Diterpenoid Alkaloids from *Consolida oliveriana*

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From the aerial parts of *Consolida oliveriana* (DC) Schröd. a new norditerpenoid alkaloid consolidine (**2**) has been isolated, in addition to the known alkaloids pubescenine (**1**), gigactonine, and delsoline and the diterpenoid alkaloid ajaconine (**4**). The structure of alkaloid **2** was established on the basis of its physical and spectroscopic data including detailed NMR studies. A detailed NMR study on ajaconine (**4**) resulted in the revision of 11 ^{13}C chemical shift assignments.

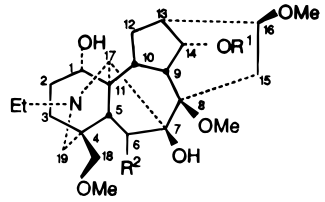
Turkish *Delphinium* and *Consolida* (Ranunculaceae) species are used externally in the treatment of rheumatic pain and sciatica and also against body lice.[1]

Phytochemical investigations of the constituents of *Consolida oliveriana* (DC) Schröd., a plant indigenous to Southeastern Turkey, have not been reported. In continuation of our studies on the alkaloidal constituents of genus *Delphinium* and *Consolida* (Ranunculaceae) native to Turkey,[2−7] we report in this paper the isolation and structure determination of the diterpenoid alkaloids of *C. oliveriana*.

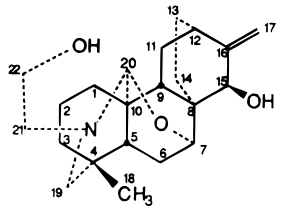
The aerial parts of *C. oliveriana* (DC) Schröd. were extracted at room temperature with 95% EtOH and the alkaloids extracted with CH_2Cl_2 at pH 10 and pH 14 to give 2.17 g of a crude alkaloidal fraction. Column chromatography of the crude alkaloidal mixture gave three major fractions A−C (see the Experimental Section). Fraction A gave two norditerpenoid alkaloids that were identified as the known compound pubescenine (**1**) and a new alkaloid consolidine (**2**). Fraction B afforded gigactonine and delsoline, and fraction C gave ajaconine (**4**).

The isolation of pubescenine from the aerial parts of *Consolida pubescens* D. C. Soo and its structure determination as **1** was first reported in 1988.[8] Pubescenine is an unusual lycoctonine-type alkaloid, in that the C-6 hydroxyl is α, unlike all others that have a C-6 β-OH group. The structure of pubescenine was determined by an X-ray analysis and ^1H and ^{13}C NMR spectroscopic evidence.[8] The C-6 epimer, 6-*epi*-pubescenine (**3**), has been isolated from *D. nuttalianum*.[9]

Consolidine (**2**), an amorphous compound, was homogeneous on TLC. The structure **2** for consolidine (14-deacetylpubescenine 14-methyl ether) was derived on the basis of its detailed spectroscopic data. Its molecular formula $C_{25}H_{41}NO_7$ was derived on the basis of its FABHRMS and the carbon-13 NMR spectra.



1 Pubescenine R^1 = Ac, R^2 = ----- OH
2 Consolidine R^1 = Me, R^2 = -----OH
3 6-*epi*-Pubescenine R^1 = Ac, R^2 = ▬OH



4 Ajaconine

The ^1H NMR spectrum showed the presence of an *N*-Et and four methoxyl groups. The ^{13}C NMR spectrum of consolidine shows 23 lines for 25 carbons of the molecule. The signal at δ 38.1 is very strong and indicates two carbons, one for a quaternary and the other overlapping signal as a methine carbon. The resonance at δ 80.5 represents a $-CH_2$ and a quaternary carbon. The quaternary carbons in the ^{13}C NMR spectrum were determined by the QUATD program.

The DEPT spectra indicated the presence of four quaternary carbons at δ 85.0, 80.5, 47.3, and 38.1, nine methines at δ 83.8, 83.2, 72.2, 70.4, 63.2, 46.2, 44.3, 43.5, and 38.1, seven methylenes at δ 80.5, 56.4, 50.5, 30.0, 29.4, 29.2, and 27.7, and five methyl carbons at δ 59.1, 57.3, 56.5, 52.5, and 13.6. The HETCOR spectrum showed 38 proton correlations with the corresponding

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Table 1. ^{1}H NMR (300 MHz) and HETCOR Data of Consolidine (**2**)[a]

position no.	^{1}H (δ)	mult	shows correlatn with ^{13}C (δ)
1	3.58	(1H, br s)	72.2 (d)
2	1.48	(2H, m)	29.2 (t)
3	1.56(β), 1.70(α)	(2H, m)	29.4 (t)
5	2.05	(1H, m)	46.2 (d)
6	4.43	(1H, br s)	70.4 (d)
9	2.15	(1H, d, J = 6.5 Hz)	44.3 (d)
10	1.78	(1H, m)	43.5 (d)
12	1.75(β), 1.98(α)	(2H, m)	30.0 (t)
13	2.38	(1H, m)	38.1 (d)
14	3.46	(1H, t, J = 4.2 Hz)	83.8 (d)
15	2.08(β), 2.50(α)	(2H, m)	27.7 (t)
16	3.35	(1H, m)	83.2 (d)
17	2.69	(1H, s)	63.2 (d)
18	3.38(b), 3.61(a)	(each 1H, AB, J = 7.5 Hz)	80.5 (t)
19	2.36(β), 2.88(α)	(each 1H, AB, J = 11 Hz)	56.4 (t)
8-OMe	3.38	(3H, s)	52.5 (q)
14-OMe	3.20	(3H, s)	57.3 (q)
16-OMe	3.35	(3H, s)	56.5 (q)
18-OMe	3.31	(3H, s)	59.1 (q)
NCH_2	2.83(α), 2.92(β)	(each 1H, m)	50.5 (t)
CH_3	1.06	(3H, t)	13.6 (q)

[a] ^{13}C NMR chemical shifts (δ) for the quaternary carbons in **2** are C-4 (38.1), C-7 (85.0), C-8 (80.5), and C-11 (47.3).

Table 2. ^{1}H−^{1}H COSY (300 MHz) and Selective INEPT Data of Consolidine (**2**)

proton	shows correlatn with	enhanced ^{13}C signal on irradn of ^{1}H assigned to
H-1	H-2α	
H-2α	H-1, H-3β	
H-3α	H-3β	
H-3β	H-3α	
H-5β	H-6β	C-7, C-11, C-18
H-6β	H-5β	C-4, C-8
H-9	H-10, H-14	
H-10	H-9, H-12α	
H-12α	H-10, H-12β, H-13	
H-12β	H-12α	
H-13	H-12α, H-14	
H-14	H-9, H-13	C-14′
H-15α	H-15β, H-16	
H-15β	H-15α, H-16	C-7
H-16	H-15α, H-15β	C-14
H-17		C-5, C-6, C-11, N-CH_2CH_3
H-18a	H-18b	C-5, C-18′
H-18b	H-18a	
H-19α	H-19β	
H-19β	H-19α	
N-$CH_{2\alpha}$	N-$CH_{2\beta}$, $-CH_3$	
N-$CH_{2\beta}$	N-$CH_{2\alpha}$	
N-CH_2CH_3	N-$CH_{2\alpha}$	N-CH_2

carbons (Table 1) except for the protons of the three hydroxyl groups.

The ^{1}H−^{1}H COSY NMR spectrum showed correlations of the protons as shown in Table 2. The presence of an ethyl group and four methoxyl groups account for six carbon atoms. Biogenetic considerations for the remaining 19 carbon atoms of the alkaloid suggested that consolidine should be a norditerpenoid and not a diterpenoid alkaloid. In this norditerpenoid alkaloid skeleton, we have an N-Et group a methine appearing at δ 2.69 (δ 63.2, C-17) and a methylene at δ 2.36, 2.88 (AB, J = 11 Hz) (δ 56.4, C-19). The methylene at C-18 at δ 80.5 (δ 3.38, 3.61, AB, J = 7.5 Hz) is attached to a methoxyl group. When CH_2-18 carries a hydroxyl group, as in lycoctonine, this carbon appears at ~68.0 ppm; other examples of alkaloids having a C-18 CH_2-OH group are scaconine (δ 68.6),[10] 6-*epi*-neolinine (δ 67.4),[9] neolinine (δ 70.9),[11] and columbianine (δ 68.3).[12] The presence of a methoxyl attached at C-18 was confirmed in a selective INEPT experiment (Table 2). When H-18a was selectively pulsed the methoxyl carbon C-18′ (δ 59.1) showed a response, three bonds away. One of the methoxyl carbons should be located at C-8 because of the upfield methyl resonance at δ 52.5, which is typical of a methoxyl at this position, e.g., deltatsine (δ 51.3),[13] delvestidine (δ 54.3),[14] and deltatsine dimethylether (δ 53.5).[13] The proton at δ 3.46 bearing an oxygen function ascribed to H-14 appears as a triplet and shows correlation with the signals at δ 2.15 (H-9) and δ 2.38 (H-13). In the HETCOR spectrum, H-14 is correlated with δ 83.8, and this downfield signal indicated that C-14 bears a methoxyl group. That the fourth methoxyl group should be located at C-16 is supported by the H-16 proton appearing at δ 3.35, which is correlated with the downfield resonance at δ 83.2. Having established the location of the four methoxyl groups at C-8, C-14, C-16, and C-18 positions, the remaining task is the placement of three hydroxyl groups. Of the three hydroxyls, one forms a quaternary carbon that is the most downfield signal at δ 85.0. This tertiary hydroxyl may be located at C-7, C-9, C-10, or C-13. The COSY correlations (Table 2) showed that C-9 and C-13 are unsubstituted (*vide supra*) and a hydroxyl group cannot be assigned to these positions. If C-10 bears a hydroxyl group C-11 would appear at δ 55.0 −56.5,[15] and there is no quaternary carbon in this range. The tertiary OH group should therefore be located at C-7. The remaining two hydroxyls may be located at C-1, C-2, C-3, C-6, C-12, or C-15. Of the remaining three singlets, δ 80.5 should be assigned to C-8, δ 47.3 to C-11, and δ 38.1 to C-4. These assignments are supported by the selective INEPT experiments (Table 2). The positions C-3 and C-15 for location of OH groups are eliminated because the ^{13}C chemical shifts for C-4 and C-8 would have been moved downfield by ~5 ppm from their present values. Since norditerpenoid alkaloids having OH groups at C-2 and C-12 positions are very rare, we located the two hydroxyls at C-1 and C-6. The C-6 hydroxyl appears to be α, since the chemical shift at δ 70.4 for this carbon is very close to that assigned to pubescenine (**1**) at δ 70.8.[8] The structure of pubescenine was established by an X-ray crystal structure determination. In the case of 6-*epi*-pubescenine (**3**) the C-6 carrying a β OH group is assigned the value δ 81.0.[9]

Fraction B gave gigactonine[15] and delsoline.[15] The ^{1}H and ^{13}C NMR chemical shift assignments for delsoline have been recently revised by us.[16]

Fraction C provided ajaconine (**4**).[17] A detailed NMR study of **4** through 1D, DEPT, COSY, HMQC, and HMBC spectra resulted in the revision of eleven ^{13}C chemical shift assignments.[17] The complete ^{1}H NMR assignments are reported, here, for the first time (Table 3).

Experimental Section

General Experimental Procedures. Melting points are corrected and were determined on a Thomas-Koffler hot stage equipped with a microscope and a polarizer.

Table 3. NMR Data[a] of Ajaconine (**4**)

position	δ ^{13}C[b]	δ ^{1}H (mult. J = Hz)	COSY	HMBC (^{1}H to ^{13}C)
1	30.0 (t)*	α, 1.55 (m)	H-2	
		β, 1.17 (m)		C-9, C-10, C-20
2	21.0 (t)	α, 2.27 (m)		C-1, C-3, C-4, C-5
		β, 1.40 (m)	H-1	C-10, C-4
3	41.1 (t)*	α, 1.58 (m)	H-3β	
		β, 1.23 (m)	H-3α	
4	33.4 (s)			
5	44.1 (d)	1.22 (s)	H-6, H-20[c]	C-4, C-19, C-20
6	26.5 (t)*	α, 2.42 (m)	H-5, H-7	
		β, 1.80 (t, 3.4)		C-4, C-5, C-7, C-8
7	72.1 (d)*	3.67 (t, 9.2)	H-6	C-5, C-9, C-20
8	41.5 (s)			
9	40.1 (d)*	1.50(m)	H-11α, H-11β	
10	35.2 (s)			
11	25.1 (t)*	α, 1.65 (m)	H-9, H-12	C-8, C-9, C-12, C-16
		β, 1.40 (m)	H-9, H-12	
12	36.7 (d)*	2.36 (t, 3.7)	H-11α, H-11β	C-11, C-15, C-16, C-17
13	26.3 (t)*	1.85 (m)		
14	26.9 (t)*	α, 2.15 (m)		
		β, 1.35 (m)		
15	75.2 (d)*	4.15 (br s)	H-17[c]	C-8, C-9, C-14, C-16, C-17
16	156.7 (s)			
17	107.9 (t)	a, 5.11 (s)	H-15α[c]	C-15, C-12
		b, 4.99 (s)		C-15, C-12
18	25.0 (q)*	0.73 (s)		C-3, C-4, C-5, C-19
19	51.4 (t)	α, 2.81 (AB 11.3)	H-19β	
		β, 2.27 (AB 11.3)	H-19α	C-3, C-4, C-5
20	87.7 (d)	4.57 (br s)	H-5[c]	C-5, C-10, C-21
21	57.9 (t)	α, 2.94 (m)	H-21β, H-22	C-19, C-20
		β, 2.83 (m)	H-21α, H-22	C-19
22	57.1 (t)	α, 3.67 (m)	H-22β, H-21	
		β, 2.82 (m)	H-22α, H-21	
15		OH-2.46 (br d)		
22		OH-4.08 (br d)		

[a] HMQC and HMBC spectra were recorded on an AMX 400 spectrometer. [b] * indicates values that are revised from those reported earlier.[17] [c] Long-range coupling.

Optical rotations were measured on a Perkin-Elmer, Model 141, polarimeter in $CHCl_3$. IR spectra were recorded in Nujol on a Perkin-Elmer Model 1420 spectrophotometer. HRMS were determined on a Fisons Auto Spec ETOFFPD FAB$^+$ mass spectrometer. ESIMS were recorded on a Perkin-Elmer SCIEX AP1-1 mass spectrometer. NMR spectra including DEPT and 2D experiments were recorded in $CDCl_3$ on Bruker AC-250, AC 300, and AMX 400 spectrometers. The pulse sequences employed for the NMR experiments were those of the standard Bruker software. Chromatographic separations on a Chromatotron[18] were carried out on rotors coated with 1 mm thick layers of Merck Al_2O_3 60 PF 254, 365 (EM 1104). All known compounds reported in this work were identified by comparing their TLC, mp, IR, and ^{1}H and ^{13}C NMR spectra with those of authentic samples. The pH measurements were carried out with DUOTEST indicator papers (Machery-Nagel, Düren, Germany).

Plant Material. The aerial parts of *C. oliveriana* (DC) Schröd. were collected and identified by one of the authors (HCÖ) (June 1994) on the outskirts of Diyarbakir in southeastern Turkey. A voucher specimen has been deposited in the Herbarium of the Faculty of Sciences and Literature, University of Dicle, Diyarbakir, Turkey.

Extraction of Crude Alkaloids. Dried and powdered aerial parts of *C. oliveriana* (600 g) were exhaustively extracted, by percolation at room temperature, with 95% EtOH and the extract evaporated *in vacuo* to give a gummy residue (86.14 g). This was dissolved in CH_2Cl_2 (500 mL) and extracted with 2% H_2SO_4 (v/v) (200 mL × 10). The acidic fraction was washed with CH_2Cl_2 (200 mL × 3). The combined acidic fraction was basified in the cold, with aqueous NaOH. Extraction with CH_2Cl_2 (pH 10) (300 mL × 10) and (pH 14) (250 mL × 5) and evaporation of the combined extracts *in vacuo* gave a crude mixture of alkaloids (2.17 g). The crude alkaloidal mixture was chromatographed on basic Al_2O_3 (E. Merck) column (5 × 57 cm) and eluted with petroleum ether with a step gradient (100 mL each) addition of EtOAc up to 100% followed by EtOH up to 20%. Fifty-five fractions (100 mL each) were collected and pooled according to their TLC pattern to give three main fractions: A (fractions 14−16, 256 mg), B (fractions 17−44, 307 mg), and C (fractions 45−55, 450 mg). Compounds were isolated from these three fractions as described below.

Isolation of Consolidine (2) and Pubescenine (1). Fraction A (256 mg) was chromatographed on a basic Al_2O_3 rotor. Ninety-seven fractions (20 mL each) were collected with a solvent gradient (in steps of 50 mL) of hexane, $CHCl_3$, and MeOH. Fraction 5, eluted with hexane/$CHCl_3$ (1:1), gave a homogeneous (TLC, Al_2O_3, 30%hexane in $CHCl_3$) amorphous solid (76 mg) with a mp range 175−182 °C, which was identified and designated as the new norditerpenoid alkaloid consolidine (**2**): $[\alpha]_D$ +11.8° (*c* 0.17); FABHRMS *m/z* 468.2943 (M + H)$^+$ calculated for $C_{25}H_{42}NO_7$ *m/z* 468.2961; IR ν_{max} 1100, 750, and 725 cm^{-1}. For ^{1}H and ^{13}C NMR assignments see Tables 1 and 2. Fraction 7 eluted with hexane/$CHCl_3$ (3:7) gave another homogeneous compound that crystallized from acetone−hexane: mp 225−228 °C (lit.[8] mp 227−229 °C); ESIMS *m/z* 496.2 (M + H)$^+$. This crystalline compound was identified as pubescenine (**1**, 10.5 mg).

Isolation of Delsoline and Gigactonine. Fraction B (300.0 mg) was further fractionated on an Al_2O_3 rotor of a Chromatotron eluting with a solvent gradient of hexane, $CHCl_3$, and methanol. Fractions 41−56 eluted with $CHCl_3$, when crystallized from MeOH, gave delsoline (40.2 mg), mp 212−214 °C (lit.[15] mp 215−216 °C). Fractions 60−67 eluted with 2% MeOH in $CHCl_3$ gave gigactonine (50.3 mg) (Me_2CO−hexane), mp 168−169.5 °C (lit.[15] mp 168−169 °C).

Isolation of Ajaconine (4). Fraction C was chromatographed on a basic Al_2O_3 rotor, and the elution was carried out with a solvent gradient comprised of hexane, $CHCl_3$, and MeOH. Fractions 32−36 eluted with $CHCl_3$: 2% MeOH gave a homogeneous compound that crystallized from acetone to afford colorless plates, mp 170−172 °C (lit.[19] mp 170−172 °C). This was identified as the diterpenoid alkaloid ajaconine (**4**).[17] For a complete assignment of the NMR data see Table 3.

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References and Notes

(1) Baytop, T. *Therapy with Medicinal Plants in Turkey (Past and Present)*; Publication no. 3255; Istanbul University: Istanbul, 1984; p 187.
(2) Ulubelen, A.; Desai, H. K.; Joshi, B. S.; Venkateswarlu, V.; Pelletier, S. W.; Meriçli, A. H.; Meriçli, F.; Özcelik, H. *J. Nat. Prod*. **1995**, *58*, 1555−1561.
(3) Ulubelen, A.; Meriçli, A. H.; Meriçli, F.; Ilarslan, R. *Phytochemistry* **1993,** *32*, 213−215; *Phytochemistry* **1992**, *31*, 1019−1021.
(4) Ulubelen, A.; Meriçli, A. H.; Meriçli, F.; Ilarslan, R.; Matlin, S. A. *Phytochemistry* **1992**, *31*, 3239−3241.
(5) Ulubelen, A.; Meriçli, A. H.; Meriçli, F. *J. Nat. Prod*. **1993**, *56*, 780−781.
(6) Ulubelen, A.; Meriçli, A. H.; Meriçli, F.; Ilarslan, R.; Voelter, W. *Phytochemistry* **1993**, *34*, 1165−1167.
(7) Ulubelen, A.; Desai, H. K.; Srivastava, S. K.; Hart, B. P.; Park, J. C.; Joshi, B. S.; Pelletier, S. W.; Meriçli, A. H.; Meriçli, F.; Ilarslan, R. *J. Nat Prod*. **1996**, *59*, 360−366.
(8) de la Fuente, G.; Acosta, R. D.; Gavin, J. A.; Lugo, R. H.; Jones, P. G. *Tetrahedron Lett*. **1988**, *29*, 2723−2726.
(9) Bai, Y.; Benn, M.; Majak, W. *Heterocycles* **1989**, *29*, 1017−1021.
(10) Hao, X. J.; Chen, S. Y.; Zhou, J. *Yunnan Zhiwu Yanjiu* **1985**, *7*, 217−224.
(11) Ross, S. A.; Desai, H. K.; Pelletier, S. W. *Heterocycles* **1987**, *26*, 2895−2904.
(12) Boido, V.; Edwards, O. E.; Handa, K. L.; Kolt, R. J.; Purushothaman, K. K. *Can. J. Chem*. **1984**, *62*, 778−784.
(13) Joshi, B. S.; Glinski, J. A.; Chokshi, H. P.; Chen, S. Y.; Srivastava, S. K.; Pelletier, S. W. *Heterocycles* **1984**, *22*, 2037−2042.
(14) Desai, H. K.; Joshi, B. S.; Pelletier, S. W. *Heterocycles* **1985**, *23*, 2483−2487.
(15) Pelletier, S. W.; Mody, N. V.; Joshi, B. S.; Schramm, L. C. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier S. W., Ed.; Wiley: New York, 1984; Vol. 2, pp 205−462.
(16) Joshi, B. S.; Desai, H. K.; Pelletier, S. W.; Newton, M. G. *J. Crystallogr. Spectrosc. Res.* **1992**, *22*, 477−483.
(17) Pelletier, S. W.; Mody, N. V. *J. Am. Chem. Soc.* **1979**, *101*, 492−494.
(18) Desai, H. K.; Trumbull, E. R.; Pelletier, S. W. *J. Chromatogr.* **1986**, *366*, 439−444.
(19) Pelletier, S. W.; Harraz, F. M.; Badawi, M. M.; Tantiraksachai, S.; Wang, F. P.; Chen, S. Y. *Heterocycles* **1986**, *24*, 1853−1865.

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