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TWO NOVEL NORDITERPENOID ALKALOIDS FROM *DELPHINIUM STAPHISAGRIA*

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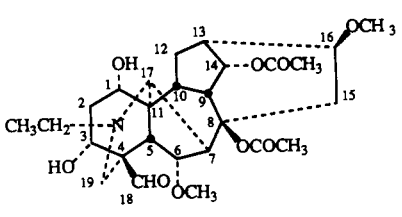
ABSTRACT.—Two novel norditerpenoid alkaloids, staphisadrine [**1**] and staphisadrinine [**2**], have been isolated from *Delphinium staphisagria*. Structures **1** and **2** were determined from physical and spectroscopic data, including DEPT and nOe difference experiments. Staphisadrine [**1**] is the first example of an aconitine-type alkaloid bearing a formyl group on C-4. Staphisadrinine [**2**] is the first norditerpenoid alkaloid that possesses a carbonyl function on C-16. Two known norditerpenoid alkaloids, pyrodelphinine [**3**] and 14-acetyl-1-*epi*-neoline [**4**], were also isolated and have not been previously reported as natural products.

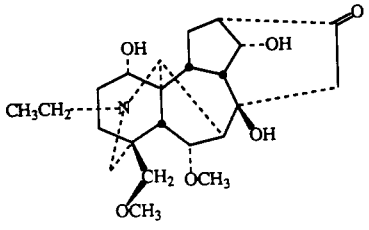
The investigation of the diterpenoid alkaloids in the seeds of *Delphinium staphisagria* L. (Ranunculaceae) has a long history with eight bis-diterpenoid and eighteen norditerpenoid alkaloids having been reported previously (1). In continuation of the study of alkaloids from the mother liquor accumulated during the isolation of delphinine we now report the isolation of two novel norditerpenoid alkaloids, staphisadrine [**1**] and staphisadrinine [**2**], along with two known alkaloids, pyrodelphinine [**3**] and 14-acetyl-1-*epi*-neoline [**4**]. The latter two alkaloids have not been reported previously as natural compounds.

RESULTS AND DISCUSSION

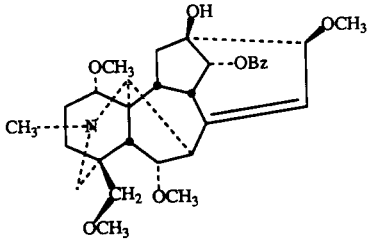
Staphisadrine [**1**] was obtained as an amorphous compound, $[\alpha]^{20}D$ 6.3° ($CHCl_3$). Its molecular formula $C_{27}H_{39}NO_9$ was deduced from its eims, $[M]^+$ *m/z* 521, and ^1H- and ^{13}C-nmr spectra. Its ir spectrum showed absorption at ν max 3420 (OH), 2820 and 1740 (CHO), 1730, 1720 and 1250 (OAc) cm^{-1}. The ^1H-nmr spectrum exhibited signals at δ 1.15 (3H, t, $J = 7.2$ Hz, N-CH_2CH_3), 1.97, 2.04 (each 3H, s, 2 × OAc),



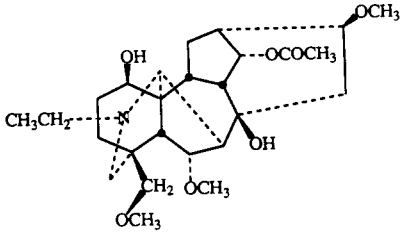
1 staphisadrine



2 staphisadrinine



3 pyrodelphinine



4 14-acetyl-1-*epi*-neoline

3.04 (1H, d, $J=6.6$ Hz, H-5β), 3.15, 3.33 (each 3H, s, 2 × OMe), 3.77 (1H, dd, $J_1=J_2=5.2$ Hz, H-1β), 3.98 (1H, d, $J=6.6$ Hz, H-6β), 4.04 (1H, dd, $J_1=5.2$ Hz, $J_2=7.6$ Hz, H-3β), 4.82 (1H, t, $J=4.6$ Hz, H-14β), and 9.39 (1H, s, -*CH*O). Its ^{13}C-nmr chemical shifts are given in Table 1. The ^{13}C-nmr spectrum exhibited 27 lines for the 27 carbon atoms of the molecule. The DEPT spectra revealed the presence of five quaternary carbons, twelve methine carbons, five methylene carbons and five methyl carbons. Of the nine oxygenated carbons in the ^{13}C-nmr spectrum of **1**, two can be accounted for in the two acetoxyl groups and seven in the oxygen atoms attached to the skeletal carbons. The only quaternary oxygenated carbon at 85.2 ppm was readily assigned to C-8 bearing an acetoxyl group. The remaining six signals are doublets. Considering the structures of norditerpenoid alkaloids isolated from this plant, only delphinine has seven skeletal oxygenated carbons, of which C-8 and C-13, having hydroxyl groups, appear as quaternary carbons. Of the seven skeletal oxygenated carbons in **1**, only one carbon is quarternary, and the other six are methine carbons. This result indicated that **1** is a neoline-type alkaloid with one more oxygenated carbon including the carbonyl carbon than in neoline [**5**].

The doublets at 82.6 and 81.2 ppm can be assigned to C-16 and C-6, respectively,

TABLE 1. ^{13}C Chemical Shifts and Assignments for Staphisadine [**1**], Staphisadrinine [**2**], Neoline [**5**], Delstaphisine [**6**], and 1-Dehydrodelstaphisine [**7**].[a]

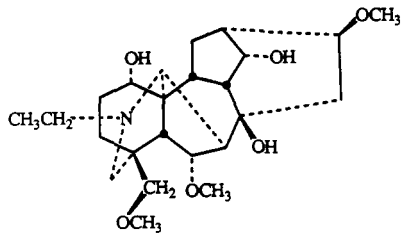
Carbon	Compound				
	1	**2**	**5**[b]	**6**[c]	**7**
C-1	72.2 d	72.3 d	72.3	71.9	213.1 s
C-2	42.3 t	29.4 t	29.5	29.3	41.7 t
C-3	70.5 d	29.9 t	29.9	29.9	39.2 t
C-4	50.6 s	38.2 s	38.2	38.1	39.3 s
C-5	47.1 d	44.6 d	44.9	43.8	52.7 d
C-6	81.2 d	83.0 d	83.3	83.8	82.9 d
C-7	47.7 d	52.2 d	52.3	48.0	48.6 d
C-8	85.2 s	73.6 s	74.3	85.7	85.8 s
C-9	42.5 d	48.2 d	48.3	43.1	43.0 d
C-10	38.7 d	43.9 d	40.7	42.9	39.0 d
C-11	48.2 s	49.6 s	49.6	49.8	60.9 s
C-12	28.4 t	29.4 t	29.8	29.0	33.4 t
C-13	43.1 d	53.2 d	44.3	43.1	43.0 d
C-14	75.3 d	74.5 d	75.9	76.0	76.1 d
C-15	38.2 t	53.5 t	42.7	41.3	41.7 t
C-16	82.6 d	212.9 s	82.3	72.9	73.2 d
C-17	62.5 d	63.5 d	63.6	62.9	63.2 d
C-18	204.4 d	80.2 t	80.3	79.7	78.5 t
C-19	53.5 t	57.1 t	57.2	56.8	54.5 t
N-CH_2	48.7 t	48.2 t	48.2	48.4	48.5 t
Me	13.0 q	12.9 q	13.0	12.7	13.3 q
C-6′	57.7 q	58.0 q	57.8	58.1	58.2 q
C-16′	56.6 q	—	56.3	—	—
C-18′	—	59.3 q	59.1	59.2	59.1 q
C=O	169.6 s, 170.7 s	—	—	169.2, 170.5	169.5 s, 170.2 s
Me	22.4 q, 21.2 q	—	—	22.3, 21.2	22.3 q, 21.7 q

[a]All the spectra were recorded in $CDCl_3$ solution; multiplicities were assigned by SFORD and DEPT experiments.

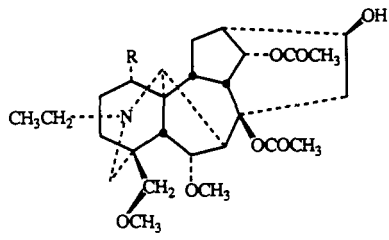
[b]Data in this column are from Pelletier *et al.* (2).

[c]Data in this column are from Pelletier and Badawi (9).

bearing methoxyl groups, an assignment supported by the corresponding methoxy methyl quartets at 56.6 and 57.7 ppm (2). The doublet at 75.3 ppm is characteristic of C-14 (2). The lower field one-proton triplet at 4.82 ppm, assignable to H-14β, provided evidence for an acetyl group at C-14.



5 neoline



6 R=α-OH, delstaphisine
7 R= =O, 1-dehydrodelstaphisine

Assignment of the doublets at 72.2 ppm and 70.5 ppm to C-1 and C-3, respectively, each bearing a hydroxyl group, was supported by the ^{1}H-nmr spectrum of **1**. Irradiation of either of the two related signals at 3.77 and 4.04 ppm resulted in the simplification of a multiplet at 2.01 ppm, and when the signal at 2.01 ppm was irradiated, the signals at 3.77 and 4.04 ppm each collapsed to give a broad singlet. This result suggests the partial structure >CH-CH$_2$-CH< and hence, the substitution pattern of C-1-R and C-3-R.

The presence of an aldehyde group in **1** is indicated by the presence of a doublet at 204.4 ppm in the ^{13}C-nmr spectrum, a singlet at 9.39 ppm in the ^{1}H-nmr spectrum, and absorptions at 2820 and 1740 cm^{-1} in the ir spectrum. . The absence of the C-18-OR signal and a significant downfield shift for the C-4 singlet in the ^{13}C-nmr spectrum suggested that the -CHO group is at C-4. Comparing the chemical shift for C-4 (50.6 ppm) in **1** with that of lycoctonal (48.2 ppm) (2) shows a downfield shift of 2.4 ppm, thus supporting the presence of a -CHO group on C-4 and an -OH group on C-3 in **1**. The DIFFNOE spectrum supported the position of the -CHO group. When the singlet at 9.39 ppm was irradiated, a significant nOe was observed for the doublet at 3.04 ppm, assignable to H-5β (3) because of its coupling with H-6β at 3.98 ppm (3). The nOe experiment indicates that the orientation of the C-4 CHO group is β.

Staphisadrinine [**2**] is an amorphous compound, $[\alpha]^{19}_D$ −19.1° ($CHCl_3$). Its molecular formula $C_{23}H_{35}NO_6$ was derived from the eims *m/z* 421 [M]$^+$ and the ^{13}C-nmr spectrum. Its ir spectrum showed ν max 3530, 3240 (OH), and 1710 (C=O) cm^{-1}. The ^{1}H-nmr spectrum revealed the following signals: δ 1.08 (3H, t, J = 7.1 Hz, N-CH$_2$C*H*$_3$), 3.33, 3.36 (each 3H, s, 2 × OMe), 3.61 (1H, brs, H-1β), 3.21, 3.67 (each 1H, d, J = 8.1 Hz, H$_2$-18), 4.25 (1H, d, J = 6.6 Hz, H-6β), 4.35 (1H, t, J = 4.7 Hz, H-14β). For ^{13}C-nmr chemical shift assignments see Table 1.

The ^{1}H- and the ^{13}C-nmr spectra of **2** are similar to those of neoline [**5**] (2), except that there are only two methoxyl groups in **2**. The ^{13}C-nmr spectrum of **2** indicates that the chemical shifts for C-16 and the methyl carbon of the methoxyl group on C-16 (82.0 and 56.3 ppm in **5**) are absent. The quaternary carbon signal at 212.9 ppm and a strong absorption at 1710 cm^{-1} in the ir spectrum of **2** indicate the presence of a ketone group on a saturated six-membered ring. Comparison of the ^{13}C-nmr spectra of **2** and **5** suggests that C-16 in **2** is a carbonyl function, and this is supported by the downfield chemical shifts for C-13 (53.2, d) and C-15 (53.5, t) caused by the presence of a C-16 carbonyl group.

In an attempt to synthesize staphisadrinine [**2**] from delstaphisine [**6**] by selective

oxidation of the hydroxyl group on C-16, an oxidation of **6** was carried out using pyridinium dichromate (PDC) in CH_2Cl_2 at $-70°$ (Me_2CO/dry ice) and then at $-10°$. The product isolated was identified as 1-dehydrodelstaphisine [**7**]. The room temperature oxidation of **6** gave a complex mixture of products. The structure of compound **7** was established on the basis of its physical and spectroscopic data. Its molecular formula $C_{27}H_{39}NO_8$ was supported by the eims *m/z* 505 $[M]^+$ and the ^{13}C-nmr spectral chemical shifts. A chemical shift at 213.1 (s) ppm showed the presence of a carbonyl group at C-1, which was established by comparing the ^{13}C-nmr chemical shifts of **7** with those of **6** (Table 1). The chemical shifts of carbons 2 and 11 (s) have moved downfield because of the presence of a carbonyl group at C-1, and the chemical shifts for C-16, C-15, C-13 have remained virtually unchanged. The selective oxidation of the 1α-OH group of **6** at low temperature in preference to the 16β-OH group in the molecule is noteworthy.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Specific rotations were determined on a Perkin-Elmer model 141 polarimeter. Ir spectra were recorded on a Perkin-Elmer model 1420 spectrophotometer. ^1H- and ^{13}C-nmr spectra were recorded on JEOL FT models FX-60 and FX-270 and Bruker WM 300 spectrometers. Mass spectra were determined on a Finnegan Quadrupole 4032 instrument. Chromatographic separations were carried out using vacuum liquid chromatography (vlc) (4) and centrifugally accelerated radial tlc using a Chromatotron (5) Model 7924T (Harrison Research, Palo Alto, California).

PLANT MATERIALS.—*D. staphisagria* seeds purchased from S.B. Penick Co., New York, were processed as described (6,7). The mother liquor (87.5 g) left after removing the major alkaloid delphinine was fractionated using a pH gradient separation to give the following fractions: fractions A and B (neutrals) 50.51 g, fraction C (pH 4.5) 18.85 g, fraction D (pH 8.0) 17.89 g, and fraction E (pH 10.0) 0.7 g.

ISOLATION OF PYRODELPHININE [**3**].—Fraction C (18.85 g, pH 4.5) was fractionated on a vlc column (SiO_2, 40 g, EM 1085) using a gradient of hexane and $CHCl_3$. Fractions eluted with hexane-$CHCl_3$ (6:4) gave a gummy residue from which delphisine (6.6 g) was obtained in crystalline form. The mother liquor (0.706 g) on repeated fractionation on an Al_2O_3 rotor (1 mm) gave pyrodelphinine [**3**] (4 mg) having identical mp, co-tlc, ir, and ^1H- and ^{13}C-nmr spectra with those of an authentic sample.

ISOLATION OF 14-ACETYL-1-*epi*-NEOLINE [**4**], STAPHISADRININE [**2**], AND STAPHISADRINE [**1**].—Fraction D (17.89 g, pH 8) was chromatographed (vlc) on a Si gel column (50.0 g) using a gradient of hexane, CH_2Cl_2, and MeOH, giving nine fractions. The combined fractions 1 and 2 (1.591 g) were rechromatographed (vlc) on an Al_2O_3 column (26.3 g) using a gradient of hexane and Et_2O. The fractions eluted with Et_2O (0.37 g) were combined and purified on an Al_2O_3 rotor (1 mm) to furnish 14-acetyl-1-*epi*-neoline [**4**] (21.0 mg). The identity of **4** was established by comparing its tlc, cotlc, ir, ^1H-nmr, ^{13}C-nmr, and eims data with those of an authentic sample of **4** (8). The fractions eluted with MeOH (0.15 g) were combined and purified on an Al_2O_3 rotor using a gradient of Et_2O and EtOH. The fractions eluted with Et_2O-EtOH (80:20) furnished amorphous staphisadrinine [**2**] (19 mg): $[\alpha]^{19}D - 19.1°$ ($c = 0.15$, $CHCl_3$), ir ν max (Nujol) 3530, 3240 (OH), 1710 (C=O) cm^{-1}; ^1H nmr ($CDCl_3$) δ 1.08 (3H, t, $J = 7.1$ Hz, N-CH_2CH_3), 3.33, 3.36 (each 3H, s, 2 × OCH_3), 3.61 (1H, brs, H-1β), 3.21, 3.67 (each 1H, d, $J = 8.1$ Hz, H_2-18), 4.25 (1H, d $J = 6.6$ Hz, H-6β), 4.35 (1H, t, $J = 4.7$ Hz, H-14β); eims *m/z* (%) $[M]^+$ 421 (4.12), $[M - OH]^+$ 404 (56.57), 388 (6.18), 372 (3.63), 358 (2.15), 334 (4.88), 237 (4.86), 45 (100); ^{13}C nmr see Table 1.

The combined third and the fourth fractions (1.036 g) from the initial nine fractions mentioned above, eluted with CH_2Cl_2-MeOH (98:2), were chromatographed (vlc) over a Si gel column (30.0 g) and eluted with hexane-$CHCl_3$ (1:1); $CHCl_3$, and $CHCl_3$-MeOH (97:3). The fraction (0.49 g) eluted with $CHCl_3$ was repeatedly purified on an Al_2O_3 rotor giving staphisadrine [**1**] (25 mg) as an amorphous compound: $[\alpha]^{20}D$ 6.3° ($c = 0.175$, $CHCl_3$); eims *m/z* (%) $[M]^+$ 521 ($C_{27}H_{39}NO_9$) (2.14), $[M - OH]^+$ 504 (0.98) $[M - OAc]^+$ 462 (2.82), 388 (2.47), 328 (2.63), 300 (1.74), 268 (1.00), 191 (1.10), 164 (3.44), 134 (3.92), 108 (2.02), 91 (4.15), 77 (2.94), 71 (7.77), 58 (9.99), 43 (100); ir ν max (Nujol) 2820, 1740 (-CHO), 1730, 1720, 1250 (OAc) cm^{-1}; ^1H nmr ($CDCl_3$) δ 1.15 (3H, t, $J = 7.2$ Hz, N-CH_2CH_3), 1.97, 2.04 (each 3H, s, 2 × $OCOCH_3$), 3.04 (1H, d, $J = 6.6$ Hz, H-5β), 3.15, 3.33 (each 3H, s, 2 × OCH_3), 3.77 (1H, dd, $J_1 = J_2 = 5.2$ Hz, H-1β), 3.98 (1H, d, $J = 6.6$ Hz, H-6β), 4.04 (1H, dd, $J_1 = 5.2$ Hz, $J_2 = 7.6$ Hz, H-3β), 4.82 (1H, t, $J = 4.6$ Hz, H-14β), 9.39 (1H, s, -CHO); ^{13}C nmr see Table 1.

OXIDATION OF DELSTAPHISINE [**6**].—To a solution of delstaphisine [**6**] (10.0 mg) in CH_2Cl_2 (30 ml), was added pyridinium dichromate (PDC) (25.0 mg), and the mixture was stirred at $-70°$ for 1 h. Tlc

indicated no change in the starting material. The temperature was raised to $-10°$, and the mixture was stirred for 4 h. When starting material disappeared on the tlc plate the mixture was filtered through a small column of Al_2O_3 (neutral, activity III), and the column was washed with Et_2O. Evaporation of the combined washings gave 1-dehydrodelstaphisine [**7**] (10.0 mg). The compound **7** (amorphous) showed: eims *m/z* (%) $[M]^+$ 505 ($C_{27}H_{39}NO_8$) (1.90), $[M-Me]^+$ 490 (6.88), $[M-OMe]^+$ 474 (1.90), $[M-OAc]^+$ 446 (3.99); ir ν max (Nujol) 3400 (OH), 1730 (C=O) cm^{-1}; ^1H nmr ($CDCl_3$) δ 1.05 (3H, t, N-CH_2CH_3), 1.95, 2.04 (each 3H, s, 2 × OAc), 3.23, 3.25 (each 3H, s, 2 × OCH_3), 4.32 (1H, d, $J = 6.6$ Hz, H-6β), 4.89 (1H, dd, $J = 4.5$ Hz, H-14β); ^{13}C nmr see Table 1.

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