DELSTAPHIGINE AND 14-O-BENZOYLDELPHONINE, NEW ALKALOIDS FROM DELPHINIUM STAPHISAGRIA LINNE'

S. William Pelletier*, Samir A. Ross[+], and Joseph T. Etse

Institute for Natural Products Research and the School of Chemical Sciences,

The University of Georgia, Athens, Georgia 30602, USA

Abstract - Delstaphigine (1) and 14-O-benzoyldelphonine (2), two new C_{19}-diterpenoid alkaloids, and chasmaconitine (4) have been isolated from the seeds of Delphinium staphisagria. The structures of these alkaloids were determined from spectral data and by correlation with alkaloids of established structures. Thus methylation of delstaphigine (1) afforded delphinine (3). 14-O-Benzoyldelphonine (2) was synthesized from both delphinine (3) and delphonine (5). Chasmaconitine (4) has not been previously isolated from a Delphinium species.

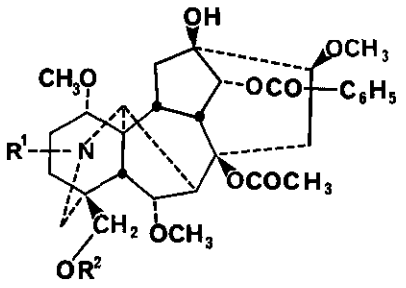
Delphinium staphisagria L. is a plant that is extraordinarily rich in alkaloids. In continuation of our phytochemical studies on this plant [1-10], we report here the isolation of two new C_{19}-diterpenoid alkaloids: delstaphigine (1) and 14-*O*-benzoyldelphonine (2). Chasmaconitine (4), an alkaloid previously reported only from *Aconitum* species[11], has also been isolated.

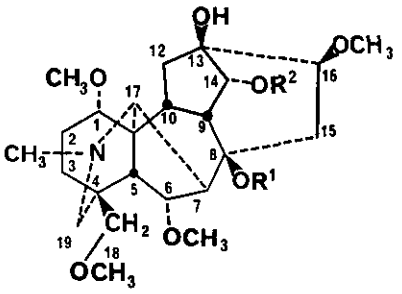
Delstaphigine (1) was obtained in an amorphous form, $[\alpha]_D^{18}$ +4.2° (c, 0.4, $CHCl_3$), and its molecular formula $C_{32}H_{43}NO_9$ was derived from the mass spectral (M^+ 585) data. The ^1H nmr spectrum exhibited the following signals: δ1.25 (3H, s, C(8)-O-$COCH_3$), 2.32 (3H, s, *N*-CH_3), 3.16, 3.29, and 3.54 (3H each, s, OCH_3), 4.91 (1H, d, = 4.5 Hz, C(14)-β-H) and multiplets between δ 7.31-8.15 (5H, aromatic protons of benzoate group). The noise-decoupled ^{13}C nmr spectrum appears in the Table. The mass spectrum showed: M^+, m/z (%) 585(0.1), 554(M^+ -OCH_3, 12), 495(3), 494(9), 224(7), 223(9), 105(89), 77(41), 58(31) and 43(100).

The ^1H nmr spectrum of delstaphigine was similar to that of delphinine[11], except for the presence of one less methoxyl group. Delstaphigine showed 3 singlets at 3.16, 3.29 and 3.54 ppm (3H each), whereas delphinine showed 2 singlets at 3.26 and 3.65 ppm (3H each) and one singlet at 3.40 ppm (6H). Also the molecular ion 585 m/z of delstaphigine is 14 mass units less than that of delphinine. Comparison of the chemical shifts of the methoxyl carbons in both

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

delstaphigine and delphinine (Table) suggested that the two signals at 56.3 and 57.6 ppm in delstaphigine can be assigned to C(1)-OCH_3 and C(6)-OCH_3, respectively. The third signal at 58.8 ppm can be attributed to either C(18)-OCH_3 or C(16)-OCH_3. Usually, the signal for C(18) in alkaloids carrying a C(18)-methoxyl group appears about 79.9-80.8 ppm.[11] If the oxygen function at C(18) had been an OH group, this signal would have been shifted upfield about 9-10 ppm, as in the case of delstaphisagrine (70.2 ppm)[6] and neolinine (70.9 ppm).[8] Thus the signal at 71.2 ppm in delstaphigine is assigned to C(18)- bearing an OH, and the signal at 58.8 ppm to the C(16)-methoxyl carbon.





1 $R^1 = CH_3$; $R^2 = H$ **DELSTAPHIGINE**

2 $R^1 = H$; $R^2 = COC_6H_5$ **14-O-BENZOYL-DELPHONINE**

4 $R^1 = CH_2CH_3$; $R^2 = CH_3$

3 $R^1 = COCH_3$; $R^2 = COC_6H_5$ **DELPHININE**

5 $R^1 = H$; $R^2 = H$ **DELPHONINE**

The tentative structure 1 can thus be derived for delstaphigine and its ^{13}C nmr data (Table) are consistent with this structure. The structure of delstaphigine was established by methylation with trimethyloxonium tetrafluoroborate[12] and a proton sponge to afford a product that was identical in all respects with an authentic sample of delphinine (3).

14-*O*-Benzoyldelphonine (2) was obtained in an amorphous form, $[\alpha]_D^{24}$ +60.1° (c, 0.2, $CHCl_3$), and its molecular formula $C_{31}H_{43}NO_8$ was derived from the mass spectral (M^+ 557) data. The ir spectrum (nujol) showed carbonyl (1720 cm^{-1}) and hydroxyl (3480 cm^{-1}) absorptions. The ^1H nmr spectrum exhibited the following signals: δ 2.23 (3H, s, N-CH_3), 3.28(6H, s, OCH_3), 3.30 and 3.38 (3H each, s, OCH_3), 4.04 (1H, dd, $J_1 = J_2 = 7$ Hz, C(6)-β-H), 5.19 (1H, d, J = 5 Hz, C(14)-β-H) and multiplets in the region between δ 7.44-8.05 (5H, aromatic protons of benzoate group). The ^{13}C nmr data are given in the Table. The mass spectrum showed: M^+, m/z (%), 557(1), 526($M^+ - OCH_3$, 98), 494 (3), 105(100), 77(40), 71(12), 45(34), 44(36), 43(11), 42(14).

The proton and ^{13}C nmr spectra of 14-*O*-benzoyldelphonine are similar to those of delphinine,[11] except for the absence of peaks for the acetate group. Also the molecular ion 557 m/z is 42 mass units less than that of delphinine (3). Alkaline hydrolysis with 5% methanolic KOH solution gave a product which was identical with delphonine (5) by tlc behavior, ir, ^{1}H and ^{13}C nmr spectra.[13,14] The tentative structure 2 can thus be derived for this new alkaloid.

The structure of 14-*O*-benzoyldelphonine (2) was confirmed by synthesis from both delphinine (3) and delphonine (5). Thus, deacetylation[15] of delphinine was effected by boiling in water under nitrogen for 4 h to afford 2. Benzoylation of 5 with benzoyl chloride and pyridine also resulted in the formation of 2. Identity of natural and synthetic samples of 2 was confirmed by tlc behavior, and ir, proton and ^{13}C nmr spectra.

Chasmaconitine (4) was isolated in a crystalline form, mp 185.5-187.5°C. The ir, mass and proton nmr data for 4 compare favorably with published values.[16-18] The ^{13}C nmr data for chasmaconitine is given in the Table. Chasmaconitine has not previously been reported in a *Delphinium* species, but has been isolated from *Aconitum chasmanthum*, *A. ferox*, *A. forestii* and *A. franchetii*.[16-18]

EXPERIMENTAL

Melting points are corrected and were taken on a Thomas-Kofler hot stage equipped with a microscope and a polarizer. Specific rotations were measured on a Perkin-Elmer model 141 polarimeter. Infrared spectra were taken on a Perkin-Elmer model 1420 spectrophotometer. ^{1}H and ^{13}C nmr spectra were recorded on JEOL FT models FX-60 and FX-90Q spectrometers in $CDCl_3$. Mass spectra were determined on a Finnegan Quadrupole 4023 instrument. For chromatographic separations on a Chromatotron,[19,20] rotors were coated with a 1 mm thick layer of aluminum oxide 60 PF 254 + 365, basic, type E, (EM reagents, Cat. No. 1104) or aluminum oxide 60 GF 254, neutral (EM reagents, Cat. No. 1092); for separation by vacuum liquid chromatography (vlc)[21], aluminum oxide 60 H 254, basic, type E, (EM reagents, Cat. No. 1085) was used. For ptlc, aluminum oxide 60 GF 254, neutral (EM reagent, Cat. No. 1092) was employed.

Isolation of Delstaphigine (1) and Chasmaconitine (4): - Recently,[7-10] we reported separation of the amorphous fraction of mother liquors of *D. staphisagria* into 6 fractions by a gradient pH extraction technique.[22] From the fraction taken at pH 4.5 we reported[8,9] the isolation of the new alkaloids 1-acetyldelphisine and delstaphidine, besides the known alkaloids delphisine and delphinine. The mother liquors after the separation of the above four alkaloids were combined (800 mg). Subsequent purification on an alumina rotor of a Chromatotron, followed by

separation on preparative tlc plates (alumina) afforded delphinine (3; 279 mg, mp 196.5-198.5°C)[23] and chasmaconitine (4, 78 mg, mp 185.5-187.5°C (ether:hexane)); $[\alpha]_D^{22}$ +11.6° (c, 0.39, absolute EtOH); ir (nujol): 3500 cm^{-1} (OH), 1720 cm^{-1} (C = O); EIMS: m/z (%) 613 (M^+, $C_{34}H_{47}NO_9$, 0.3), 584(12), 582 (M^+ -OCH_3, 47), 554(6), 553(2), 522(14), 508(1), 448(2), 105(100), 77(34), 71(13), 58(17), 45(42), 43(59); ^1H nmr ($CDCl_3$): δ 1.08 (3H, t, J = 7 Hz, N-CH_2-CH_3), 1.27 (3H, s, $OCOCH_3$), 3.14, 3.25, 3.27, 3.52 (3H each, s, OCH_3), 4.90 (1H, d, J = 4.6 Hz, C(14)-β-H), multiplets between δ 7.41-8.10 (5H); for ^{13}C nmr data see the Table.

The pH 8 fraction (19.96 g) was chromatographed (vlc) on silica gel. Elution was performed with hexane, hexane: $CHCl_3$, and then $CHCl_3$:CH_3OH mixtures in order of increasing polarity. Fractions eluted with $CHCl_3$:CH_3OH (98:2 and 96:4) were combined (4.37 g) and chromatographed (vlc) on alumina. Elution was performed with hexane, hexane:ether, and then ether:$CHCl_3$ mixtures in order of increasing polarities. Fractions eluted with hexane:ether (35:65) were combined (0.56 g); subsequent purification on an alumina rotor of a Chromatotron (twice) followed by separation on a preparative tlc plate (alumina) afforded delstaphigine (1, 14 mg).

Methylation of Delstaphigine (1) - To 6 mg of 1 in 2 ml of CH_2Cl_2 was added 6 mg of proton sponge [1,8-bis-(dimethylamino)naphthalene] and 8 mg of trimethyloxonium tetrafluoroborate and the mixture was kept at room temperature for one day. Ice water (10 ml) was added and the reaction mixture was rendered alkaline with $NaHCO_3$. The mixture was extracted with 3 x 10 ml of $CHCl_3$. The combined extracts were dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue (4.9 mg) was crystallized from acetone:hexane to give 3 mg of delphinine (3), mp 197.5-199.5°C. The synthetic and natural samples of delphinine (3) were identical by tlc behavior, mp, mixed mp and ir spectra.

Isolation of 14-*O*-Benzoyldelphonine (2) - The amorphous powder from the mother liquors of *D. staphisagria* (8 g) was chromatographed on a silica column (180 g, Kieselgel 60, 70-230 mesh ASTM, Art. #7734). Elution was performed with CH_2Cl_2-EtOH in order of increasing polarity. Fractions eluted with CH_2Cl_2-EtOH (70:30) were combined (868 mg) and chromatographed (vlc) on alumina. The fraction eluted with hexane-EtOH (98:2)(185 mg) was purified on an alumina rotor of a Chromatotron to give 25 mg of 2 (see text for physical properties).

Alkaline Hydrolysis of 14-*O*-Benzoyldelphonine (2) - To 10 mg of 2 in 5 ml of CH_3OH was added 5 ml of 5% methanolic KOH solution. The mixture was kept at room temperature for 18 h. Methanol was distilled and 10 ml of H_2O was added. The solution was extracted with 4 x 15 ml of $CHCl_3$. The chloroform extract was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to give 8 mg of a residue which was identical with delphonine (5) by

^{13}C nmr chemical shifts and assignments for delstaphigine (1), 14-*O*-benzoyldelphonine (2), delphinine (3) and chasmaconitine (4).

Carbon	1	2	3	4
1	84.7	85.4	84.9	84.9
2	26.4	26.2	26.3	26.2
3	35.2	34.8	34.7	34.8
4	39.8 s	39.6 s	39.3 s	39.1 s
5	49.9	48.5	48.8	49.5 [a]
6	82.7	82.5	83.0 [a]	83.0 [b]
7	47.5	52.8	48.2	49.1
8	85.6 s	73.7 s	85.4 s	85.6 s
9	45.0	49.1	45.1	45.1
10	41.2	42.4	41.0	41.0
11	50.3 s	50.5 s	50.2 s	50.2 s
12	35.5	36.4	35.7	35.8
13	74.9 s	76.3 s	74.8 s	74.9 s
14	78.8	80.5	78.9	78.8
15	39.4	41.9	39.3	39.2
16	83.7	83.4	83.7 [a]	83.7 [b]
17	63.3	63.4	63.3	61.9
18	71.2	80.5	80.2	80.4
19	56.5 [a]	56.6 [a]	56.1	53.7
N-CH_3	42.6	42.4	42.3	-
N-CH_2	-	-	-	49.1 [a]
CH_3	-	-	-	13.4
1′	56.3 [a]	56.4 [a]	56.1	56.0
6′	57.6	57.6	57.6	57.7
16′	*58.8*	*58.3*	*58.6*	*58.7*
18′	-	59.3	58.9	59.1
(8)-O-CO	170.0 s	-	169.4 s	169.7 s
CH_3	21.6	-	21.4	21.5
(14)-O-COφ				
CO	166.5 s	167.0 s	166.0	166.3 s
1′	130.3 s	130.2 s	130.4	130.3 s
2′,6′	128.6	128.6	128.4	128.5
3′,5′	*129.8*	*129.8*	*129.6*	*129.7*
4′	133.1	133.1	132.8	133.0

a and b - The assignments may be interchanged in any vertical column.

tlc behavior, ir, proton and ^{13}C nmr spectra.[13,14]

Conversion of Delphinine (3) to 14-*O*-Benzoyldelphonine (2) - About 30 mg of delphinine (3) suspended in 20 ml of distilled water was refluxed under N_2 for 4 h. The solution was extracted with 4 x 20 ml $CHCl_3$. The $CHCl_3$ extract was dried over anhydrous Na_2SO_4 and was distilled under reduced pressure. The residue (27.5 mg) was purified on an alumina rotor of a Chromatotron to give 20 mg of 2. The synthetic and natural samples were identical by tlc behavior, ir, mass, ^1H and ^{13}C nmr spectra.

Alkaline Hydrolysis of Delphinine (3) to Delphonine (5) - To 155 mg of 3 in 10 ml of CH_3OH was added 10 ml of 5% methanolic KOH solution and the mixture was left overnight under N_2 at room temperature. The reaction mixture was worked up by the above procedure to give 119 mg of delphonine (5).

Conversion of Delphonine (5) to 14-*O*-Benzoyldelphonine (2) - One ml of benzoyl chloride and 0.3 ml of pyridine were added to 30 mg of 5 in 5 ml of dry benzene and kept at room temperature for 24 h. Twenty-five ml of iced water was added and the reaction mixture was rendered alkaline with $NaHCO_3$. The mixture was extracted with four 25 ml portions of $CHCl_3$. The combined extracts were dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was purified on an alumina rotor of a Chromatotron to give 18 mg of 2. The synthetic and natural samples were identical by tlc behavior, ir, mass, ^1H and ^{13}C nmr spectra.

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