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NEW ALKALOIDS FROM *DELPHINIUM STAPHISAGRIA*

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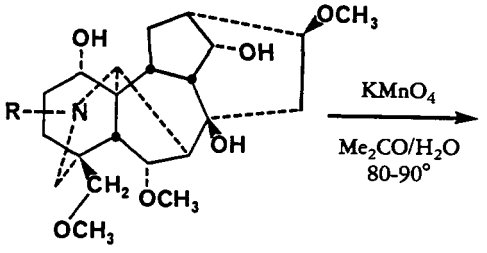
ABSTRACT.—Delstaphinine [**1**], a new C_{19}-diterpenoid alkaloid, and 1-dehydrodelphisine [**7**] have been isolated from the seeds of *Delphinium staphisagria*. The structures have been determined with the aid of ^1H- and ^{13}C-nmr spectroscopy and were confirmed by synthesis from neoline and delphisine, respectively. 1-Dehydrodelphisine has not been isolated from a natural source previously.

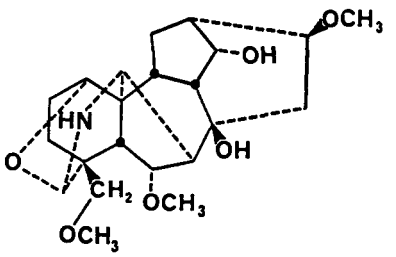
The seeds of *Delphinium staphisagria* L. (Ranunculaceae), when extracted with ligroin, yield an alkaloid fraction of which delphinine (1) is the major component. The mother liquor accumulated during the isolation of a large quantity of delphinine furnished an amorphous fraction from which delphisine (2), delphidine (3), delphirine (1-epineoline) (4), and several bisditerpenoid alkaloids have been isolated (5-7). Recently we reported the isolation of three new alkaloids, delstaphisine, delstaphisagrine, and delstaphisagnine from this amorphous fraction (8).

In this paper we report separation of an amorphous fraction from the seeds of *D. staphisagria* by a combination of gradient pH separation, vacuum liquid chromatography (vlc), preparative tlc, and centrifugally accelerated, radial, tlc ("Chromatotron") to give a new C_{19}-diterpenoid alkaloid, delstaphinine [**1**], and the alkaloid, 1-dehydrodelphisine [**7**]. The latter has not been reported in nature before but had been prepared by CrO_3 oxidation of delphisine (2).



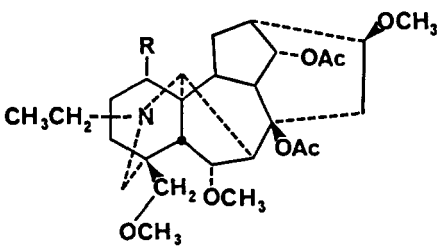
3 R=CH_2CH_3 neoline
4 R=H *N*-deethylneoline

KMnO4
Me_2CO/H_2O
80-90°

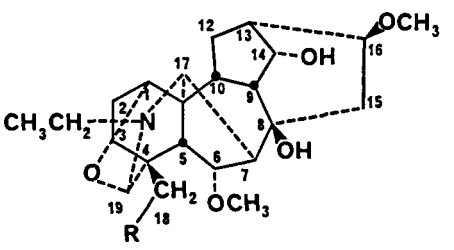


5 *N*-deethyldelstaphinine

EtBr
Me_2CO
K_2CO_3



6 R=α−OH delphisine
7 R=O 1-dehydrodelphisine



1 R=OCH_3 delstaphinine
2 R=H pentagynine

RESULTS AND DISCUSSION

Delstaphinine [**1**] was obtained in an amorphous form $[\alpha]^{28}D+47.7°$ ($CHCl_3$), and its molecular formula $C_{24}H_{37}NO_6$ was deduced from the mass spectral (M^+, 435), ^1H-, and ^{13}C-nmr data. The ^1H-nmr spectrum exhibited the following signals: δ 1.13 (3H, t, J=7 Hz, N-CH_2-CH_3), 3.31 (6H, s, 2× OCH_3), 3.36 (3H, s, OCH_3), 3.58 (1H, s, 19-H), 3.69 (1H, m, w/2=7 Hz, C(1)-β*H*), 4.04 [1H, s, C(6)-β*H*], and 4.21 [1H, dd, J_1=J_2=4.5 Hz, C(14)-β*H*].

The noise decoupled ^{13}C-nmr spectrum of delstaphinine [**1**] exhibited 23 signals for the 24 carbon atoms of the molecule (Table 1). The mass spectrum showed a molecular ion M^+, *m/z* 435 (2%), 420 (M^+ -CH_3, 12%), 404 (M^+ -OCH_3, 42%), 390 (M^+ -CH_2OCH_3, 10%), 379 (M^+ -C_3H_4O, 82%), 364 [M^+ -CH_3]-C_3H_4O, 4%), 348 ([M^+ -OCH_3]-C_3H_4O, 54%), 152 (50%), 85 (65%), and 45 (100%).

Structure **1** was deduced for delstaphinine from its spectral data and comparison of its data with those of the neoline group of aconitine-type C_{19}-diterpenoid alkaloids (9) and with those of pentagynine [**2**], gadesine, and 18-methoxygadesine (10).

The SFORD spectrum of delstaphinine exhibited 3 singlets at 40.2, 48.0, and 71.4 ppm. The two upfield signals (40.2 and 48.0 ppm) are assigned to the non-oxygenated quaternary carbons 4 and 11, respectively. The downfield signal at 71.4 ppm is assigned to the only oxygenated quaternary C(8). Six triplets appear at 75.3 (C-18), 50.0 (N-CH_2-), 40.2 (C-15), 29.7 (C-3), 27.7 (C-12), and 22.6 (C-2) ppm.

TABLE 1. ^{13}C Chemical Shifts and Assignments for Delstaphinine [**1**], Pentagynine [**2**], Neoline [**3**], *N*-Deethylneoline [**4**] and *N*-Deethyldelstaphinine [**5**]

Carbon	Compounds				
	1	**2**	**3**	**4**	**5**
1	69.2 d	68.8 d[a]	72.3	72.3	69.4
2	29.7 t	30.2 t[b]	29.5[c]	29.4[c]	27.6
3	22.6 t	23.0 t[b]	29.9[c]	30.0[c]	22.3
4	40.2 s	38.3 s	38.2	38.4	46.7 s
5	36.8 d	37.3 d	44.9	44.9	36.9
6	84.2 d	84.3 d	83.3	82.9	83.1
7	56.4 d	56.9 d	52.3	49.2	53.1
8	71.4 s	73.6 s	74.3	74.2	71.1 s
9	49.8 d	52.6 d	48.3	47.9	53.0
10	38.9 d	39.0 d	40.7	40.4	39.2
11	48.0 s	47.5 s	49.6	49.7	49.6 s
12	27.7 t	28.7 t	29.8[c]	28.9[c]	27.2
13	46.8 d	45.7 d	44.3	44.1	46.8
14	75.5 d	75.5 d	75.9	75.7	75.5
15	40.2 t	39.0 t	42.7	42.4	40.1
16	81.7 d	82.2 d	82.3	81.7	81.7
17	60.8 d	61.7 d	63.3	60.0	61.7
18	75.3 t	20.2 q	80.3	80.2	75.3
19	87.8 d	91.2 d[a]	57.1	58.4	84.1
N-CH_2	50.1 t	47.8 t	48.2	—	—
CH_3	14.2 q	14.4 q	13.0	—	—
6′	57.8 q[c]	58.0 q	57.8	57.9[c]	58.2[c]
16′	56.4 q[c]	56.4 q	56.3	56.3[c]	56.5[c]
18′	60.5 q	—	59.1	59.3	58.9

[a]The published values for gadesine, 18-methoxygadesine, and pentagynine have been reversed (16).
[b]G. de la Fuente, private communication, November 8, 1983.
[c]Values within any vertical column may be interchanged.

The ^{13}C-nmr spectrum also showed the presence of 5 oxygenated CH doublets at 87.8, 84.2, 81.7, 75.5, and 69.2 ppm, and 3 methoxyl functions as quartets at 60.5, 57.8, and 56.3 ppm. The downfield doublets at 84.2 and 81.7 ppm are assigned to C(6) and C(16) bearing methoxyl groups. The corresponding methoxyl methyl quartets are those occurring at 57.8 and 56.3 ppm, respectively. The third quartet at 60.5 ppm is assigned to the methoxyl group attached to the only oxygenated methylene C(18), which is represented by the most downfield triplet at 75.3 ppm.

Beside the three methoxyl functions, two of the six oxygen atoms of the molecule exist as two hydroxyl groups. One is attached to the only oxygenated quaternary C(8), indicated by the singlet at 71.4 ppm, and the second is attached to C(14), which resonates as a doublet at 75.5 ppm.

The last oxygen atom in the molecule is attached to C(1) and C(19) forming an internal ether bridge. C(1) and C(19) are represented by the two remaining doublets, the most downfield doublet at 87.8 and the upperfield doublet at 69.2 ppm, respectively. In neoline, C(1) bearing an OH group resonates at 72.3 ppm, and the C(19) methylene signal occurs at 57.1 ppm. The presence of the C(1)-O-C(19) ether bridge in delstaphinine was also confirmed by the existence of a peak at *m/z* 379 (82%) in its mass spectrum, caused by loss of an acrolein molecule from the molecular ion (11). The ir spectrum of **1** showed absorptions at 990 and 895 cm^{-1} characteristic of a carbinolamine ether structure (12).

The structure of delstaphinine [**1**] was confirmed by synthesis from neoline [**3**]. In principle, this objective should be attainable by a one-step oxidation of neoline in such a manner that the C(1)-hydroxyl group is converted to a C(1)-O-C(19) carbinolamine ether. However, oxidation of neoline by treatment with $KMnO_4$ in Me_2CO/H_2O solution at room temperature for 24 h resulted only in dealkylation of the *N*-ethyl group to give *N*-deethylneoline [**4**] as the main product. Compound **4** exhibited a ^{13}C-nmr spectrum (Table 1) similar to that of neoline (9), except for the absence of signals due to the *N*-CH_2CH_3 carbons. The signal characteristic of the methyl protons of the *N*-ethyl group was also absent. *N*-Deethylneoline [**4**] was reconverted to neoline by boiling under reflux with EtBr in Me_2CO solution in the presence of dry K_2CO_3 for 3 h.

Under more vigorous conditions neoline [**3**] was oxidized to the dealkylated carbinolamine ether, *N*-deethyldelstaphinine [**5**] [warming in aqueous Me_2CO solution (H_2O bath, 70-80°) with $KMnO_4$]. Compound **5** exhibited a ^{13}C-nmr spectrum similar to that of delstaphinine [**1**] except for the absence of signals due to the carbons of the *N*-ethyl group. The ^{1}H-nmr spectrum of **5** is also similar to that of delstaphinine [**1**] except for the absence of the triplet due to the methyl protons of the *N*-ethyl group. Conversion of **5** to **1** was effected by boiling with EtBr in Me_2CO solution in presence of dry K_2CO_3 for 3 h. Identity of the synthetic and natural delstaphinine was confirmed by tlc behavior and ^{1}H-nmr spectra.

Investigation of a "neutral" fraction left after extraction of delphinine mother liquors with 2% H_2SO_4 showed that it still contained basic material. Reextraction with dilute H_2SO_4 and basification gave an alkaloid fraction that was separated by a Chromatotron and preparative tlc plates to give a crystalline fraction identified as 1-dehydrodelphisine [**7**], mp 168-170°. Identity was established by synthesis from delphisine [**6**]. 1-Dehydrodelphisine has not previously been reported in nature.

Table 1 gives the ^{13}C chemical shifts and assignments for delstaphinine [**1**], pentagynine [**2**], neoline [**3**], *N*-deethylneoline [**4**], and *N*-deethyldelstaphinine [**5**].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are corrected and were determined on a Thomas-Kofler hot stage equipped with a microscope and a polarizer. Optical rotations were measured on a Perkin-Elmer model 141 polarimeter. Ir spectra were recorded on a Perkin-Elmer model 1420 spec-

trophotometer. ^{1}H-nmr spectra were recorded on Varian EM-390 and JEOL model FX-90 Q spectrometers. ^{13}C-nmr spectra were recorded on JEOL FT models FX-60 and FX-90 Q spectrometers. Mass spectra were recorded on a Finnegan Quadrupole 4023 mass spectrometer. For chromatographic separations on a Chromatotron (13), rotors were coated with a 1 mm thick layer of Al_2O_3 60 GF-254 neutral (type E, EM reagents, cat. no. 1092), for column and vlc (14,15), Al_2O_3 neutral, activity 3 (EM reagents, cat. no. 1077), and for preparative tlc, Al_2O_3 60 HF-254 basic (type E, EM reagents, cat. no. 1094).

ISOLATION OF DELSTAPHININE.—A fraction of 5.7 g obtained at pH 4.0 by a gradient pH separation of 50.0 g of the amorphous mixture of alkaloids (2-4, 17) from the mother liquor of alkaloids from seeds of *D. staphisagria* (commercial sample obtained from S.B. Penick, New York, NY) was chromatographed on a column of neutral Al_2O_3. Elution was carried out with hexane, 5-15% Me_2CO in hexane, and 1-5% EtOH in 15% Me_2CO in hexane. Fractions 46 (300 mg) and 47 (125 mg), eluted with 1% EtOH in 15% Me_2CO in hexane, were designated as fractions A and B, respectively.

Fraction A was chromatographed (vlc) on Al_2O_3. Elution was performed with 1% EtOH in hexane. Fractions 6-9 were combined to give 50 mg of a residue as Fraction A1.

Fraction B was chromatographed (vlc) on Al_2O_3, and elution was performed with 1% EtOH in hexane. Fractions 5-8 were combined to give 35 mg of a residue as Fraction B1.

Fractions A1 and B1 were similar on tlc plates and were combined (85 mg) as fraction C. This fraction was rechromatographed (vlc) on Al_2O_3. Elution was carried out with CH_2Cl_2, 0.5% MeOH in CH_2Cl_2, and 5.0% MeOH in CH_2Cl_2. Fractions 11-13, eluted with 5% MeOH in CH_2Cl_2, were combined (35 mg) as fraction D. This fraction was chromatographed (preparative tlc) on two plates of Al_2O_3, using 1% MeOH in CH_2Cl_2 as an eluent. The major zone was extracted to give 22 mg of delstaphinine [**1**], amorphous, $[\alpha]^{28}D$ +47.7° (*c*, 1.1, $CHCl_3$). For ^{13}C-nmr data see Table 1.

ISOLATION OF 1-DEHYDRODELPHISINE [**7**].—A fraction of 14.4 g of "neutral" material left after extraction with 2% H_2SO_4 was redissolved in 200 ml of $CHCl_3$ and reextracted with 4×250 ml of 2% H_2SO_4. The acidic extracts were combined and made basic with cold, aqueous Na_2CO_3 solution, then extracted with 4×150 ml of $CHCl_3$. The $CHCl_3$ extract was dried over anhydrous Na_2SO_4 and distilled under vacuum to give 350 mg of residue, designated as fraction A. This was chromatographed on a Chromatotron using an alumina rotor, 1 mm thick. Elution was performed with 1-5% EtOH in hexane. Fraction 3 (33 mg), eluted with 1% EtOH in hexane, was chromatographed (preparative tlc) on two plates of Al_2O_3 using Et_2O-hexane (3:2) as an eluent. The upper zone was extracted to give 10 mg of 1-dehydrodelphisine which crystallized from Me_2CO/Et_2O, mp 168-170°; ^{1}H nmr δ 1.10 (3H, t, J=7 Hz, N-CH_2-CH_3), 1.97 and 2.02 (3H, each s, 2 OCOCH_3), 3.27 (9H, s, 3 OCH_3), 4.00 [1H, dd, J_1=1 Hz, J_2=7 Hz, C(6)-β*H*] and 4.84 [1H, dd, J_1=J_2=4.5 Hz, C(14)-β*H*]. This compound proved to be identical with a synthetic sample prepared by oxidation of delphisine (see below).

PREPARATION OF 1-DEHYDRODELPHISINE [**7**] FROM DELPHISINE [**6**].—Cornforth reagent (CrO_3-pyridine-H_2O) was prepared by the gradual addition of a solution of 0.1 g of CrO_3 in H_2O (0.1 ml) to pyridine (1.0 ml) with stirring and cooling in ice. This reagent (0.3 ml) was added gradually to a solution of delphisine (54 mg) in pyridine (5 ml) with stirring and cooling in ice. The reaction mixture was left overnight and then chromatographed (vlc) over 2 g of Al_2O_3. Elution with 1% EtOH in hexane gave 40 mg of 1-dehydrodelphisine which was crystallized from Me_2CO/Et_2O, mp 168-170°. The synthetic and natural samples of 1-dehydrodelphisine were identical by mixture mp, tlc, ^{1}H-, and ^{13}C-nmr spectra (2).

OXIDATION OF NEOLINE [**3**] TO *N*-DEETHYLNEOLINE [**4**].—To 45 mg of neoline in 30 ml of Me_2CO-H_2O (1:1) was added a solution of $KMnO_4$ (35 mg) in 20 ml of Me_2CO-H_2O (1:1). The mixture was stirred at room temperature for 19 h, then excess $KMnO_4$ was decomposed with about 15 mg of $NaHSO_3$ and 3 drops of 3% H_2SO_4. The Me_2CO was removed under reduced pressure and 50 ml of H_2O was added. The solution was basified with aqueous Na_2CO_3 solution and extracted with 3×50 ml of $CHCl_3$. The $CHCl_3$ extract was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give 23 mg of residue which was chromatographed on two plates of Al_2O_3 using 3% MeOH in CH_2Cl_2 as an eluent. The major zone was cut and extracted to give 19 mg of **4** as an amorphous residue: ^{13}C nmr (see Table 1); ^{1}H nmr δ 3.32, 3.34, and 3.36 (each 3H, s, OCH_3), 3.70 [1H, m, w/2=7 Hz, C(1)-β*H*], and 4.22 [1H, dd, J_1=J_2=4.5 Hz, C(14)-β*H*].

CONVERSION OF *N*-DEETHYLNEOLINE [**4**] TO NEOLINE [**3**].—To a solution of *N*-deethylneoline (**3**, 5 mg) in MeOH-Et_2O (1:1, 30 ml) was added 0.3 g of anhydrous K_2CO_3 and 0.1 ml of EtBr. The mixture was boiled under reflux for 3 h. The solvent was concentrated under vacuum, 20 ml of H_2O was added, and then the mixture was extracted with 3×50 ml of $CHCl_3$. The $CHCl_3$ extract was dried over anhydrous Na_2SO_4 and distilled to give 5 mg of residue. This was chromatographed on one plate of Al_2O_3 using 3% MeOH in CH_2Cl_2 as an eluent. The major zone was cut and extracted to give 3 mg of a residue which was identical with neoline by tlc behavior and ir spectra.

OXIDATION OF NEOLINE [3] TO *N*-DEETHYLDELSTAPHINIINE [5].—To a solution of neoline (55 mg) in Me_2CO (30 ml) was added $KMnO_4$ (55 mg) in 60 ml of H_2O-Me_2CO (1:5). The mixture was kept for 1 h at room temperature with occasional shaking, and then an additional amount of $KMnO_4$ (55 mg) in 60 ml of H_2O-Me_2CO (1:5) was added, and the mixture was warmed by immersing in a water bath heated to (80-90°) for 10 min. The Me_2CO was removed under reduced pressure, H_2O (50 ml) was added, and excess $KMnO_4$ was decomposed by addition of about 20 mg of $NaHSO_3$ and 2 drops of 2% H_2SO_4. The mixture was cooled in an ice bath, made basic with aqueous Na_2CO_3 solution, and extracted with 4×50 ml of $CHCl_3$. The $CHCl_3$ extract was dried over anhydrous Na_2SO_4 and distilled under vacuum to give 32 mg of a residue, designated as residue A. Then the aqueous layer was extracted again with 4×30 ml of $CHCl_3$, and the extract was dried and evaporated under reduced pressure to give 12 mg of a residue, designated as residue B.

Residue A was chromatographed on a 1 mm alumina rotor using a Chromatotron, and elution was performed with 2% MeOH in CH_2Cl_2 as an eluent. Fractions 4-7 were combined (26 mg) and was chromatographed on two plates of Al_2O_3, using 3% MeOH in CH_2Cl_2 as an eluent. The major zone was extracted to give 17 mg of homogenous material similar to residue B. These were combined to give 29 mg of pure *N*-deethyldelstaphinine [5], amorphous. ^{13}C nmr see Table 1; ^1H nmr δ 3.32, 3.35, and 3.36 (each 3H, s, OCH_3), 3.58 [1H, s, (19)-H], 3.80 [1H, m, C(6)-βH], and 4.21 [1H, dd, $J_1=J_2=4.5$ Hz, C(14)-βH].

CONVERSION OF *N*-DEETHYLDELSTAPHINIINE [5] TO DELSTAPHINIINE [1].—To 7 mg of **5** in Me_2CO (30 ml) was added 30 mg of anhydrous K_2CO_3 and 0.2 ml of EtBr. The mixture was boiled under reflux for 3 h. The solvent was decanted and evaporated under reduced pressure to give 7 mg residue. This residue was chromatographed on a plate of Al_2O_3 using 3% MeOH in CH_2Cl_2 as an eluent. The major zone was cut and extracted to give 4 mg of amorphous product which was identical with delstaphinine [1] by tlc, ir, and ^1H nmr.

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