DELTATSINE, A NEW C_{19}-DITERPENOID ALKALOID FROM *DELPHINIUM TATSIENENSE* FRANCH

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Abstract — The isolation and structure elucidation of a novel alkaloid deltatsine (2A) from the roots of Delphinium tatsienense Franch is described. The structure was established on the basis of spectroscopic data and a chemical correlation with delcosine (11).

From the roots of the Chinese species *Delphinium tatsienense* Franch[1,2] we have isolated a new alkaloid designated as *deltatsine*. The alkaloid, $C_{25}H_{41}NO_7$, is amorphous but homogeneous by tlc. Tne ^{13}C nmr spectrum of deltatsine showed 24 lines for 25 carbon atoms and off-resonance partial decoupling experiments gave the multiplicity of each signal (Table 1, 2A). The signal at 39.9 ppm was attributed to two carbon atoms as it corresponded to about twice the intensity of proton-bearing carbon atoms. Four methoxyls and an ethyl group in the molecule (^1H nmr) indicate that deltatsine belongs to the C_{19}-diterpenoid alkaloids. Almost all the alkaloids of this class bear a hydroxyl or a methoxyl group on C(1), C(8), C(14) and C(16). As no tertiary C-methyl group is observed in the ^1H nmr or ^{13}C nmr spectrum, C(18) bears an oxygen function. This conclusion is also supported by the triplet at 78.6 ppm assigned to the C(18)-methylene carbon which bears a methoxyl group. In the case of a CH_2OH group on C(18), the signal appears about 66.5-68.5 ppm.[3] The remaining three oxygens of the seven present in the molecule could be located on any of the carbons, such as C(3), C(13), C(15) (only in the case of aconitine type), C(7) (only in the case of lycoctonine type) C(9) and/or C(10). Two of the singlets appearing at 37.1 and 48.6 ppm can be assigned to the quaternary carbon atoms C(4) and C(11). The other two singlets at 91.2 ppm and 81.2 ppm due to carbons bearing OH/OMe groups could be located on any of the carbons C(7), C(8), C(9), C(10) or C(13). The C(10)-position can be excluded, as C(11) appears at 48.6 ppm and not around 55-56 ppm as would be expected.[4] In the case of aconitine-type C_{19}-diterpenoid alkaloids bearing a hydroxyl group at C(13), the methylene triplet at C(12) is observed in the region 33.5-38.0 ppm.[5,6] As there are three methylene triplets in the upfield region at 27.2, 28.5 and 29.3 ppm, an OH group at C(13) can be ruled out. All the compounds having an OH group at C(9) are known to exhibit a singlet about 77.5-78.5 ppm.[7] As no singlet appears in this region, an oxygen function at C(9) can be excluded. Deltatsine should therefore bear an oxygen function at C(7) and C(8) and these carbon resonances are observed at 91.2 and 81.2 ppm, respectively. Deltatsine belongs to

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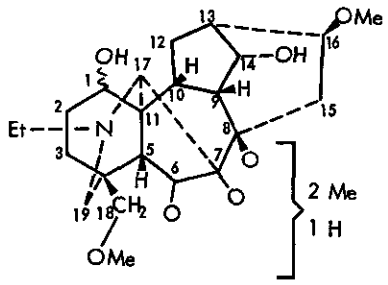
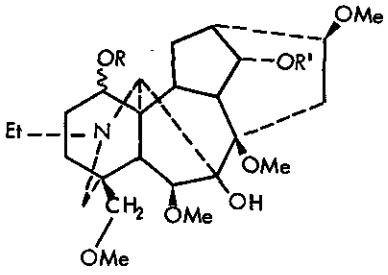
the lycoctonine type and hydroxyl functions at C(3) and C(15) can therefore be ruled out. On the basis of these data, deltatsine appears to bear oxygen functions at C(1), C(6), C(7), C(8), C(14), C(16) and C(18). A methoxyl group at C(1) is unlikely because of the methylene triplets at 27.2 and 29.3 ppm assigned to C(2) and C(3), respectively. All the C_{19}-diterpenoid alkaloids bear a methoxyl group at C(16) and this carbon appears around 79.5-84 ppm. The downfield doublet at 82.4 ppm should be assigned to C(16).



1



2: R = R' = H

4: R = H; R' = Me

6: R = Ac; R' = Me

8: R = R' = Me

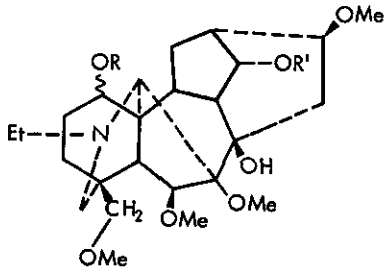
The chemical shift of C(14) bearing a methoxyl group appears about 83.5-85.0 ppm. Since there is no signal in this region, C(14) must be substituted by a hydroxyl group and the methine doublet at 74.7 ppm is ascribed to this carbon atom. This evidence leads to the partial structure **1** for deltatsine. The remaining two methoxyl groups can be located at 6,7-, 6,8-, or 7,8- positions. A methoxyl rather than a hydroxyl group should be placed at C(6), because the downfield signal at 90.6 can only be assigned to this carbon bearing a methoxyl group. These data lead to two alternative structures **2** and **3** for the alkaloid.

When methylated with methyl iodide and sodium hydride deltatsine afforded the monomethyl and dimethyl ether as amorphous compounds. Acetylation of the monomethyl ether gave a monoacetate which showed a one-proton apparent triplet at δ 4.75 (J=8 Hz). The large coupling constant is consistent with the C(1)-proton which makes a dihedral angle of ~ 180° (chair form) with one of the C(2)-methylene protons. On the basis of the alternative structures **2** or **3** for deltatsine, the monomethyl ether can be formulated as **4** or **5** and its acetate as **6** or **7**. In the ^{13}C nmr spectrum of the monomethyl ether, the disappearance of the signal at 74.7 ppm attributed to C(14), and the appearance of a new signal at 84.1 ppm support this conclusion.

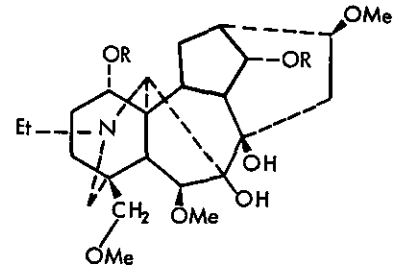
Deltatsine dimethyl ether shows six methoxyl groups (^1H NMR) and its ^{13}C NMR spectrum shows 26 lines for 27 carbon atoms of the molecule. These data are consistent with either structure **8** or **9** for the dimethyl ether. Compound **9** is an amorphous product occurring naturally in *Delphinium dictyocarpum*[8] and has also been prepared by methylation of lycoctonine.[9] The ^{13}C NMR spectral values reported for **9** *do not agree* with those observed for deltatsine dimethyl ether. Delcosine-7-methyl ether (**3**), mp 206-208°C, was prepared by acetylation of 14-acetyldelcosine[10] to 1,14-diacetyldelcosine (**10**), followed by methylation and hydrolysis. As

the TLC and the ^{13}C spectra of **3** *are not identical* with those of deltatsine, the latter must therefore have structure **2**.

With a view to correlate deltatsine with a known compound, it was heated with 3M sulfuric acid to afford a crystalline compound, mp 203-204°C. This was shown to be identical in all respects with delcosine **11**, establishing structure **2A** for deltatsine. This reaction which probably proceeds by the formation of a tertiary carbonium ion, is useful for dealkylation at the C(8)-position.[11] The hydroxyl group at C(1) of deltatsine must be α-oriented as shown in **2A** since the structure of delcosine has been confirmed by an X-ray crystallographic investigation[12] and also by its conversion to delsoline[13] which has been correlated with lycoctonine.[14] It follows that compounds **4**, **6** and **8** must also have a C(1)-oxygen function in the α-configuration.

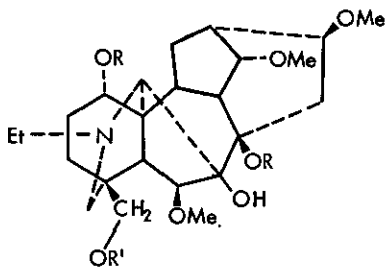


3: R = R' = H
5: R = H; R' = Me
7: R = Ac; R' = Me
9: R = R' = Me

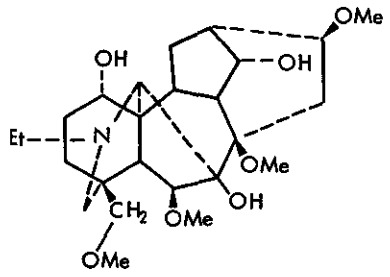


10: R = Ac
11: R = H

A correlation of the ^{13}C nmr spectrum of deltatsine (**2A**) and its methyl ethers (**4**) and (**8**) with delsoline (**12**),[15] delcosine (**11**)[16], delcosine-7-methyl ether (**3**), 7,18-di-*O*-methyllycoctonine (**9**)[9], the aminoalcohol (**13**) obtained by hydrolysis of septentrionine[13] and 1,14-diacetyl delcosine (**10**) is shown in Table 1.



12: R = H; R' = Me
13: R = Me; R' = H



2A

Table 1. Carbon-13 Chemical Shifts and Assignments for Delsoline (**12**), Delcosine (**11**), Delcosine-7-methyl ether (**3**), Deltatsine (**2A**), Deltatsine monomethyl ether (**4**), Deltatsine dimethyl ether (**8**), 7-18-Di-*O*-methyllycoctonine (**9**), Aminoalcohol of septentrionine (**13**), 1,14-Diacetyldelcosine (**10**).

Carbon	12	11	3	2A	4	8	9	13	10
1	72.6	72.7	72.8	72.3 d	72.3	82.9	82.9	83.2	77.3
2	27.2	27.5	27.4	27.2 t	27.0	25.6	30.0	25.5	27.4
3	29.3	29.4	29.5	29.3 t	29.7	31.7	32.3	31.2	32.3
4	37.4	37.6	37.7	37.1 s	37.2 s	38.1 s	38.0	38.7	38.1
5	43.9	44.0	43.6	39.9 d	39.0	40.6	50.4	40.8	43.5
6	90.4	90.1	88.0	90.6 d	91.2	91.4	85.5	91.4	90.1
7	87.8	87.9	90.7	91.2 s	91.2 s	89.7 s	92.8	89.7	88.4
8	78.5	78.1	84.8	81.2 s	82.1 s	80.6 s	80.0	80.6	77.3
9	44.9	45.3	44.2	48.9 d	49.1	52.0	44.5	52.1	50.6
10	43.3*	45.3*	45.4	45.2 d	44.9	46.7	45.6	46.7*	44.9
11	49.3	48.9	49.6	48.6 s	49.1 s	47.4 s	49.0	47.2	49.6
12	30.5	29.4	30.7	28.5 t	29.4	27.9	26.2	27.9	28.4
13	37.7*	39.4*	38.0	39.9 d	36.7	38.0	38.9	37.9*	37.6
14	84.5	75.8	77.7	74.7 d	84.1	83.5	84.5	83.6	75.4
15	33.5	34.5	33.7	30.9 d	30.2	28.1	33.5	27.9	33.7
16	82.9	82.0	83.1	82.4 d	83.2	83.2	84.2	82.9	82.8
17	66.0	66.3	66.0	66.5 d	66.1	66.7	66.4	66.9	63.8
18	77.3	77.4	78.6	78.6 t	78.8	79.4	78.1	68.8	77.7
19	57.2	57.1	57.6	57.3 t	57.6	54.3	52.4	53.5	53.2
N-CH_2	50.3	50.4	50.3	50.3 t	50.4	51.9	51.4	51.9	47.8
CH_3	13.5	13.7	13.5	13.7 q	13.7	15.0	14.0	14.5	14.0
1'						55.5	55.8	55.7	
6'	57.2	57.4	57.4	59.2 q	59.5	59.5	56.3	60.6	57.4
7'			57.4				55.5		
8'				51.3 q	50.8	53.5		53.5	
14'	57.9				57.6	57.6	57.7	57.7	
16'	56.3	56.4	56.3	56.3 q	56.4	56.3	56.0	56.6	56.2
C18'	59.1	59.1	59.3	59.3 q	59.5	59.5	59.0		59.0
C=O(1')									170.2
CH_3									21.9
C=O(14')									171.6
CH_3									21.4

*The published assignments for C(10) and C(13) have been reversed.

EXPERIMENTAL

IR spectra were taken on a Perkin-Elmer Model 1430 spectrophotometer. ^1H nmr spectra were taken on Perkin-Elmer EM-390, 90 MHz and Brucker WM-300 MHz (Aspect 2000 data system) spectrometers; ^{13}C nmr spectra on JEOL FT model FX-60 and FX-90 Q spectrometers in $CDCl_3$ solution with TMS as an internal reference. Mass spectra were determined on Finnegan Quadrupole 4023 and Hitachi RMU-7L (SS 200 data system) instruments. Melting points are corrected.

Isolation of deltatsine (2A). The crude alkaloid fraction E_1 (15 g) isolated from the roots of *D. tatsienense* (10.9 kg)[1] at pH 8 was chromatographed on alumina (Act. III; 700 g) and eluted with toluene containing increasing amounts of methanol (0.4-2.5%). Fractions (500 ml each) were collected and the separation monitored by t.l.c. Fractions 32-36 consisted of pure deltatsine (1.03 g) obtained as an amorphous powder, $[\alpha]_D^{20}$ +28.6° (c, 2.4 EtOH); Found: C, 62.5; H, 8.7; $C_{25}H_{41}NO_7.H_2O$ requires: C, 61.9; H, 8.9%. MS, m/z (M^+, 467, 5%), 452(15), 436(30), 420(30), 404(8), 390(10), 376(15), 178 (10), 114(30), 91(25), 71(50), 58(100); HRMS, 467.2948; calc. for $C_{25}H_{41}NO_7$, 467.2883. ^1H nmr spectrum (300 MHz): δ 1.08 (3H, t, J=7.3 Hz, N-CH_2-CH_3), 2.64 (2H, AB type, J=8.9 Hz, C(19)-H), 3.14, 3.30 (2H, AB type J=8.9 Hz, C(18)-H), 3.36, 3.38, 3.39, 3.46 (each 3H, s, OCH_3), 3.60 (1H, brs, C(1)-β-H), 3.80 (1H, s, C(6)-α-H), 4.00 (1H, t, J=4.5 Hz, C(14)-β-H). See Table 1 for ^{13}C nmr spectrum.

Methylation of deltatsine (2A) to the methyl ethers (4) and (8). Deltatsine (150 mg; 0.3 m mole), methyl iodide (140 mg; 10 m mole), sodium hydride (0.36 g, 5 m mole) and dioxane (5 ml) were sealed in a glass tube and heated in an oil bath at 115-120°C for 70 h. The residue (190 mg) obtained by evaporation of dioxane was chromatographed on alumina (14 g, Act. III) and eluted with hexane containing increasing amounts of acetone to give the dimethyl ether **8** (47 mg; t.l.c. Al_2O_3, hexane:acetone 9:1, Rf 0.62) and the monomethyl ether **4** (62 mg; Rf 0.54). The monomethylether **4** showed in the ^1H nmr (C_5D_5N): δ 1.02 (3H, t, J=7.5 Hz, N-CH_2-CH_3), 3.33, 3.41, 3.41, 3.46, 3.51 (each 3H, s, OCH_3). For ^{13}C nmr spectrum see Table 1.

To a solution of **4**, (35 mg) in CH_2Cl_2 (5 ml), acetic anhydride (0.1 ml) and pyridine (0.1 ml) were added and kept at r.t. for 16 h. Usual work up gave the amorphous acetate **6**; ^1H nmr: δ1.06 (3H, t, J=7 Hz, N-CH_2-CH_3), 2.03 (3H, s, OAc), 3.40 (9H, s, 3 x OCH_3), 3.50 (6H, s, 2 x OCH_3), 4.70 (1H, t, J=8 Hz, C(1)-H). The dimethyl ether (8) showed in the ^1H nmr: δ 1.05 (3H, t, J=7.5 Hz, N-CH_2-CH_3), 3.26, 3.38 (each 3H, s, OCH_3), 3.39, 3.53 (each 6H, s, 2 x OCH_3). For ^{13}C nmr spectrum see Table 1.

1,14-Diacetyldelcosine (10). To a solution of 14-acetyl delcosine[10] (102 mg) in pyridine (1.4 ml), acetic anhydride (1.4 ml) was added and the mixture was allowed to stand at r.t. for 8 h. The usual workup afforded a residue (105 mg) that was chromatographed over alumina and crystallized from ether-hexane to afford **10** (86 mg), mp 115-116°C. ^1H nmr: δ 1.02 (3H, t, N-CH_2-CH_3), 2.00 (6H, s, 2 x OAc), 3.22, 3.25, 3.38 (each 3H, s, OCH_3), 3.85 (1H, s, C(6)-α-H), 4.63 (2H, t, C(1)-H; C(14)-H).

Delcosine-7-methyl ether (3). To a suspension of potassium hydride (162 mg) in dimethylsulfoxide (162 mg) taken in a three-neck flask kept under argon, was added a solution of 1,14-diacetyldelcosine (200 mg) in dimethylsulfoxide (1.0 ml). The reaction mixture was stirred at r.t. for 15

min, methyl iodide (0.65 ml) was added at 0-5°C and the mixture was stirred at r.t. for 2 h. The usual workup afforded (3) which was recrystallized from benzene, mp 206-208°C. T.l.c. (Al_2O_3; toluene: 20% acetone). Rf 0.10; (Cf. **2A**, Rf 0.14); M^+ m/z 467. ^1H nmr: δ1.04 (3H, t, N-CH_2-CH_3), 3.28, 3.30, 3.32, 3.37 (each 3H, s, OCH_3). See Table 1 for the ^{13}C nmr spectrum.

Preparation of delcosine (11) from deltatsine (2). A solution of deltatsine (45 mg) in 3M aq sulfuric acid (4 ml) was heated on a steam bath for 5 h. The reaction mixture was basified with 2N NaOH, extracted with $CHCl_3$ (40 ml x 4), dried over anhyd. Na_2SO_4 and the solvent evaporated to give a residue (45 mg). The latter was chromatographed by PTLC(0.5 mm Al_2O_3; $CHCl_3$ + 1.5% MeOH) and the band corresponding to delcosine was collected (13 mg). This was then passed through a column of Al_2O_3 (2 g) and eluted with hexane, hexane:CH_2Cl_2 (8:2), CH_2Cl_2 and $CHCl_3$. The chloroform fraction on evaporation afforded a crystalline product (9 mg), mp 203-204°C, identical in its t.l.c. behavior, mixture mp and ir spectra with those of an authentic sample of delcosine (**11**).

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