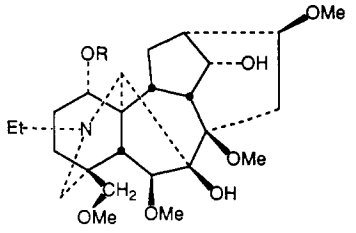
7 R^1=  , R^2=O, R^3=Me

10 R^1=  , R^2=O, R^3=Me

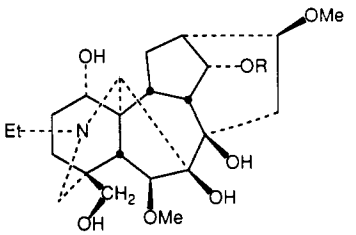
8 R^1=Me, R^2=O, R^3=Me

11 R^1=  , R^2=H_2, R^3=Me

[1]On leave from the Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt.



2 R=Me
3 R=H

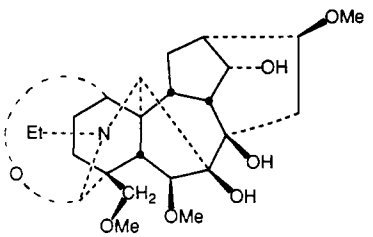


4 R=Me
5 R=H

known alkaloids (2,3) which are new to this plant are delectine [**1**], 14-deacetylambiguine [**2**], deltatsine [**3**], gigactonine [**4**], takaosamine [**5**], and 18-methoxygadesine [**6**]. The two new alkaloids are assigned structures of 19-oxoanthranoyllycoctonine [**7**] and 19-oxodelphatine [**8**].

Three of the alkaloids, delsoline, delcosine, and anthranoyllycoctonine [**11**], isolated from the leaves of this plant were previously reported, and deltaline [**12**], delpheline [**13**], and gigactonine [**4**] are new to this plant. All the known alkaloids isolated from the stems and leaves were identified by comparing their mp's, tlc, and ^{1}H- and ^{13}C-nmr spectra with those reported (2). Interestingly, deltaline [**12**] and delpheline [**13**], alkaloids bearing the 7,8-methylenedioxy group, appeared to be absent in the stems or seeds of this plant.

The new alkaloid 19-oxoanthranoyllycoctonine [**7**] was amorphous, $[\alpha]_D$ +58.1°. The molecular formula $C_{32}H_{44}N_2O_9$ was derived on the basis of its eims *m/z* 600 $[M]^+$ and its ^{13}C-nmr spectral data. Its ir spectrum showed absorption at 3450 (OH), 3350 (NH_2), 1690 (anthranilate C=O), and 1620 (lactam) cm^{-1}. The ^{1}H-nmr spectrum indicated the presence of an *N*-ethyl group at 1.14 ppm (3H, t, J = 7.2 Hz, *N*-CH_2CH_3), four methoxyl groups at 3.24, 3.35, 3.38, and 3.44 ppm (each 3H, s), two hydroxyl groups at 3.42 and 3.98 (each 1H, s, exchanges with D_2O), a primary amino group at 5.57 ppm (2H, br s, exchanges with D_2O), and aromatic protons at 6.67–7.78 ppm. Its ^{13}C-nmr spectrum exhibited thirty-two signals for thirty-two carbon atoms present in the molecule, and DEPT experiments revealed eight quaternary carbons, thirteen methines, six methylenes, and five methyl carbons. Most of the chemical shifts for **7** are consistent with the assignments of norditerpenoid alkaloid lactams recorded earlier (4,5). The chemical shift assignments for C-10 and C-13 have been made in conformity with earlier findings (6). Finally, the structure of compound **7** was confirmed by synthesis from both ajacine [**9**] and anthranoyllycoctonine [**11**]. Oxidation of **9** with OsO_4 afforded 19-oxoajacine [**10**]. Refluxing **10** with 4% aqueous HCl at 100 ° for 6 h gave **7**. Also oxidation of **11** with OsO_4 yielded **7** in a 92.5% yield. Identity of the natural and synthetic 19-oxoanthranoyllycoctonine was confirmed by tlc behavior, ir, mass, ^{1}H-nmr, and ^{13}C-nmr spectra.



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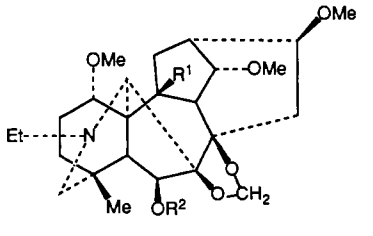

12 R^1=OH, R^2=Ac
13 R^1=R^2=H

The alkaloid 19-oxodelphatine [**8**], $C_{26}H_{41}NO_8$, was isolated as an amorphous solid, $[\alpha]_D$ +32.8°; eims *m/z* 496 $[M+1]^+$. Its ^{1}H-nmr spectrum showed the presence of the methyl of an *N*-ethyl group at δ 1.10 ppm (3H, t, $J=7.2$ Hz) and five methoxyl groups at 3.19, 3.32, 3.36, 3.43, and 3.44 ppm (each 3H, s). The ^{13}C-nmr spectrum of **8** exhibited twenty-six lines for twenty-six carbon atoms of the molecule. The DEPT spectra showed the presence of five quaternary carbons, nine methines, six methylenes, and six methyl carbons. The ^{13}C chemical shift assignments are consistent with the lactam structure **8** assigned for this alkaloid (4,5). Compound **8** was prepared earlier (7) by $KMnO_4$ oxidation of delphatine.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Spectra were recorded on the following instruments: ir Perkin-Elmer model 1420; ^{1}H nmr Bruker WM 300; ^{13}C nmr JEOL FT models FX 60 and FX 270 (for DEPT); and ms Finnigan Quadrupole model 4023. Optical rotations were measured on a Perkin-Elmer model 141 polarimeter. Chromatographic separations were carried out using vacuum liquid chromatography (vlc) (8) and on a "Chromatotron" (9) with rotors coated with 1-mm-thick layers of Al_2O_3 (EM 1104-3) or Si gel (EM 7741).

PLANT MATERIAL.—The plants of *D. ajacis* were cultivated in September 1988, in the Experimental Station of the Faculty of Pharmacy, Assiut University, Assiut, Egypt and collected during the flowering stage in April 1989. Seeds were supplied and the plants were identified by Professor Naeem E. El-Keltawy, Faculty of Agriculture, Assiut University. A voucher specimen (no. 105) has been deposited in the herbarium of the Department of Pharmacognosy of Assiut University.

ISOLATION OF ALKALOIDS FROM THE STEMS.—Air dried and powdered stems (1055 g) of *D. ajacis* were defatted with hexane (3 × 2.5 liters) and then extracted at room temperature with 80% EtOH (9 × 3.5 liters). The 80% EtOH extract was passed over DOWEX 50W X8; H^+ (300 g) until all the basic compounds were retained on the column (10). The column was basified with 10% NH_4OH solution, and the resin was extracted with CH_2Cl_2 in a Soxhlet extractor. The crude alkaloidal fraction (3.56 g) was again purified through an acid-base extraction procedure to give a foam of the alkaloidal mixture (2.03 g, 0.19%). A portion of the mixture (1.71 g) was fractionated on a vlc (Al_2O_3, 66.5 g, EM 1085), eluting with a gradient of hexane, Et_2O, and MeOH. In all, sixteen fractions (200 ml each) were collected.

Fractions 2 and 3 (eluted with 20% and 30% Et_2O/hexane) were combined (166.0 mg) and purified twice on an Al_2O_3 rotor (hexane/Et_2O gradient) to give 14-deacetylambiguine [**2**] (10.2 mg) (2).

Fractions 5 and 6 (188.1 mg, 50% Et_2O/hexane) were further fractionated on an Al_2O_3 rotor with a gradient of hexane/Et_2O and EtOH. This fractionation gave four main fractions A (90.2 mg), B (33.0 mg), C (17.1 mg), and D (23.3 mg). Fraction A was separated on a Si gel rotor ($CHCl_3$/MeOH gradient) to give delphatine (13.1 mg) (1). Fraction B was separated on an Al_2O_3 rotor (hexane/Et_2O gradient) to give delsoline (11.0 mg) (1). Fraction C (Et_2O/5% EtOH) was chromatographed on a small column of Al_2O_3 (neutral, activity III) to afford 19-oxodelphatine [**8**] (17.0 mg): $[\alpha]_D$ +32.8° ($c=0.307$, $CHCl_3$), eims *m/z* (%), $[M+H]^+$ 496 (8.8), $[M-Me]^+$ 480 (33.7), $[M-Me-H_2O]^+$ 462 (9.7), 71 (71.1), 45 (100); ir (Nujol) ν max 3440 (OH), 1630 (lactam C=O) cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 1.10 (3H, t, $J=7.2$ Hz, *N*-CH_2CH_3), 3.19, 3.32, 3.36, 3.43, and 3.44 (each 3H, s, 5 × OMe), 3.66 (1H, dd, $J_1=J_2=4.5$ Hz, H-14β), 3.82 (1H, s, H-6α) 3.40, 4.12 (each 1H, s, exchanges with D_2O, 2 × OH), 3.63–3.72 (2H, AB q, $J=9.6$ Hz, H-18); ^{13}C nmr ($CDCl_3$) δ 81.5 d (C-1), 24.7 t (C-2), 29.4 t (C-3), 47.6 s (C-4), 48.3 d (C-5), 91.4 d (C-6), 86.0 s (C-7), 76.7 s (C-8), 42.8 d (C-9), 45.0 d (C-10), 49.4 s (C-11), 28.7 t (C-12), 37.7 d (C-13), 83.7 d (C-14), 33.0 t (C-15), 82.2 d (C-16), 63.2 d (C-17), 74.1 t (C-18), 171.1 s (C-19), 43.5 t (*N*-CH_2Me), 12.0 q (*N*-CH_2CH_3), 55.2 q (C-1′), 57.9 q (C-6′), 58.0 q (C-14′), 56.4 q (C-16′), 58.9 q (C-18′).

Fraction 7 of the vlc fractionation (72.0 mg, eluted with 70% Et_2O-hexane), when purified on an Al_2O_3 rotor gave more of delsoline (38.1 mg).

Fraction 9 (108.0 mg, Et_2O) was fractionated on an Al_2O_3 rotor. The fractions eluted with 10% EtOH in Et_2O gave delectine [**1**] (8.0 mg) (2) when crystallized from Me_2CO/hexane.

Fraction 8 (128.0 mg, Et_2O) was purified on an Al_2O_3 rotor. The fractions eluted with 20–30% hexane in Et_2O were combined with the fractions eluted with Et_2O in fraction 9 and purified twice on an Al_2O_3 rotor to furnish anthranoyllycoctonine (17.2 mg) (1). The fractions collected with 10% EtOH in Et_2O gave a homogeneous amorphous compound (6.1 mg) that was identified as 19-oxoanthranoyllycoctonine [**7**]: $[\alpha]_D$ +58.1° ($c=0.078$, $CHCl_3$); ir (Nujol) ν max 3450 (OH), 3350 (NH_2), 1690 (anthranilate -C=O), 1620 (lactam -C=O) cm^{-1}; eims *m/z* (%) $[M]^+$ 600 (for $C_{32}H_{44}N_2O_9$) (4.5), $[M-Me]^+$ 583 (10.0), $[M-Me-H_2O]^+$ 567 (4.9), $[M-$anthranoyl$]^+$ 464 (1.1), 137 (11.5), 120 (100); ^{1}H nmr

($CDCl_3$) δ 1.14 (3H, t, J = 7.2 Hz, N-CH_2CH_3), 3.24, 3.35, 3.38, 3.44 (each 3H, s, 4 × OMe), 3.42, 3.98 (each 1H, s, exchanges with D_2O, 2 × OH), 4.49, 4.79 (2H, AB q, H-18), 5.57 (br s, exchanges with D_2O, -NH_2), 6.67, 7.29, 7.78 (2H, 1H, 1H, m, aromatic); ^{13}C nmr ($CDCl_3$) δ 81.4 d (C-1), 25.1 t (C-2), 29.6 t (C-3), 47.6 s (C-4), 49.5 d (C-5), 91.9 d (C-6), 86.0 s (C-7), 76.6 s (C-8), 45.3 d (C-9), 42.8 d (C-10), 49.1 s (C-11), 28.5 t (C-12), 37.7 d (C-13), 83.6 d (C-14), 33.2 t (C-15), 82.1 d (C-16), 63.2 d (C-17), 66.1 t (C-18), 170.1 s (C-19), 43.7 t (N-CH_2CH_3), 12.0 q (N-CH_2CH_3), 55.2 q (C-1′), 57.9 q (C-6′), 58.6 q (C-14′), 56.4 q (C-16′), anthranoyl ester carbons 167.5 s (-C=O), 110.3 s (C-1″), 150.9 s (C-2″), 116.9 d (C-3″), 134.2 d (C-4″), 116.1 d (C-5″), 130.6 d (C-6″).

Fraction 11 (186.1 mg, 2% EtOH in Et_2O) was fractionated on an Al_2O_3 rotor. The fraction eluted with 5% MeOH in Et_2O crystallized from Me_2CO to give gigactonine [**4**] (49.2 mg) (2).

Fractions 12 (115.0 mg, 5% MeOH-Et_2O) and 13 (162.1 mg, 10% MeOH/Et_2O) were combined and repeatedly crystallized from EtOH to furnish delcosine (169.3 mg) (1).

The combined fractions 14 and 15 (246.1 mg, 20% MeOH/Et_2O) were purified on a Si gel rotor by eluting with a gradient of $CHCl_3$ and MeOH. Fractions eluted with 2% MeOH/Et_2O afforded delcosine. Fractions eluted with 3 and 4% MeOH/Et_2O were combined and purified on an Al_2O_3 rotor using a gradient of MeOH and Et_2O. Takaosamine [**5**] (50.0 mg) (2) was isolated as colorless plates from the fractions eluted with 2% MeOH/Et_2O.

The impure fractions from all the above separations were combined (662.0 mg) and purified twice on an Al_2O_3 rotor with a gradient of hexane, Et_2O, and EtOH. In all, sixteen fractions (A–P) were collected. Fraction D gave delphatine (7.0 mg), E gave delsoline (19.1 mg), K gave delcosine (51.0 mg), and G + H gave gigactonine [**4**] (24.1 mg). Fractions A–C (50% Et_2O/hexane) on further purification on an Al_2O_3 rotor gave deltatsine [**3**] (6.2 mg) (11). Fraction F [EtOH-Et_2O (1:1)] was purified twice, first on an alumina rotor and then on a Si gel rotor to give browniine (16.2 mg) (1). Fractions I–J were purified on a Si gel rotor to give 18-methoxygadesine [**6**] (11.3 mg) (2).

ISOLATION OF ALKALOIDS FROM THE LEAVES.—Air-dried and powdered leaves (2 kg) of *D. ajacis* were defatted with hexane (3 × 4 liters) and exhaustively extracted with 80% EtOH (5 × 4 liters). A mixture of crude alkaloids was isolated from the extracts on a cation exchange resin (DOWEX 50W X8, H^+). The crude alkaloidal fraction was separated into 3 groups by a pH gradient extraction technique (10). These three groups were: group 1 (pH 4.5, 1.37 g), group 2 (pH 8, 0.21 g), and group 3 (p H 12, 0.03 g).

Group 1 (1.37 g) was chromatographed (vlc) on Si gel and eluted with a gradient of hexane, $CHCl_3$, and EtOH; nine fractions (100 ml) each were collected.

Fractions 5 (120.0 mg, 2% EtOH/$CHCl_3$) was purified twice on an Al_2O_3 rotor to afford delcosine (131.1 mg) and anthranoyllycoctonine (14.2 mg).

Fraction 6 (354.1 mg, 4% EtOH/$CHCl_3$) was purified twice on an Al_2O_3 rotor to furnish delcosine (11.8 mg), anthranoyllycoctonine (22.3 mg), deltaline [**12**] (17.8 mg), delsoline (35.9 mg), and delpheline [**13**] (13.2 mg).

Fraction 8 (198.0 mg, EtOH) was purified twice on an Al_2O_3 rotor and crystallizd from Me_2CO to afford gigactonine [**4**] (15.5 mg).

CONVERSION OF AJACINE [**9**] TO 19-OXOAJACINE [**10**].—To 125 mg of **9** in 4 ml of pyridine was added 160 mg of OsO_4 in 4 ml of *p*-dioxane; the mixture was stirred at room temperature for 20 h (12). A solution of $NaHSO_3$ (300 mg of $NaHSO_3$ in 2 ml of H_2O and 4 ml of pyridine) was added to the reaction mixture and stirred for 2 h. The mixture was extracted with CH_2Cl_2 (3 × 30 ml). The CH_2Cl_2 extracts were combined, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The residue was dissolved in $CHCl_3$ (50 ml) and extracted with 1.5% aqueous H_2SO_4 (3 × 20 ml). The $CHCl_3$ extract was dried over anhydrous Na_2SO_4 and evaporated to give 96 mg of residue which was purified on a small alumina column (2 g Al_2O_3, neutral type) to give 89 mg of **10**: ir (Nujol) 3450, 3300 cm^{-1} (OH), 1692, 1682 cm^{-1} (C=O), 1630 cm^{-1} (lactam), 1585, 1525, 1515 cm^{-1} (C=C); mass *m/z* $[M]^+$ 642 ($C_{34}H_{46}N_2O_{10}$) (2.9), 480 (2), 466 (2.5), 162 (31), 137 (4.7), 120 (34.4), 119 (8.1), 92 (8.4), 71 (32.4), 45 (18.6), 43 (100); ^{1}H nmr ($CDCl_3$) δ 1.13 (3H, t, J = 7 Hz, N-CH_2-CH_3), 2.24 (3H, s, OCOCH_3), 3.24, 3.30, 3.35, 3.38 (3H each, s, 4 × OCH_3), 3.52, 3.93 (1H each, s, 2 × OH), 3.68 (1H, t, J = 4.5 Hz, H-14β), 4.54 and 4.83 (1H each, AB q, H-18), 7.10 and 7.57 (1H each, t, J = 8 Hz, H-4″ and H-5″), 7.95 and 8.71 (1H each, d, J = 8 Hz, H-3″ and H-6″), 11.0 (1H, br s, N*H*); ^{13}C nmr ($CDCl_3$) δ 81.0 d (C-1), 24.9 t (C-2), 29.5 t (C-3), 47.3 s (C-4), 49.4 d (C-5), 91.7 d (C-6), 85.9 s (C-7), 76.5 s (C-8), 45.0 d (C-9), 42.6 d (C-10), 48.8 s (C-11), 28.3 t (C-12), 37.4 d (C-13), 83.4 d (C-14), 33.1 t (C-15), 81.9 d (C-16), 63.2 d (C-17), 67.1 t (C-18), 169.6 s (C-19), 43.6 t (N-CH_2-CH_3), 11.9 q (N-CH_2-CH_3), 55.2 q (C-1′), 57.9 q (C-6′), 58.5 q (C-14′), 56.3 q (C-16′), anthranoyl ester carbons 167.0 s (C=O, 114.4 s (C-1″), 141.7 s (C-2″), 120.5 d (C-3″), 134.8 d (C-4″), 122.4 d (C-5″), 130.0 d (C-6″), 169.1 s and 25.4 q (NH-CO-Me).

CONVERSION OF 19-OXOAJACINE [**10**] TO 19-OXOANTHRANOYLLYCOCTONINE [**7**].—To 46

mg of **10** was added 10 ml of 4% aqueous HCl solution, and the mixture was refluxed at 100° for 6 h. The reaction mixture was cooled and extracted with $CHCl_3$ (5 × 10 ml). The $CHCl_3$extracts were combined and evaporated to give 45 mg of residue which was factionated on an alumina rotor of a Chromatotron to afford 11 mg of **7** and 17 mg of starting material **10**.

CONVERSION OF ANTHRANOYLLYCOCTONINE [**11**] TO 19-OXOANTHRANOYLLYCOCTONINE [**7**].—To 20 mg of **11** in 2 ml of pyridine was added 26 mg of OsO_4 in 1.5 ml of *p*-dioxane. The reaction mixture was stirred at room temperature for 20 h, then worked up by the above method to afford 18.5 mg of **7**. The synthetic and natural samples of 19-oxoanthranoyllycoctonine [**7**] were identical by tlc behavior, ir, mass, ^{1}H-nmr and ^{13}C-nmr spectra.

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