NEW ALKALOIDS FROM *DELPHINIUM STAPHISAGRIA* Linné

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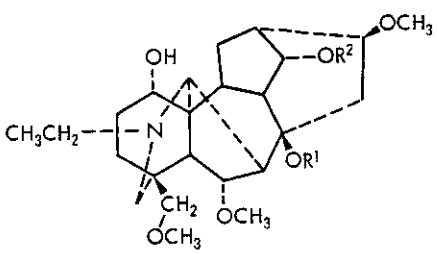
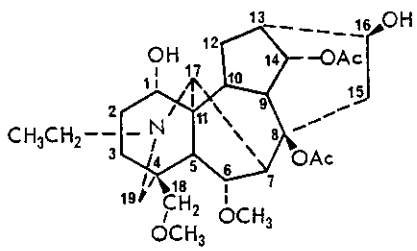
Abstract - Three new C_{19}-diterpenoid alkaloids, delstaphisine (3), delstaphisagrine (4) and delstaphisagnine (5) have been isolated from the seeds of Delphinium staphisagria by a combination of gradient pH extractions and chromatographic techniques, including vacuum liquid chromatography (vlc) and centrifugally accelerated, radial, thin-layer chromatography ("Chromatotron"). The structures have been determined with the aid of proton and carbon-13 nmr spectroscopy. Delstaphisine (3) is the first reported aconitine-type, C_{19}-diterpenoid alkaloid bearing a C(16)-hydroxyl group.

The seeds of *Delphinium staphisagria* L., on extraction with ligroin yield an alkaloidal fraction of which delphinine[1] is the major component. The mother liquors accumulated during the isolation of a large quantity of delphinine furnished an amorphous fraction from which delphisine (**1**)[2], delphidine (**2**)[3], delphirine (1-epineoline)[4], and several novel bis-diterpenoid alkaloids have been isolated.[5-7] In this paper we report separation of the amorphous fraction by a combination of gradient pH separation, vacuum liquid chromatography (vlc), preparative tlc (of the fraction obtained at pH 6.0) and centrifugally accelerated, radial, thin-layer chromatography ("Chromatotron") to give three new C_{19}-diterpenoid alkaloids, *delstaphisine* (**3**), *delstaphisagrine* (**4**), and *delstaphisagnine* (**5**).



Delphisine, 1 $R^1 = R^2 = Ac$
Delphidine, 2 $R^1 = Ac$; $R^2 = OH$
Delstaphisagnine, 5 $R^1 = OH$; $R^2 = Ac$
Neoline, 7 $R^1 = R^2 = H$



Delstaphisine, 3

Delstaphisine (**3**) was obtained as colorless plates, mp 182-184°C, $[\alpha]_D^{25}$ -11.0° (c, 1.35,

EtOH), and its molecular formula $C_{27}H_{41}NO_8$ was deduced from the mass spectral (M^+, 507.4), proton and carbon-13 nmr data. The proton nmr spectrum exhibited the following signals: δ 1.16 (3H, t, J = 7 Hz, N-CH_2-CH_3), 2.00 and 2.06 (each 3H, s, OCOCH_3), 3.30 and 3.33 (each 3H, s, OCH_3), 4.05 (1H, dd, J_1 = 1 Hz, J_2 = 7 Hz, C(6)-β-H) and 4.85 (1H, dd, J_1 = J_2 = 4.5 Hz, C(14)-β-H).

The noise decoupled ^{13}C nmr spectrum of **3** exhibited 26 signals for the 27 carbon atoms of the molecule (Table 1). The fragmentation pattern in the mass spectrum is similar to that of delphisine (**1**) and delphidine (**2**). The molecular ion 507.4 m/z is 14 mass units less than the molecular ion in the mass spectrum of delphisine (**1**) (521.3 m/z). Most of the high molecular fragments of delstaphisine (**3**) are 14 mass units less than the corresponding fragment in delphisine (**1**; in parenthesis): 507(521), 490(504), 448(462), 430(444), 420(434), 416(430), 404-(418), 388(402), 376(390), 342(356) m/z. The spectra of delphisine (**1**) and delstaphisine (**3**) contain the following identical fragments: 298, 237, 236, 224, 208, 178, 164, 147, 122, 108, 91, 71, 58, 44 and 43 (100%) m/z.

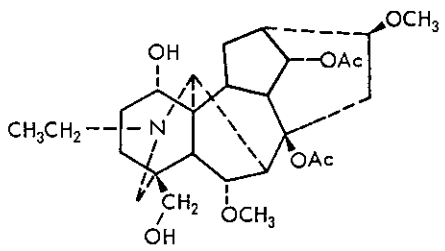
Structure **3** was deduced for delstaphisine from the spectral data and from its comparison with the neoline group of aconitine-type C_{19}-diterpenoid alkaloids. Of the two acetate groups observed in the proton nmr spectrum at δ 2.00 and 2.06, one is attributed to a C(14) α-acetate group. This assignment is supported by presence of a signal at δ 4.85 (dd, J_1 = J_2 = 4.5 Hz) that is characteristic of the C(14)-acetate. The C(14)-β H appears at δ 4.80 in delphisine (**1**), 4.73 in 1-acetyldelphisine, 4.86 in 1-*epi*-delphisine, and 4.80 in 1-acetyl-1-*epi*-delphisine.[8] In the ^{13}C spectrum, C(14) appeared as a doublet at its regular position of 76.0 ppm. The SFORD spectrum showed the presence of five singlets at 170.5, 169.6, 85.7, 49.8 and 38.1 ppm. The downfield signals at 170.5 and 169.6 are assigned to the two acetate carbonyl carbons, whereas the upfield signals at 49.8 and 38.1 ppm are due to the non-oxygenated quaternary carbons C(11) and C(4), respectively. The remaining signal at 85.7 ppm is assigned to the only oxygenated quaternary carbon at C(8).

Substitution of an acetoxyl for a hydroxyl group at C(8) in delphidine (**2**) and delphisine (**1**) produces a downfield shift (α-effect) of 11.1 and 11.5 ppm respectively, relative to C(8) in neoline (**7**).[8] The acetate signal at 169.6 observed for delstaphisine (**3**) is therefore assigned to C(8), as indicated by the presence of a singlet at 85.7 ppm in the ^{13}C nmr spectrum with a downfield difference (α-effect) of 11.4 ppm, relative to a shift of 74.3 ppm for C(8) in neoline — in good agreement with the expected α-effect.

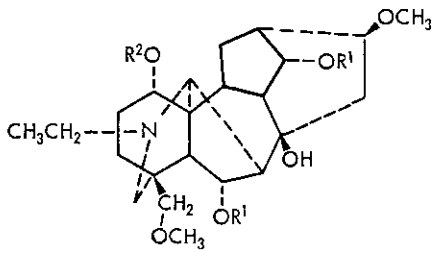
Delstaphisine (**3**) possesses two methoxyl functions, the protons of which showed signals in the ^1H nmr spectrum at δ 3.14 and 3.20 (each 3H, s), while the carbons were detected as two quartets at 59.2 and 58.1 ppm in the ^{13}C nmr spectrum. The signal at 59.2 ppm is assigned to the C(18)-methoxyl carbon, since this assignment is the usual one for the C(18)-methoxyl carbon in all C_{19}-diterpenoid alkaloids carrying a C(18)-methoxyl group; this assignment is supported by the existence of a triplet at 79.7 ppm representing the only oxygenated methylene carbon at C(18). If the oxygen function at C(18) had been an OH group, this triplet should have been shifted upfield about 10 ppm, as in the case of alkaloid **4** (see below).

The assignment of the OCH_3 signal at 58.1 (q) ppm to the methoxyl carbon at C(6), and the doublet at 83.8 ppm to C(6) bearing the methoxyl function relies on the following analysis: The ^{13}C chemi-

cal shift of C(6)-methoxyl occurs between 57.2 ppm and 58.2 ppm in all aconitine-type C_{19}-diterpenoid alkaloids having C(6)-OCH_3 and the signal due to the C(16)-methoxyl carbon occurs at ~ 56 ppm in all C_{19}-diterpenoid alkaloids, except those carrying an oxygen substituent at C(13) or C(15).[8] On the basis of above literature precedent the methoxyl signal at 58.1 (q) ppm, was assigned to C(6)-OCH_3, while the only signal present at 56.8 ppm was a triplet attributed to the C(19)-methylene carbon, indicating the absence of a C(16)-methoxyl group.



Delstaphisagrine, **4**



Foresticine, **6** $R^1 = H$; $R^2 = CH_3$

Senbusine A, **8** $R^1 = R^2 = H$

In the proton nmr spectrum, the presence of a β-proton at C(6) is indicated by the existence of a doublet of doublets, (1H, J_1= 1 Hz, J2 = 7 Hz) at δ 4.05. An experiment of ^{13}C-^1H-selective decoupling in which the signal at δ 4.05 in the proton nmr spectrum of delstaphisine (**3**) was decoupled, resulted in decoupling of the C(6) signal in the ^{13}C spectrum and gave rise to a sharp singlet at 83.8 ppm. The proton signal at δ 4.05 must be H(6) based on the observed chemical shift and coupling constants; therefore the carbon signal at 83.8 ppm is due to C(6). Comparison of the C(6) chemical shifts observed for delphisine (**1**), and neoline (**7**) [with a C(6) α-methoxyl] with those observed for foresticine (**6**)[9] and senbusine A (**8**)[10] [with a C(6) α OH] show the expected β-effect of ~ 11 ppm downfield shift with the substitution of a OCH_3 for a OH. The chemical shift of 83.8 ppm for delstaphisine shows the 11 ppm downfield shift expected for a C(6) substituted with methoxyl.

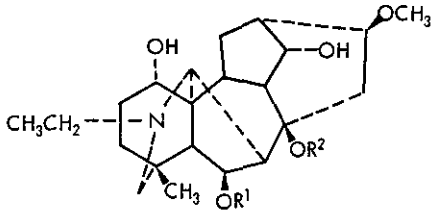
The presence of a C(1)-OCH_3 group in alkaloid **3** is ruled out by the occurrence of C(2) and C(3) signals as triplets at 29.3 ppm and 29.9 ppm [SFORD, ^{13}C nmr], respectively, and the signal of C(1) bearing an α-OH as a doublet at 71.9 ppm. This interpretation is based on a comparison with chasmanine (1-methoxyneoline), where the C(2)-signal occurs at 26.0 ppm and the C(3)-signal at 35.2 ppm. The downfield shift of 3.3 ppm for C(2) and the upfield shift of 5.3 ppm for C(3) in alkaloid **3** relative to chasmanine are comparable with the net downfield β-effect of 3.5 ppm and the net upfield γ-effect of 5.4 ppm exerted by the C(1) α-OH on the chemical shift of C(2) and C(3), respectively, in neoline (**7**) relative to chasmanine.[8]

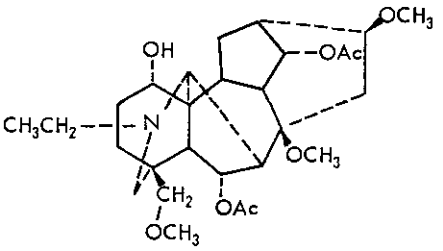
Among the aconitine-type and lycoctonine-type of alkaloids, there are only two examples of alkaloids having a hydroxyl group at C(1) in the β-configuration, viz: delphirine (1-epineoline) and talatizidine.[11] The general range of the chemical shift for C(1) with an α-OH is 72.0 -73.0 ppm except where a C(10)-OH or a C(2)-C(3) double bond is present.[8] The value for C(1) in delphirine

with a β-OH is 69.0 ppm.[8] Since the value for C(1) in **3** is 71.9 ppm, the C(1)-OH is assigned an α-configuration.

In the ^{13}C nmr spectrum of **3**, the region between 44 and 56 ppm shows the presence of three signals at: 48.4 ppm (triplet), attributed to the methylene carbon of *N*-ethyl; 49.8 ppm (singlet), attributed to C(11) (quaternary carbon); 48.0 ppm (doublet), assigned to C(7). The chemical shift for C(7) is comparable to that found in similar compounds as shown in Table 1 and as expected, differs in a predictable manner from compounds such as neoline.

In "Alkaloid B" (**9**)[12] from *Delphinium bicornis* with C(6) and C(8) bearing a β-OH, C(7) occurs at 54.8 ppm. In "Alkaloid A" (**10**)[12], having a 6-β-acetoxyl-8-β-methoxyl grouping, the C(7) signal appears at 48.0 ppm. The occurrence of the chemical shift of C(5) at 43.8 ppm in **3**, compared with delphisine (44.1 ppm) suggests similar methoxyl substitution at adjacent C(6).





Alkaloid B, **9** $R^1 = R^2 = H$

Alkaloid A, **10** $R^1 = Ac$; $R^2 = CH_3$

11

An alternative structure **11**, with C(6) bearing an acetate group and C(8) a second methoxyl function, is ruled out by comparing the ^{13}C nmr spectral data of **3** with those values reported for "Alkaloid A" (**10**).[12] In "Alkaloid A" (**10**), the chemical shift of the quaternary C(8), bearing a methoxyl group, is reported at 79.9 ppm and the C(8)-methoxyl at 52.8 ppm; the C(16)-methoxyl carbon resonates at its usual position (56.4 ppm). Also, the occurrence of the signal at δ 5.74 in the proton nmr spectrum of foresticine-6,14-diacetate[9] and the signal at δ 5.66 in senbusine A 1,6,14-triacetate[10] is assigned to the C(6)-β proton; by comparison, in **3** only the C(14)-β proton occurs at δ 4.85, while the other acetate group is attached to quaternary C(8).

In the ^{13}C nmr spectrum of **3**, the signal attributed to C(15) appeared as a triplet at 41.3 ppm. In the related neoline group of C_{19}-diterpenoid alkaloids, bearing an acetoxyl group at C(8), C(15) occurs at: 38.5 ppm in delphisine (**1**); 38.4 ppm in delphidine (**2**); 38.2 ppm in 1-epidelphisine; 37.7 ppm in delphisine-1-acetate. In alkaloid **3**, in which C(8) bears an acetate function, the observed downfield shift (~ 3 ppm) for the resonance of the C(15) methylene triplet is attributed to the β-effect of the non-methylated OH at C(16).

The possibility that C(16) is non-oxygenated and that C(15) bears the hydroxyl function is excluded because, in such a case, the quaternary C(8) bearing an acetate group should resonate at 92 ppm.

Because of the β-effect caused by a neighbouring C(15)-hydroxyl group, C(8) bearing an acetate occurs at: 92.1 ppm in isodelphinine[13]; 92.0 ppm in aconitine[8]; 91.8 ppm in mesaconitine[8]; 92.5 ppm in anhydroaconitine[8]; 92.0 ppm in deoxyaconitine[8].

The alternative possibility of the location of the hydroxyl group on C(13), C(9), C(10) or C(7) instead of C(16) is ruled out because of the non-existence of any quaternary oxygenated singlets other than the one at 85.7 ppm assigned to C(8)-$OCOCH_3$. The occurrence of the chemical shift of C(2) and C(3) at 29.3 ppm and 29.8 ppm, respectively, in **3** rules out the possibility of the hydroxyl group being attached to C(3).

Delstaphisine (**3**) is thus the first reported aconitine-type, C_{19}-diterpenoid alkaloid having a C(16)-hydroxyl group.

Delstaphisagrine (**4**) was obtained in an amorphous form, $[\alpha]_D^{23}$ +3.8° (c, 0.6, EtOH), and its molecular formula $C_{27}H_{41}NO_8$ was derived from the mass spectral (M^+ 507.3), proton and ^{13}C nmr data. The proton nmr spectrum exhibited the following signals: δ 1.13 (3H, t, J = 7 Hz, N-CH_2CH_3), 1.94 and 2.0 (each 3H, s, $OCOCH_3$), 3.24 and 3.30 (each 3H, s, OCH_3), 4.05 (1H, dd, J_1= 1 Hz, J_2 = 7 Hz, *C(6)-β-H) and* 4.80 (1H, dd, $J_1 = J_2$ = 4.5 Hz, C(14)-β-H).

The noise decoupled ^{13}C nmr spectrum of **4** exhibited 25 signals for the 27 carbon atoms of the molecule (Table 1). The fragmentation pattern in the mass spectrum of **4** is similar to that of delphisine (**1**). The molecular ion, 507.3 m/z, is 14 mass units less than the molecular ion in the mass spectrum of delphisine (**1**) (521.3 m/z). Each of the high molecular fragments of delstaphisagrine is 14 mass units less than the corresponding fragment in delphisine (**1**) (in parenthesis): 507(521), 490(504), 474 (488), 448(462), 430(444), 416(430), 404(418), 388(402), 223 (237), 222(236), 210(224) m/z. The spectra of delphisine and delstaphisagrine (**4**) contain the following identical fragments: 298, 192, 164, 148, 147, 122, 108, 91, 71, 58, 44 and 43 (100%) m/z.

The structure of **4** was determined from its spectral data and from comparison of these data with those of the neoline group of aconitine-type C_{19}-diterpenoid alkaloids. As in the case of alkaloid **3**, two acetate groups observed in the proton nmr spectrum at δ 1.94 and 2.00 are attributed to C(14) and C(8). The secondary 14-acetate is confirmed by the presence of the signal at δ 4.80 corresponding to C(14)-β-H. The tertiary 8-acetate is indicated by the occurrence of the quaternary C(8)-singlet at 85.8 ppm and the acetyl signals at 169.7 ppm (singlet) and 22.4 ppm (quartet).

The two methoxyl signals at δ 3.24 and 3.30 in the proton nmr spectrum correspond to C(6) and C(16), as indicated by the two doublets at 83.7 and 82.6 ppm for C(6) and C(16), and two quartets at 58.2, 56.6 ppm for C(6)-OCH_3 and C(16)-OCH_3, respectively. The doublet at 83.7 ppm is analogous to that found for delstaphisine (**3**) at 83.8 ppm. The C(16)-methoxyl is indicated by the signal at 38.4 ppm for C(15) relative to 41.3 ppm as found for delstaphisine (**3**), the expected β effect of ~ 3 ppm.

The presence of C(1)-α-OH is shown by the occurrence of C(2) and C(3) signals at 29.5 and 29.8 ppm as triplets in the ^{13}C nmr [SFORD] spectrum. The location of an OH group on C(18) is deduced by the change of the chemical shift of the only oxygenated C(18) methylene triplet from ~ 80.0 to

70.2 ppm and absence of the OCH_3 quartet at ~ 59.0 ppm in the ^{13}C nmr spectrum.

Delstaphisagnine (**5**) was obtained in an amorphous form, $[\alpha]_D^{25}$ +20.0° (c, 0.85, EtOH), and its molecular formula $C_{26}H_{41}NO_7$ was deduced from the mass spectral (M^+ 479.5), proton and carbon-13 nmr data. The proton nmr spectrum exhibited the following signals: δ 1.10 (3H, t, J=7 Hz, N-CH_2-CH_3), 2.00 (3H, s, OCOCH_3), 3.25, 3.27 and 3.30 (each 3H, s, OCH_3), 4.06 (1H, dd, J_1= 1 Hz, J_2= 7 Hz, C(6)-β-H) and 4.80 (1H, dd, J_1=J_2 = 4.5 Hz, C(14)-β-H).

The noise decoupled ^{13}C nmr spectrum of **5** showed 26 signals due to 26 carbon atoms in the molecule (Table 1). The structure **5** was derived from the spectral data and from its comparison with the neoline group of aconitine-type C_{19}-diterpenoid alkaloids. The acetate group observed in the proton nmr spectrum at δ 2.00 is attributed to the C(14)-α-acetate group. This assignment is supported by the presence of a signal at δ 4.80 (dd, J_1 = J_2 = 4.5 Hz) that is characteristic of the C(14)-acetate. For comparison, the C(1)-β-proton in neoline-1-acetate[2], appears at δ 4.8 as an ABX type quartet (J_{AX} = 10 Hz and J_{BX} = 6 Hz). The SFORD spectrum of **5** showed the presence of four singlets at 170.4, 74.6, 49.7 and 38.0 ppm. The downfield signal at 170.4 is assigned to the acetate carbonyl carbon, while the upfield signals at 49.7 and 38.0 ppm are attributed to the non-oxygenated quaternary carbons, C(11) and C(4), respectively. The remaining signal at 74.6 ppm is assigned to the only oxygenated quaternary carbon, C(8). C(8) bearing a hydroxyl in neoline (**7**) appears at 74.3 ppm, whereas C(8) bearing an acetoxyl group occurs at 85 ppm as in the case of delphisine (**1**), delphidine (**2**), delstaphisine (**3**) and delstaphisagrine (**4**).

In the ^{13}C nmr spectrum of **5**, the region between 44 and 56 ppm shows the presence of three signals at: 48.2 ppm (triplet), attributed to the methylene carbon of *N*-ethyl; 49.7 ppm (singlet), attributed to quaternary C(11); 52.6 ppm (doublet) assigned to C(7). The corresponding resonance for C(7) in neoline (**7**) having a 6α-methoxyl-8-hydroxyl moiety is located at 52.3 ppm. The three methoxyl signals at δ 3.25, 3.27 and 3.30 in the proton nmr spectrum correspond to those substituted at C(6), C(16) and C(18). In the ^{13}C nmr spectrum of **5** the methoxyl carbons at C(16), C(6) and C(18) are represented with three quartets at 56.1, 57.9 and 59.1 ppm, respectively. The presence of a C(1)-α-OH is shown by the occurrence of signals for C(2) and C(3) at 29.5 and 30.0 ppm, respectively, as triplets in the ^{13}C nmr spectrum. Structure **5** for staphisagnine was confirmed by alkaline hydrolysis of staphisagnine to neoline (**7**).

Table 1 gives the carbon-13 chemical shifts and assignments for delstaphisine (**3**), delstaphisagrine (**4**), delphisine (**1**), neoline (**7**), delstaphisagnine (**5**), delphidine (**2**), senbusine A (**8**), alkaloid B (**9**) and foresticine (**6**).

ACKNOWLEDGMENT

We are grateful to Dr. John Wunderlich for running model decoupling experiments on delphisine (**1**). We thank Drs. Hitesh Chokshi and John Wunderlich for reviewing this manuscript and making helpful suggestions.

Table 1. Carbon-13 Chemical Shifts* and Assignments for Delstaphisine (**3**), Delstaphisagrine (**4**), Neoline-type Bases: Delphisine (**1**), Neoline (**7**), Delstaphisagnine (**5**), Delphidine (**2**), Senbusine A (**8**), Alkaloid B (**9**) and Foresticine (**6**).

Carbon	3	4	1	7	5	2	8	9	6
1	71.9 d	71.9 d	72.1	72.3	72.0 d	72.0	72.1	72.0	85.7**
2	29.3 t	29.5 t	29.5	29.5	29.5 t	29.5	29.2	29.7	25.8
3	29.9 t	29.8 t	30.1	29.9	30.0 t	29.9	29.8	32.2	34.8
4	38.1 s	39.1 s	38.1	38.2	38.0 s	38.2	37.9	32.8	39.1
5	43.8 d	45.2 d	44.1	44.9	44.5 d	46.1	48.2	46.1	49.3
6	83.8 d	83.7 d	84.2	83.3	83.3 d	84.1	72.6	72.0	71.9
7	48.0 d	47.6 d	48.3	52.3	52.6 d	48.2	55.4	54.8	54.3
8	85.7 s	85.8 s	85.8	74.3	74.6 s	85.4	75.6	76.0	74.0
9	43.1 d	43.1 d	43.3	48.3	46.2 d	44.0	45.6	50.2	48.9
10	42.9 d	38.4 d	38.5	40.7	36.6 d	40.8	40.6	40.0	38.7
11	49.8 s	49.8 s	49.8	49.6	49.7 s	49.9	48.2	48.4	50.6
12	29.0 t	29.4 t	29.2	29.8	29.3 t	29.5	29.9	29.7	28.8
13	43.1 d	43.1 d	43.3	44.3	43.3 d	44.0	44.2	44.4	45.6
14	76.0 d	75.6 d	75.5	75.9	77.1 d	75.0	75.4	76.0	75.3
15	41.3 t	38.4 t	38.5	42.7	42.6 t	38.4	42.2	42.2	39.4
16	72.9 d	82.6 d	82.7	82.3	81.9 d	82.4	82.4	82.4	82.8
17	62.9 d	63.8 d	62.7	63.6	63.3 d	63.0	63.5	64.9	62.6
18	79.7 t	70.2 t	79.8	80.3	80.1 t	79.8	80.3	27.4	80.8
19	56.8 t	56.6 t	56.8	57.2	57.9 t	56.8	57.1	61.8	54.3

Table 1. continued

Carbon	3	4	1	7	5	2	8	9	6
N-CH_2	48.4 t	48.4 t	48.0	48.2	48.2	48.4	49.7	48.4	50.4
CH_3	12.7 q	12.9 q	12.9	13.0	13.0	12.7	12.9	13.0	13.5
6'	58.1 q	58.2 q	58.0	57.8	57.9	58.1	--	--	--
16'	--	56.6 q	56.5	56.3	56.1	56.6	56.3	56.3	56.4
18'	59.2 q	--	59.0	59.1	59.1	59.1	59.2	--	59.2
C=O (8')	169.6 s	169.7 s	169.3	--	--	169.9	--	--	--
CH_3	22.3 q	22.4 q	22.2	--	--	22.5	--	--	--
C=O (14')	170.5 s	170.7 s	170.4	--	170.4	--	--	--	--
CH_3	21.2 q	21.3 q	21.1	--	21.3	--	--	--	--

* in ppm downfield to TMS, solvent deuterochloroform

** Chemical shift of methoxyl at C(1) occurs at 56.1 ppm

EXPERIMENTAL

Melting points are corrected and were taken on a Thomas-Kofler hot stage equipped with a microscope and a polarizer. Infrared spectra were taken on a Perkin-Elmer model 1420 spectrophotometer. ^{1}H nmr spectra were taken on a Perkin-Elmer EM-390, 90 MHz spectrometer; ^{13}C nmr spectra on JEOL model FX-60 and FX-90Q spectrometers in $CDCl_3$ solution with TMS as an internal standard. Mass spectra were determined on a Finnegan Quadrupole 4023 instrument.

A fraction of 3.5 gm, obtained at pH 6.0 by a gradient pH separation of 50.0 gm of the amorphous mixture of alkaloids from the mother liquor of alkaloids from *Delphinium staphisagria*, was chromatographed (vlc)[14] on 60 gm of neutral alumina (Merck aluminum oxide 90; 70-230 mesh ASTM; activity 3) and the following fractions were collected:

Fraction	Solvent	g
A, 1-3	10% EtOAc/hexane	0.040
A, 4-7	10% EtOAc/hexane	0.048
A, 8-17	10% EtOAc/hexane	0.400
A, 18-27	25% EtOAc/hexane	0.400
A, 28-34	EtOAc/hexane (1:1)	0.140
A, 35-37	EtOAc	1.400
A, 38-40	10% MeOH/CH_2Cl_2	1.060

Fractions A, 8-17, 400 mg, was chromatographed (vlc) on 30 gm of neutral alumina (Merck aluminum oxide 90; 70-230 mesh ASTM; activity 3) and the following fractions were collected:

Fraction	Solvent	g
B, 1-19	15% EtOAc/hexane	0.123
B, 20-30	25% EtOAc/hexane	0.090
B, 31	10% MeOH/$CHCl_3$	0.170

Fractions B, 20-30, 90 mg, was chromatographed on alumina plates using 1.5% MeOH/CH_2Cl_2 as an eluent. The major band was extracted to give 38 mg of residue designated as B1.

Fractions A, 18-27, 400 mg, was chromatographed (vlc) on 30 gm of neutral alumina (Merck aluminum oxide 90; 70-230 mesh ASTM; activity 3) and the following fractions were collected:

Fraction	Solvent	g
C, 1-3	hexane	
C, 4-10	0.5% EtOH/hexane	0.070
C, 11-26	1.0% EtOH/hexane	0.190
C, 27-36	2.0% EtOH/hexane	0.135

Fractions C, 12-19, were combined (110 mg) and chromatographed on alumina preparative plates, using 1.5% MeOH in CH_2Cl_2 as an eluent. Two bands were cut and extracted to give: C2 (48 mg) and C3 (15 mg).

Fractions C, 20-26, were combined (80 mg) and chromatographed on alumina plates using 1.5% MeOH in CH_2Cl_2 as an eluent. The two major bands were extracted to give C4 (38 mg) and C5 (17 mg).

Fractions A, 28-34, were combined (140 mg) and chromatographed on alumina preparative plates, using 2% MeOH in EtOAc as an eluent. The major band was extracted to give A1 (85 mg). This residue was rechromatographed on alumina plates using 1.5% MeOH in CH_2Cl_2 as an eluent. Two zones were extracted to give A2 (40 mg) and A3 (18 mg).

Delstaphisine (3):
Fractions C3(15 mg), C5 (17 mg) and A3 (18 mg) had similar proton and ^{13}C nmr spectra and were combined (50 mg) and crystallized from acetone/hexane to give colorless plates of delstaphisine (3), mp 182-184°C, $[\alpha]_D^{25}$ -11.0° (c, 1.35, EtOH).

Fractions B1, C2, and C4 were similar and, although homogenous on alumina tlc plates using EtOAc/1-2% MeOH or 1-2% MeOH in CH_2Cl_2, were resolved into two overlapping components on silica gel tlc plates using EtOAc as a solvent system. Fraction B1 (38 mg) was chromatographed on preparative silica gel plates using EtOAc as an eluent. The lower zone was extracted to give 25 mg designated as B2. The upper zone was extracted to give B3 (7 mg).

Fraction C2 (48 mg) was chromatographed on silica gel plates using EtOAc as an eluent. The lower band was extracted to give C6 (30 mg). The upper band was extracted to give C7 (10 mg). Fraction C4 (38 mg) was chromatographed on silica gel plates using EtOAc as solvent system. The lower band was extracted to give C8 (20 mg). The upper band was extracted to give C9 (9 mg).

Delphidine (2):
Fractions B2, C6, C8 and A2 proved to be identical (tlc and proton nmr); they were combined, (115 mg) and identified as delphidine (**2**). The proton nmr spectrum of **2** showed signals at δ 1.13 (3H, t, J = 7 Hz, N-CH_2-CH_3), 2.00 (3H, s, $OCOCH_3$), 3.26, 3.31 and 3.35 (3H each, s, OCH_3). For ^{13}C nmr data see Table 1.

Delstaphisagrine (4):
Fractions B3, C7 and C9 were identical (tlc and proton nmr); they were combined (23 mg) and designated as delstaphisagrine (**4**), amorphous, $[\alpha]_D^{23}$ +3.8° (c, 0.6, EtOH). For ^{13}C nmr data see Table 1.

Delstaphisagnine (5):
Fraction A (4-7) (48 mg) was chromatographed on an alumina-covered rotor of a "Chromatotron"[15] with EtOAc/hexane (1:1) and 20 ml fractions were collected. Fraction 3 (15 mg) was chromatographed on one plate of alumina, using ether/$CHCl_3$ (1:1) as an eluent. The lower zone was cut and extracted to give 6 mg of residue. The residue from fractions 4-5 (13 mg) was chromatographed on one plate of alumina, using ether/$CHCl_3$ as an eluent. The lower zone was cut and extracted to give 5 mg. of residue. The two lower zones from fractions 3 and 4-5 were similar on tlc alumina plates using ether, ether/$CHCl_3$ (1:1) and EtOAc/hexane (1:1) as eluents. They also have similar proton nmr spectra and were combined (11 mg) to give delstaphisagnine (**5**), amorphous, $[\alpha]_D^{25}$ +20.0° (c, 0.85, EtOH). For ^{13}C nmr data see Table 1.

Hydrolysis of staphisagnine (5) to neoline (7): To an aqueous methanolic (10% H_2O) solution (10 ml) of potassium carbonate (10 mg) was added **5** (5 mg) and the solution was stirred at room temperature overnight. After removal of the solvent under vacuum the residue was dissolved in

water (20 ml) and extracted four times with chloroform. The extracts were combined and dried over sodium sulfate, and the solvent was removed under vacuum leaving 5 mg of residue. The residue was chromatographed on an alumina plate using 4% MeOH in CH_2Cl_2 as an eluent. The main band was extracted to give 3 mg of neoline (**7**). The identity was confirmed by comparison of proton nmr spectra and behavior on tlc plates.

Attempts to correlate delstaphisine (**3**) and delstaphisagrine (**4**) with delphisine (**1**) by methylation experiments were unsuccessful. Selective demethylation of delphisine to afford either **3** or **4** was also unsuccessful.

REFERENCES

1. W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **127**, 36 (1939).
2. S. W. Pelletier, Z. Djarmati, S. Lajsic, and W. H. De Camp, *J. Amer. Chem. Soc.*, **98**, 2617 (1976).
3. S. W. Pelletier, J. K. Thakkar, N. V. Mody, Z. Djarmati, and J. Bhattacharyya, *Phytochemistry*, **16**, 404 (1977).
4. S. W. Pelletier and J. Bhattacharyya, *Tetrahedron Lett.*, 4679 (1976).
5. W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **141**, 67 (1941).
6. S. W. Pelletier, N. V. Mody, Z. Djarmati, I. V. Micovic, and J. K. Thakkar, *Tetrahedron Lett.*, 1055 (1976).
7. S. W. Pelletier, Z. Djarmati, and N. V. Mody, *Tetrahedron Lett.*, 1749 (1976).
8. S. W. Pelletier and Z. Djarmati, *J. Amer. Chem. Soc.*, **98**, 2626 (1976).
9. S. W. Pelletier, C. Z. Ying, B. S. Joshi, and H. K. Desai, *J. Nat. Prod.*, **47**, 474 (1984).
10. C. Konno, M. Shirasaka and H. Hikino, *J. Nat. Prod.*, **45**, 128 (1982).
11. S. W. Pelletier, N. V. Mody, K. I. Varughese, J. A. Maddry and H. K. Desai, *J. Amer. Chem. Soc.*, **103**, 6536 (1981).
12. S. W. Pelletier, N. V. Mody, A. J. Jones and M. H. Benn, *Tetrahedron Lett.*, 3025 (1976).
13. S. W. Pelletier, N. V. Mody and N. Katsui, *Tetrahedron Lett.*, 9027 (1977).
14. S. W. Pelletier, B. S. Joshi and H. K. Desai, "Techniques for Isolation of Alkaloids", in *Advances in Medicinal Plant Research*, Editors: A.J. Vlietinck and R.A. Dommisse, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 1985, pp. 153-196.
15. H. K. Desai, B. S. Joshi, A. M. Panu and S. W. Pelletier, *J. Chromatogr.*, **322**, 223 (1985).

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