TWO NEW DITERPENOID ALKALOIDS FROM DELPHINIUM PACIFIC GIANT AND REVISED ^{13}C-NMR ASSIGNMENT OF DELPHELINE

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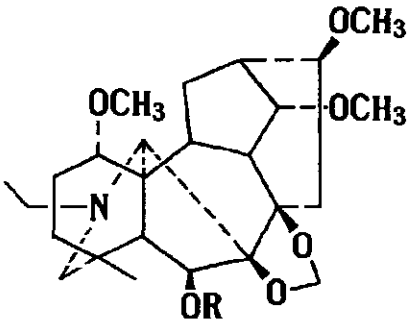
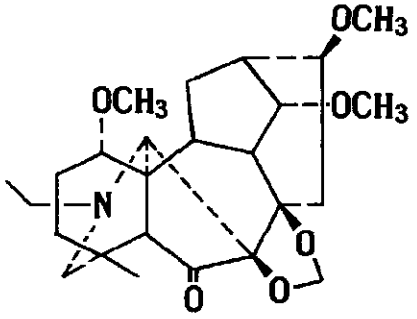
Abstract -- Two new C_{19}-diterpenoid alkaloids, paciline (1) and pacinine (2), and a known alkaloid, delpheline (3) were isolated from Delphinium pacific giant. Structures of those alkaloids were determined on the basis of their spectral data and chemical correlation with delpheline (3). Paciline (1) was afforded by methylation of delpheline (3) and pacinine (2) was afforded by oxidation of delpheline (3). ^{13}C-Chemical shifts assigned to C-1, C-9, C-10, C-14, and C-16 of delpheline (3) were revised on the basis of 2D nmr measurement.

Many C_{19}-diterpenoid alkaloids have been isolated from plants of Aconitum and Delphinium genera. We started on an investigation of the alkaloidal components of Delphinium species using the seeds of Delphinium pacific giant Mix. which were available for garden flower in Japan.[1]

We wish to report in this paper, the isolation of two new alkloids designated as paciline (1) and pacinine (2), together with a known alkaloid, delpheline (3)[2], from the seeds of Delphinium pacific giant Mix. Alkaloid 3 was determined by comparison of spectral data with those in the literature.[2]

Alkaloid 1, amorphous, $[\alpha]_D$ -7.2°, showed the following properties. The molecular formula, $C_{26}H_{41}NO_6$, was derived from hrms spectrum (M^+ 463.2943, calcd 463.2933). The ^{1}H-nmr spectrum of 1 revealed the presence of an angular methyl group at δ 0.92 (s), a methyl of an N-ethyl group at δ 1.04 (t, J=7.2 Hz), four methoxyl groups at 3.25, 3.34, 3.35, and 3.43 (each s), two carbinyl methines at δ 3.59 (s, C_6-αH) and 3.63 (t, J=5.0 Hz, C_{14}-βH), and a methylenedioxy group at δ





1 : R = CH_3
3 : R = H
4 : R = $COCH_3$

2

Table I. ^{13}C-Chemical Shifts and Assignments for Paciline (1), Pacinine (2), Delpheline (3)[2], and 6-Acetyldelpheline (4).[3]

Carbon	1	2	3	4	Carbon	1	2	3	4
1	81.9	82.3	82.9	82.0	15	34.9	32.8	33.5	33.8
2	27.0	26.7	26.9	26.9	16	81.6	81.3	81.9	81.6
3	37.0	37.6	36.9	36.7	17	64.1	62.2	63.7	64.2
4	33.5	34.8	33.9	33.8	18	26.0	24.4	25.3	25.4
5	57.1	60.6	55.5	55.9	19	56.9	56.8	57.3	56.7
6	89.8	215.9	79.3	78.4	N-CH_2	50.1	49.8	50.2	50.3
7	92.2	89.9	92.8	91.8	CH_3	14.0	13.5	13.9	13.9
8	83.4	82.4	84.6	83.3	1'	55.2	55.6	56.2	55.3
9	40.0	41.6	40.3	39.8	6'	58.9	--	--	--
10	48.4	47.4	47.9	48.2	14'	57.7	57.7	57.8	57.6
11	50.5	46.1	50.4	50.2	16'	56.1	56.2	56.8	56.1
12	27.9	27.4	28.1	27.9	C=O	--	--	--	170.0
13	38.6	38.1	37.9	38.6	CH_3	--	--	--	21.7
14	83.4	81.9	83.1	83.3	OCH_2O	93.4	94.9	92.9	93.4

a; Previous assignments[2,3] of C-1, C-9, C-10, C-14, and C-16 in 3 and 4 were revised according to the explanation described in text.

5.07 (2H, s). These spectra suggested that the compound 1 was a C_{19}-diterpenoid derivative. The ^{1}H-nmr spectrum of 1 was very similar to that of delpheline (3), with exception for the presence of one more methoxyl group. Paciline (1) showed four methoxyl signals at δ 3.25, 3.34, 3.35, and 3.43 (each 3H), whereas delpheline (3) showed three signals at δ 3.26, 3.35, and 3.43 (each 3H). The molecular ion (m/z 463) of paciline (1) was also observed more 14 mass units than that of delpheline (3). Comparison of the chemical shifts of the methoxyl groups in both ^{13}C-nmr spectra of paciline (1) and delpheline (3) suggested that the three signals at 55.2, 56.1, and 57.7 ppm in paciline (1) could be assigned to C_1-OCH_3, C_{16}-OCH_3, and C_{14}-OCH_3, respectively. The fourth signal at 58.9 ppm was assigned to C_6-OCH_3 since the C-6 resonance was shifted downfield (+10.5 ppm). The structure of paciline (1) was deduced to be 6-methyldelpheline and was confirmed by methylation of delpheline (3) with methyl iodide and sodium hydride in dimethyl sulfoxide. The methylation product was identical to paciline (1) in terms of the ir and nmr spectra, and tlc behaviors.

Alkaloid 2 named pacinine, mp 133-135.5°C, $[\alpha]_D$ -58°, showed the following properties. The molecular formula $C_{25}H_{37}NO_6$ was derived from hrms spectral data (M^+ 447.2607, calcd 447.2620). The ir spectrum showed carbonyl absorption (1740 cm^{-1}). The ^{1}H-nmr spectrum showed the following resonances; δ 0.92 (3H, s, 18-CH_3), 1.06 (3H, t, J=7.3 Hz, N-CH_2CH_3), 3.31, 3.35, and 3.39 (each 3H, s, OCH_3), 3.64 (1H, t, J=5.0 Hz, C_{14}- H), 5.08 and 5.52 (each 1H, s, -OCH_2O-). These spectra suggested that pacinine (2) was a new C_{19}-diterpenoid alkaloid. The ^{1}H-nmr spectrum of 2 was similar to that of delpheline (3), except for the absence of C-6 methine. The ir absorption and ^{13}C-nmr signal at 215.9 ppm (s, C-6) suggested the presence of a ketone moiety. The observed molecular ion (m/z 447) of pacinine (2) was 2 mass units less than that of delpheline (3). The structure of pacinine (2) was assigned to be 6-dehydrodelpheline, and was confirmed by oxidation of delpheline (3) with silver oxide.[4] The oxidation product was identical in all respects with a natural product (2).

During our structure investigation on C_{19}-diterpenoid alkaloids containing methylenedioxy group at C-7 and C-8, the previous assignment of the chemical shifts for C-9 and C-10 in delpheline (3) was recognized to be revised. ^{13}C-Chemical shift assignments for delpheline (3) were reported by S. W. Pelletier, et al.[2] and the assignment has been cited in many report on the alkaloids. We have found that the chemical shifts assigned to C-9 and C-10 of delpheline (3) were

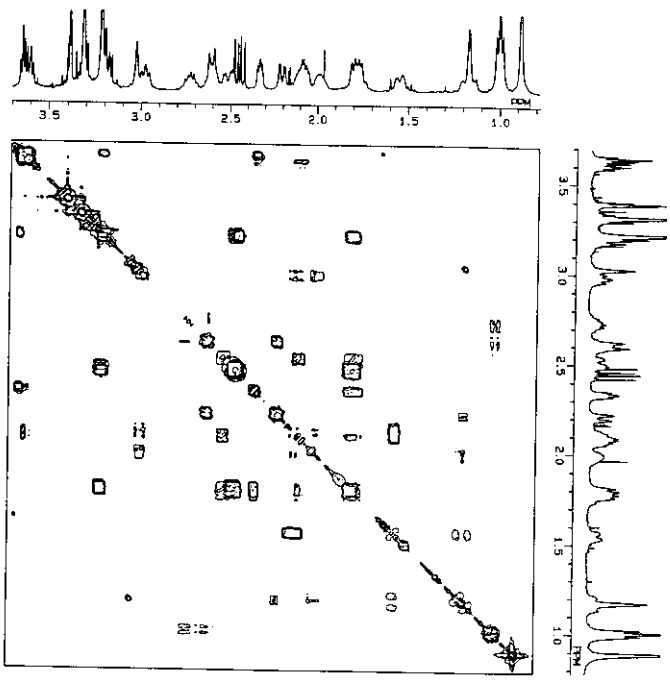


Fig. 1. The HOMO-COSY Spectrum of Delpheline (3).

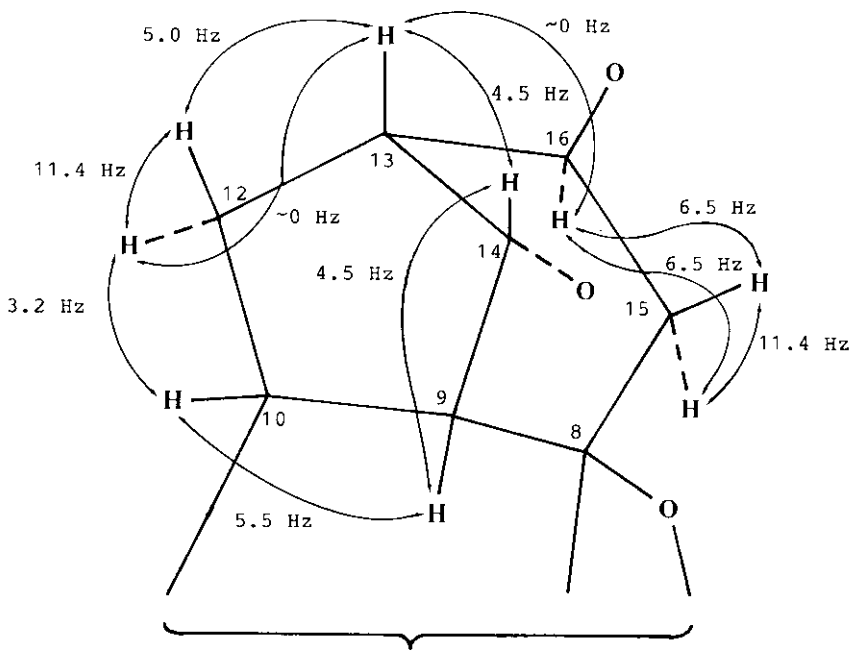


Fig. 2. Coupling Interactions of the C and D Rings in Delpheline (3).

Table II. ^{1}H-Nmr Chemical Shifts of Delpheline (3).

Proton	δ (ppm)		J (Hz)	Proton	δ (ppm)		J (Hz)
1	2.98	dd	6.1, 8.5	16α	3.20	t	6.5
2α	2.10	m		17	3.03	s	
2β	1.99	m		18	0.89	s	
3α	1.19	m		19	2.21	d	11.5
3β	1.55	dd	2.8, 9.7		2.61	d	11.5
5	1.17	s		N-C$\underline{H}_2$	2.61	dq	6.0, 10.1
6α	4.16	s			2.72	dq	6.0, 10.1
9	3.61	dd	4.5, 5.5	C$\underline{H}_3$	1.01	t	7.2
10	2.08	m		1'-OC$\underline{H}_3$	3.22	s	
12α	2.52	dd	3.2, 11.4	14'-OC$\underline{H}_3$	3.39	s	
12β	1.79	m		16'-OC$\underline{H}_3$	3.31	s	
13	2.34	dd	4.5, 5.0	OC$\underline{H}_2$O	5.01	s	
14β	3.65	t	4.5		5.09	s	
15α	1.79	dd	6.5, 11.4				
15β	2.45	dd	6.5, 11.4				

much different (ca. 8 ppm) from the previous assignment on the basis of 2D nmr measurement. The HOMO-COSY spectrum of 3 was shown in Fig. 1.
The characteristic signal of C_{14}-H which was readily recognized at δ 3.65 as triplet ($\underline{J}$=4.5 Hz) was coupled with three protons at δ 3.61 (dd, $\underline{J}$=4.5, 5.5 Hz), 3.20 (t, $\underline{J}$=6.5 Hz) and 2.34 (dd, $\underline{J}$=4.5, 5.0 Hz). The signal at δ 3.20 assignable to C_{16}-H seemed to be coupled to the C_{14}-H with W-type long-range coupling (below 1 Hz). The remaining two protons at δ 3.61 and 2.34 were assigned as C_9-H and C_{13}-H, respectively, from the correlation as shown in Fig. 2. Since both diheadral angles for C_{13}-H-C_{12}-αH and C_{13}-H-C_{16}-H were approximately 90°, their protons were not coupled with each other. The coupling constant between C_{10}-H and C_{12}-βH was not determined precisely because of overlapping signals of C_2-αH and C_{15}-αH, respectively. Chemical shifts of all protons in 3 were determined on the basis of the HOMO-COSY spectral data and measurements of coupling constant, and the assignments were listed in Table II. The chemical shifts of all carbons

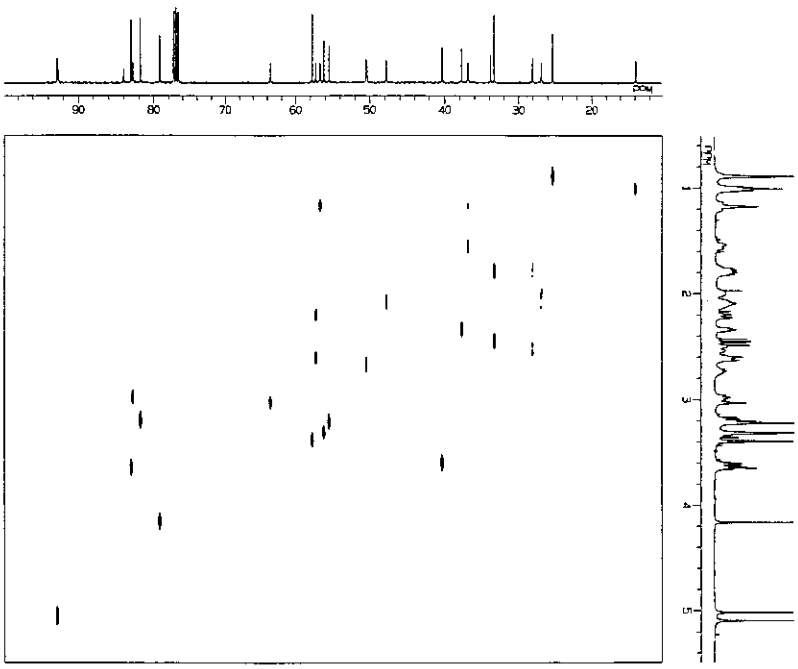


Fig. 3. The hetero-COSY Spectrum of Delpheline (3).

were determined by C-H correlation in the hetero-COSY spectrum (Fig. 3), and the revised assignment was listed in the Table I. On the basis of the assignment, the chemical shifts of C-9, 40.3 ppm, and C-10, 47.9 ppm, should be reversed in the previous assignment. Furthermore, three carbinyl methines of C-1, C-14, and C-16, should be also revised. These revised assignments led us to reinvestigate on other methylenedioxy-contaning C_{19}-diterpenoid alkaloids and the review will be reported in the future.

EXPERIMENTAL

All melting points are uncorrected. Optical rotations were measured with a JASCO DIP-4 polarimeter. Ir spectra in KBr disks were taken with a JASCO IRA-2 spectrophotometer. Nmr spectra were measured in $CDCl_3$ solution with a JOEL FX-100 and GX-270 spectrometers using TMS as an internal standard. ^{1}H and ^{13}C-nmr, HOMO-COSY, and hetero-COSY spectra of delpheline (3) were measured with a JEOL GX-400 spectrometer. Ms and hrms were measured with a Shimadzu LKB-9000B, JOEL JMS-

D300, and JMS-DX303 mass spectrometers.

Isolation procedure---The seeds (4 kg) of Delphinium pacific giant Mix. were ground. They were defatted with petroleum ether (36 l) and then extracted with ethanol (40 l) at room temperature and the solution was evaporated in vacuo to give the extract, which was partitioned between 1% HCl (200 ml) and petroleum ether (200 ml x 2). The aqueous layer was made alkaline (pH 8.5) with 28% ammonia and extracted with $CHCl_3$ (250 ml x 3) followed by evaporation of the $CHCl_3$ extract to give the crude alkaloid-A (42 g). The remaining aqueous water layer was made alkaline (pH 12) with 20% NaOH and extracted with $CHCl_3$ (250 ml x 3) to give the crude alkaloid-B (5 g). The first petroleum ether layer was evaporated to give a residue, which was partitioned again between petroleum ether (300 ml) and 1% HCl (500 ml x 2). The aqueous layer was extracted by gradient pH separation with $CHCl_3$ to give the crude alkaloid-C (pH 8.5, 564.5 g) and D (pH 12, 111.7 g). The crude alkaloid-A (42 g) was chromatographed on silica gel with a mixture of hexane and ether saturated with 28% ammonia. The content of hexane was decreased gradually. Purification by repeated column chromatography gave two new alkaloids, paciline (1, 14 mg) and pacinine (2, 15 mg), and a known alkaloid, delpheline (3, 331 mg).

Paciline (1) -- Amorphous. $[\alpha]_D$ = -7.2° (c=1.0, $CHCl_3$). Hrms (m/z): Calcd for $C_{26}H_{41}NO_6$ 463.2933, Found 463.2943. Ir (ν, cm^{-1}): 2850, 1070. Ms (m/z): 463 (M^+), 448 (M^+-15), 432 (M^+-OCH_3, base peak). ^{1}H-Nmr (δ): 0.92 (3H, s), 1.04 (3H, t, J=7.2 Hz), 3.25 (3H, s), 3.34 (3H, s), 3.35 (3H, s), 3.43 (3H, s), 3.59 (1H, s), 3.63 (1H, t, J=5.0 Hz), 5.07 (2H, s). ^{13}C-Nmr spectrum was shown in Table I.

Methylation of delpheline (3) -- A mixture of delpheline (3, 50 mg), NaH (100 mg), CH_3I (1 ml), and DMSO (2 ml) was stirred for 2 h at room temperature. The reaction mixture was diluted with water, and made alkaline with 28% ammonia, and extracted with $CHCl_3$ (40 ml x 5). The organic layer was worked up in usual manner to afford a residue, which was purified by preparative-tlc (solvent: ether saturated with 28% ammonia) to give paciline (1, 28.4 mg) in 55% yield. The ir and nmr spectra, tlc behavior of 1 were identical with those of the natural compound.

Pacinine (2) -- mp 133-135.5°C, $[\alpha]_D$ = -58° (c=1.0, MeOH). Hrms (m/z): Calcd for $C_{25}H_{37}NO_6$ 447.2620, Found 447.2607. Ir (ν, cm^{-1}): 1740, 1075. Ms (m/z): 447 (M^+), 416 (M^+-OCH_3, base peak). ^{1}H-Nmr (δ): 0.92 (3H, s), 1.06 (3H, t, J=7.3 Hz), 3.31 (3H, s), 3.35 (3H, s), 3.39 (3H, s), 3.64 (1H, t, J=5.0 Hz), 5.08 (1H, s), 5.52 (1H, s). ^{13}C-Nmr spectrum was shown in Table I.

Oxidation of delpheline (3) -- A mixture of delpheline (3, 41.9 mg), silver oxide (215 mg), ethanol (2 ml), and water (0.5 ml) was refluxed for 7 h. The reaction mixture was filtrated and evaporated to afford a residue, which was purified by column chromatography on silica gel (solvent: $CHCl_3$ saturated with 28% ammonia) to give pacinine (2, 39.8 mg) in 95% yield. Melting point and the ir and nmr spectra, and tlc behavior of 2 were identical with those of the natural compound.

Delpheline (3) -- mp 221.5-224°C. Hrms (m/z): Calcd for $C_{25}H_{39}NO_6$ 449.2777, Found 449.2764. Ir (ν, cm^{-1}): 3500, 1075. Ms (m/z): 449 (M^+), 418 (M^+-OCH_3, base peak). ^{1}H- and ^{13}C-nmr spectra were shown in Table II and I, respectively.

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