CONSOLARINE, A NOVEL NORDITERPENOID ALKALOID FROM *CONSOLIDA ARMENIACA*

Ali H. Meriçli,[a] Filiz Meriçli,[a] Ayhan Ulubelen,[a,b] Haridutt K. Desai,[c] Balawant S. Joshi,[c] S. William Pelletier*,[c,d] Seçkin Özden,[e] and Mustafa Küçükislamoglu[f]

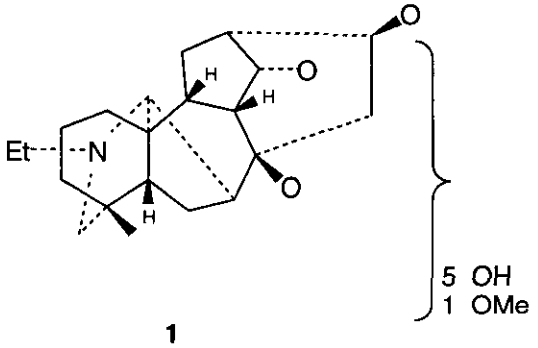
[a]Faculty of Pharmacy, University of Istanbul, 34452, Istanbul, Turkey, [b]TUBITAK, Marmara Research Center, Department of Chemistry, P. O. Box 21, 41470, Gebze, Kocaeli, Turkey, [c]Institute for Natural Products Research, [d]Institute for Natural Products Research and Department of Chemistry, The University of Georgia, Athens, Georgia 30602-2556, U. S. A., [e]Faculty of Pharmacy, University of Ankara, Tandogan 06100, Ankara, Turkey, and [f]Faculty of Education, Technical University of Karadeniz, Trabzon, Turkey

(Dedicated to Professor Koji Nakanishi on the occasion of his 75th Birthday)

Abstract–From the aerial parts of *Consolida armeniaca*, (Stapf. Ex Huth.) Schröd. a new norditerpenoid alkaloid named *consolarine* has been isolated along with the known alkaloids ajadelphinine, gigactonine, and lycoctonine. The structure of consolarine (**2**) was established on the basis of ^{1}H, ^{13}C, DEPT, homonuclear ^{1}H COSY, HETCOR, NOESY, and COLOC NMR spectral studies.

Except for the isolation of four acylated anthocyanin pigments from the flowers of *Consolida armeniaca* (Stapf. Ex Huth.) Schröd.,[1] no phytochemical work appears to have been carried out earlier on this plant. In continuation of our studies on the alkaloids of Turkish *Delphinium*, and *Consolida* species,[2-9] an investigation of the aerial parts of *C. armeniaca* led to the isolation of a novel norditerpenoid alkaloid *consolarine*. The crude alkaloid isolated from *C. armeniaca* at pH 10 was purified on an Al_2O_3 column by VLC and six fractions (A-F) were collected. By chromatographic separation of the third fraction on an Al_2O_3 rotor, an amorphous homogeneous alkaloid designated as consolarine was isolated. The EIMS and the HRMS indicated the molecular ion at m/z 409 suggesting the formula $C_{22}H_{35}NO_6$ for the alkaloid. The NMR spectra showed that the alkaloid contains an *N*-ethyl group (δ_C 13.6, q; δ_H, 1.12, 3H, t, $J = 7.2$ Hz; δ_C, 50.9 t; δ_H, 2.92, 2.99, 2H, AB J_{gem} = 11.0 Hz) and a methoxyl group (δ_C 57.0, q; δ_H 3.36, 3H, s) accounting for three carbons. Biogenetic

considerations and the molecular formula $C_{22}H_{35}NO_6$ indicated that consolarine is a norditerpenoid alkaloid. As there are no carbonyl functionalities, ether oxygens or methylenedioxy groups, the alkaloid should contain five hydroxyl groups and one methoxyl group. The ^1H and ^{13}C NMR spectra (Table 1) indicated the presence of a tertiary methyl (δ_C 30.2 q; δ_H, 1.27, 3H, s) group. As no other functional groups are discernible in the IR or the NMR spectra, a partial structure (**1**) can be written for consolarine.



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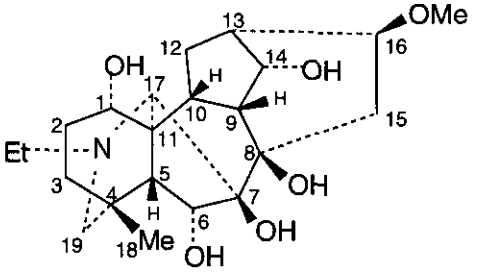


2 Consolarine

The quaternary carbon signals at δ 33.7, 47.5 and 77.5 can be readily assigned to C-4, C-11 and C-8, respectively.[10] The fourth quaternary carbon signal at δ 82.8 remains to be assigned. This carbon bearing a hydroxyl group can be located at either C-5, C-7, C-9, C-10 or C-13. The positions C-5, and C-10 can be discounted as the adjacent carbons C-4 and C-11 would have shown downfield shifts of ~ 4-6 ppm from their normal positions as in bonvalotine and bonvalol,[11] having a C-5 OH; and deltamine and dictyocarpinine[12,13] having a C-10 OH groups, respectively. The carbon signal at δ 75.2 (δ_H 4.12, 1H, t, J = 3.5 Hz) is clearly assigned to C-14 bearing an α hydroxyl group, not having any substituents on the adjacent carbons C-9 and C-13. There are numerous examples in support of this argument.[9] Hence, the tertiary hydroxyl group should be located on the remaining position at C-7, consistent with the chemical shift of this quaternary carbon at δ 82.8.

The problem of locating the two secondary hydroxyls thus remains. One of the hydroxyls is present at C-1 (δ_C 72.2 d; δ_H, 3.63, 1H, br s, $W_{1/2}$ = 4.5 Hz). This proton shows a correlation with the protons of H-2 (δ_C 28.9 t; δ_H, 1.50, 1H, m) in the COSY spectrum and with H-12 in the NOESY spectra (Table 2). The H-2 proton (δH, 1.50) in turn shows a correlation with one of the H-3 protons (δ_C 34.6 t; δ_H, 1.50, 1.80, 2H, m) in the COSY. The H-1 proton shows correlation with C-10 (δ_C 43.4) and C-11 (δ_C 47.5) in the COLOC spectrum (Figure 1). The remaining hydroxyl group should therefore be located at C-6, in preference to C-12 or C-15, which are the only other methylene groups. The H-15 protons (δ_C, 36.2, t; δ_H, 1.90, 2.85, 2H, m) shows COSY and NOESY correlations with H-16 (δ_H, 3.30, 1H, m). The proton signal for C-15 (δ_C 36.2 t; δ_H, 1.90, 2.85, 2H, m) is correlated with C-8 (δ_C 77.5) in the COLOC. The H-16 proton shows a correlation with H-13 (δ_C, 39.9 d; δ_H, 2.25,, 1H, d d) and a W-type coupling with H-14.

Table 1. ^{1}H and ^{13}C Chemical shifts assignments of consolarine (**2**) and dihydrogadesine (**3**) (in $CDCl_3$)

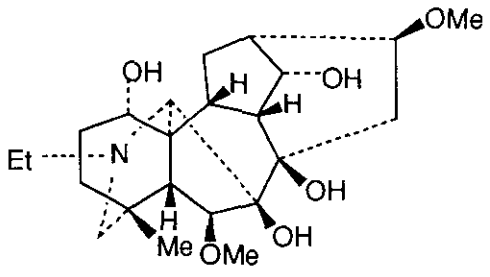
Carbon	δ (ppm) (**2**)	(**3**)	Proton (**2**)	δ (ppm)		*J* (Hz)
1	72.2 (*d*)*	72.8	1b	3.63#	br *s*	$w_{1/2}$ = 4.5
2	28.9 (*t*)	29.3	2	1.50	m	
3	34.6 (*t*)	32.2	$3_{a,b}$	1.50, 1.80	*m*	
4	33.7 (*s*)	33.1	4	-	-	
5	50.1 (*d*)	50.6	5	2.00	*d*	$J_{5,6}$= 6.7
6	69.8 (*d*)	91.0	6_b	4.49	*d*	$J_{6,5}$ = 6.7
7	82.8 (*s*)	87.9	7	-	-	
8	77.5 (*s*)	78.1	8	-	-	
9	46.8 (*d*)	45.3	9	2.20	*m*	
10	43.4 (*d*)	44.1	10	1.85	*m*	
11	47.5 (*s*)	49.2	11	-	-	
12	29.6 (*t*)	29.3	12a,b	1.30, 1.70	*m*	
13	39.9 (*d*)	39.4	13	2.25	*d d*	$J_{13,12b}$ = 4.9
14	75.2 (*d*)	75.7	14	4.12	*t*	$J_{14,9}$ = 3.5
						$J_{13,14}$ = 3.5
15	36.2 (*t*)	34.5	$15_{a,b}$	1.90, 2.85	*m*	
16	82.1 (*d*)	82.1	16	3.30	*m*	
17	63.7 (*d*)	65.7	17	2.80	s	
18	30.2 (*q*)	27.7	18	1.27	s	
19	60.3 (*t*)	60.8	19a,b	2.36, 2.82	AB	J_{gem} = 11.0
N-$\underline{C}H_2$	50.9 (*t*)	50.3	N-$C\underline{H}_{2a,b}$	2.92, 2.99	*m*	J_{VIC} = 7.2
CH_3	13.6 (*q*)	13.7	$C\underline{H}_3$	1.12	*s*	
C-6'	-	58.0	-	-	-	
C-16'	57.0 (*q*)	56.3	OCH_3	3.36	*s*	
			6-OH	3.30	br *m*	

* Multiplicity deduced by DEPT

Carbon showing long-range correlation with indicated protons deduced by HETCOR

Table 2. $^1H,^1H$ *correlations and nOe's of consolarine* (**2**)

Observed H	Correlations (COSY)	nOe's (NOESY)
H-1$_\beta$	H-2	H-12$_a$
H-2	H-1$_\beta$, H-3	-
H-3$_a$	H-3$_b$, CH_3-18	*N*-CH_2CH_3
H-3$_b$	H-3$_a$	
H-5	H-6	H-6, CH_3-18
H-6	H-5	H-5, H-9
H-9	H-10, H-13, H-14	H-6
H-10	H-9, H-12$_b$	-
H-12$_a$	H-12$_b$, H-13	H-1$_\beta$
H-12$_b$	H-10, H-12$_a$	-
H-13	H-9, H-12$_a$, H-14, H-16 (W)	H-14, H-16
H-14	H-9, H-13, H-16	H-13, H-16
H-15$_a$	H-15$_b$, H-16	H-15$_b$
H-15$_b$	H-15$_a$	H-15$_a$, H-16
H-16	H-13, H-14, H-15$_a$, H-15$_b$	H-13, H-14, H-15$_b$,
H-17	-	H-16
CH_3-18	H-3$_b$	H-5
H-19$_a$	-	CH_3-18, H-19$_b$
H-19$_b$	-	CH_3-18, H-19$_a$
N-$CH_2C\underline{H}_3$	-	H-3$_a$
N-$C\underline{H}_2CH_3$	-	CH_3-18, *N*-$CH_2C\underline{H}_3$



3 *Dihydrogadesine*

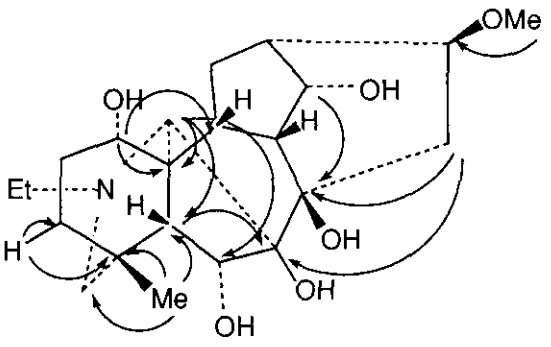


Figure 1
COLOC Correlations of consolarine **2**

Thus, the five hydroxyl groups of the alkaloid are located at C-1, C-6, C-7, C-8 and C-14 and the methoxyl is at C-16 in the partial structure (**1**). In most of the lycoctonine-type norditerpenoid alkaloids, the hydroxyl group at C-6 is in a β position and *H*-C-6 is α and the Dreiding model reveals the

H(5β)-H(6α) dihedral angle ~ 90°. The chemical shift for H-6 normally appears at ~ δ 4.5, corresponding with 6-αH and $J_{5\beta,6\alpha}$ = ~ 0 Hz. In the present alkaloid, the H-6 signal (δ_C 69.8 d; δ_H, 4.49, 1H, d, J = 6.7 Hz) clearly shows that the hydroxyl group is in an α- position. In the aconitine-type alkaloids, where the C-6 methoxyl is in an α-position, e.g., acoforesticine,[14] acoforestinine,[14] and flavaconidine,[15] C-6-βH appears at ~ δ 4.1 as a doublet, showing a coupling of ~ 6 Hz with H-5. The coupling with H-7 is negligible as the dihedral angle is ~ 90°. Consolarine indicates similarity to the C-6α hydroxyl group of pubescenine (δ_C 70.8 d; δ_H, 4.45, 1H, d, J = 6.8 Hz, H-6β),[16] as against 6-*epi*-pubescenine where H-6 appears as a singlet showing no coupling with H-5.[17] A Dreiding model shows that H-6α hydroxyl group is in close proximity to the C-18 methyl group (δ_C 30.2 q; δ_H, 1.27, 3H, s). In a 1D difference nOe experiment, the C-18 methyl group showed an nOe to the C-6 α hydroxyl group which appears at δ 3.30. A plausible explanation for the downfield shifts of the C-18 methyl in the ^{13}C and ^{1}H spectra (δ_C 30.2; δ_H, 1.27), from the normal values (δ_C 25.0-26; δ_H, 0.8-1.1) lies in the deshielding influence of the 6-α-hydroxyl group on the C-18 methyl. On the basis of these data, structure (**2**) has been assigned to consolarine. Consolarine is the only example of a lycoctonine-type norditerpenoid alkaloid bearing a C-18 methyl and C-6 α OH groups. The structure of consolarine (**2**) resembles dihydrogadesine (**3**) (see Table 1 for ^{13}C NMR spectra) except for a β-methoxyl at C-6 instead of a C-6 α–hydroxyl group.[18]

By chromatographic separation of fractions (A) and (E) on an Al_2O_3 rotor, the known alkaloids ajadelphinine,[19] lycoctonine[10] and gigactonin[20] were isolated.

EXPERIMENTAL

General Experimental Procedures.– IR spectra were recorded in $CHCl_3$ on a Perkin-Elmer Model 983 spectrophotometer. HRMS were determined on a VG Zap Spec instrument and Perkin-Elmer SCIEX AP1-1 mass spectrometer. NMR spectra including DEPT and 2D experiments, were recorded in $CDCl_3$ on a Bruker AC-250 and AC 300 spectrometers. The pulse sequences employed for the NMR experiments were those of the standard Bruker software. ^{1}H and ^{13}C NMR and COLOC spectra were determined on Bruker AC 200L instrument. Optical rotations were determined on Opt. Act Ltd AA-5 polarimeter. Chromatographic separations on a Chromatotron were carried out on rotors coated with 1 mm thick layers of Merck Al_2O_3 60 PF 254, 365 (EM 1104).

Plant Material.– The aerial parts of *Consolida armeniaca* were collected by one of the authors (M. K.) and identified by (A. H. M. and F. M.) (August 1996) near the Black sea between Gümüshane-Bayburt 30 km from Bayburt at an elevation of 1650 m. A voucher specimen is deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul ISTE 72679.

Extraction of Crude Alkaloids.– Dried and powdered aerial parts of *C. armeniaca* (2.5 kg) were exhaustively extracted by percolation at rt with 95% EtOH. Evaporation (*in vacuo*) of the combined extracts gave a gummy residue which was dissolved in CH_2Cl_2 (500 mL) and extracted with 2% H_2SO_4 (200 mL x 10). The acidic extract was washed with CH_2Cl_2 (200 mL x 3) and then basified

to pH 10 with cold aq. 10% NaOH. Extractions with CH_2Cl_2 (250 mL x 5) and evaporation of the combined extracts in *vacuo* gave a crude mixture of alkaloids (2.5 g).

Purification of the Alkaloidal Mixture.– The crude alkaloidal mixture was chromatographed by VLC[21] on an Al_2O_3 column. The eluting solvent was a gradient of hexane, EtOAc and MeOH and six fractions (A-F) were collected. These were separated on Al_2O_3 rotors of a Chromatotron. Fraction (A) was chromatographed on an Al_2O_3 rotor and gradient eluted with hexane, EtOAc, MeOH to afford ajadelphinine (30 mg) as an amorphous product. The identity was established by comparison of the TLC, ^1H and ^{13}C NMR spectra with an authentic sample. From the second fraction (B) (163 mg), consolarine (**2**) was obtained as an amorphous compound (103 mg), $[\alpha]_D$ + 0.65° (c, 0.77, $CHCl_3$). EIMS: m/z 409 (M^+, 75%), 394 (11), 376 (30), 353 (35), 145 (30), 122 (28), 58 (72). HRMS: Found, 409.2450; Calculated. for $C_{22}H_{35}NO_6$, 409.24643. IR (nujol): ν_{max} 3280, 2920, 2880, 1705, 1640. 1565, 1450, 1400, 1300, 1220, 1162, 1170, 1100 cm^{-1}. For ^1H and ^{13}C NMR spectra, see Table 1. The fraction (E) was chromatographed on an Al_2O_3 rotor to afford lycoctonine (190 mg) and gigactonine (25 mg). The alkaloids were identified by comparison of the TLC, ^1H and ^{13}C NMR spectra with those of authentic samples.

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