

## CONSOLARINE, A NOVEL NORDITERPENOID ALKALOID FROM *CONSOLIDA ARMENIACA*

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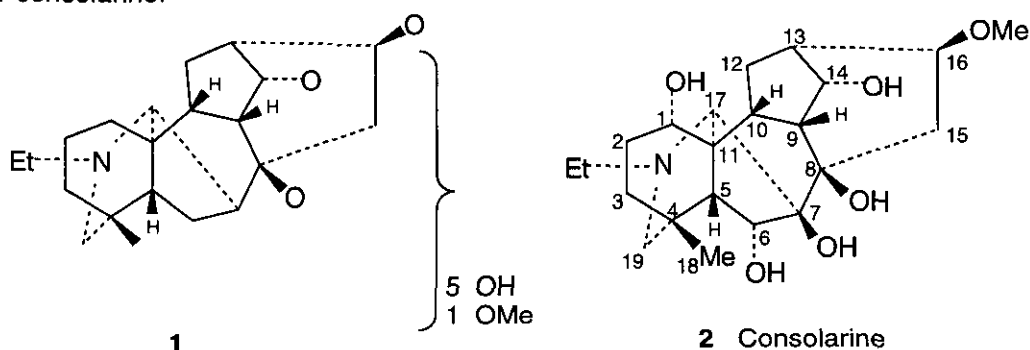
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(Dedicated to Professor Koji Nakanishi on the occasion of his 75<sup>th</sup> Birthday)

**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 <sup>1</sup>H, <sup>13</sup>C, DEPT, homonuclear <sup>1</sup>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.,<sup>1</sup> 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,<sup>2-9</sup> 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<sub>2</sub>O<sub>3</sub> column by VLC and six fractions (A-F) were collected. By chromatographic separation of the third fraction on an Al<sub>2</sub>O<sub>3</sub> 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<sub>22</sub>H<sub>35</sub>NO<sub>6</sub> for the alkaloid. The NMR spectra showed that the alkaloid contains an *N*-ethyl group ( $\delta_c$  13.6, q;  $\delta_H$ , 1.12, 3H, t,  $J = 7.2$  Hz;  $\delta_c$ , 50.9 t;  $\delta_H$ , 2.92, 2.99, 2H, AB  $J_{gem} = 11.0$  Hz) and a methoxyl group ( $\delta_c$  57.0, q;  $\delta_H$  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 ( $\delta_c$  30.2 q;  $\delta_H$ , 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.



The quaternary carbon signals at  $\delta$  33.7, 47.5 and 77.5 can be readily assigned to C-4, C-11 and C-8, respectively.<sup>10</sup> The fourth quaternary carbon signal at  $\delta$  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,<sup>11</sup> having a C-5 OH; and deltamine and dictyocarpinine<sup>12,13</sup> having a C-10 OH groups, respectively. The carbon signal at  $\delta$  75.2 ( $\delta_H$  4.12, 1H, t,  $J = 3.5$  Hz) is clearly assigned to C-14 bearing an  $\alpha$  hydroxyl group, not having any substituents on the adjacent carbons C-9 and C-13. There are numerous examples in support of this argument.<sup>9</sup> 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  $\delta$  82.8.

The problem of locating the two secondary hydroxyls thus remains. One of the hydroxyls is present at C-1 ( $\delta_c$  72.2 d;  $\delta_H$ , 3.63, 1H, br s,  $W_{1/2} = 4.5$  Hz). This proton shows a correlation with the protons of H-2 ( $\delta_c$  28.9 t;  $\delta_H$ , 1.50, 1H, m) in the COSY spectrum and with H-12 in the NOESY spectra (Table 2). The H-2 proton ( $\delta_H$ , 1.50) in turn shows a correlation with one of the H-3 protons ( $\delta_c$  34.6 t;  $\delta_H$ , 1.50, 1.80, 2H, m) in the COSY. The H-1 proton shows correlation with C-10 ( $\delta_c$  43.4) and C-11 ( $\delta_c$  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 ( $\delta_c$ , 36.2, t;  $\delta_H$ , 1.90, 2.85, 2H, m) shows COSY and NOESY correlations with H-16 ( $\delta_H$ , 3.30, 1H, m). The proton signal for C-15 ( $\delta_c$  36.2 t;  $\delta_H$ , 1.90, 2.85, 2H, m) is correlated with C-8 ( $\delta_c$  77.5) in the COLOC. The H-16 proton shows a correlation with H-13 ( $\delta_c$ , 39.9 d;  $\delta_H$ , 2.25, 1H, d d) and a W-type coupling with H-14.

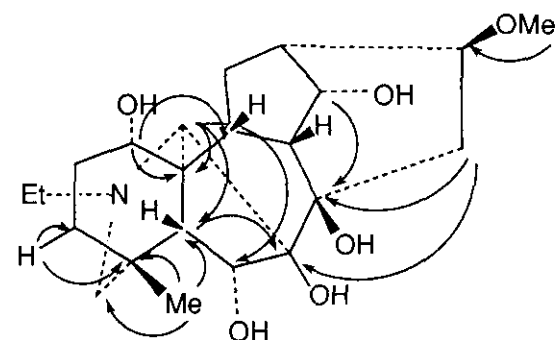
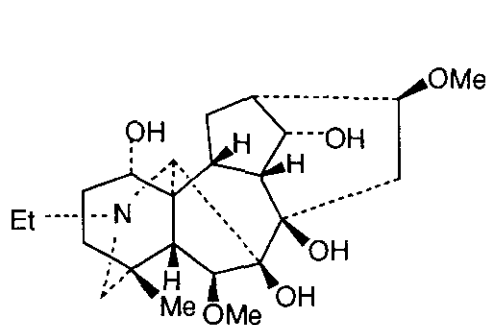
Table 1.  $^1\text{H}$  and  $^{13}\text{C}$  Chemical shifts assignments of consolarine (2) and dihydrogadesine (3) (in  $\text{CDCl}_3$ )

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

<sup>a</sup> Multiplicity deduced by DEPT<sup>#</sup> Carbon showing long-range correlation with indicated protons deduced by HETCOR

Table 2.  $^1\text{H}$ ,  $^1\text{H}$  correlations and  $n\text{Oe}$ 's of consolarine (2)

Observed H	Correlations (COSY)	$n\text{Oe}$ 's (NOESY)
H-1 $\beta$	H-2	H-12 $_a$
H-2	H-1 $\beta$ , H-3	-
H-3 $_a$	H-3 $_b$ , CH <sub>3</sub> -18	<i>N</i> -CH <sub>2</sub> CH <sub>3</sub>
H-3 $_b$	H-3 $_a$	-
H-5	H-6	H-6, CH <sub>3</sub> -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 <sub>3</sub> -18	H-3 $_b$	H-5
H-19 $_a$	-	CH <sub>3</sub> -18, H-19 $_b$
H-19 $_b$	-	CH <sub>3</sub> -18, H-19 $_a$
<i>N</i> -CH <sub>2</sub> CH <sub>3</sub>	-	H-3 $_a$
<i>N</i> -CH <sub>2</sub> CH <sub>3</sub>	-	CH <sub>3</sub> -18, <i>N</i> -CH <sub>2</sub> CH <sub>3</sub>



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 lycotoxine-type norditerpenoid alkaloids, the hydroxyl group at C-6 is in a  $\beta$  position and *H*-C-6 is  $\alpha$  and the Dreiding model reveals the

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

By chromatographic separation of fractions (A) and (E) on an Al<sub>2</sub>O<sub>3</sub> rotor, the known alkaloids ajadelphinine,<sup>19</sup> lycoctonine<sup>10</sup> and gigactonin<sup>20</sup> were isolated.

## EXPERIMENTAL

**General Experimental Procedures.**— IR spectra were recorded in CHCl<sub>3</sub> 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<sub>3</sub> on a Bruker AC-250 and AC 300 spectrometers. The pulse sequences employed for the NMR experiments were those of the standard Bruker software. <sup>1</sup>H and <sup>13</sup>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<sub>2</sub>O<sub>3</sub> 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 Gümüşhane-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<sub>2</sub>Cl<sub>2</sub> (500 mL) and extracted with 2% H<sub>2</sub>SO<sub>4</sub> (200 mL x 10). The acidic extract was washed with CH<sub>2</sub>Cl<sub>2</sub> (200 mL x 3) and then basified

to pH 10 with cold aq. 10% NaOH. Extractions with CH<sub>2</sub>Cl<sub>2</sub> (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<sup>21</sup> on an Al<sub>2</sub>O<sub>3</sub> column. The eluting solvent was a gradient of hexane, EtOAc and MeOH and six fractions (A-F) were collected. These were separated on Al<sub>2</sub>O<sub>3</sub> rotors of a Chromatotron. Fraction (A) was chromatographed on an Al<sub>2</sub>O<sub>3</sub> 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, <sup>1</sup>H and <sup>13</sup>C NMR spectra with an authentic sample. From the second fraction (B) (163 mg), consolarine (2) was obtained as an amorphous compound (103 mg), [ $\alpha$ ]<sub>D</sub> + 0.65° (c, 0.77, CHCl<sub>3</sub>). EIMS: m/z 409 (M<sup>+</sup>, 75%), 394 (11), 376 (30), 353 (35), 145 (30), 122 (28), 58 (72). HRMS: Found, 409.2450; Calculated. for C<sub>22</sub>H<sub>35</sub>NO<sub>6</sub>, 409.24643. IR (nujol):  $\nu$ <sub>max</sub> 3280, 2920, 2880, 1705, 1640, 1565, 1450, 1400, 1300, 1220, 1162, 1170, 1100 cm<sup>-1</sup>. For <sup>1</sup>H and <sup>13</sup>C NMR spectra, see Table 1. The fraction (E) was chromatographed on an Al<sub>2</sub>O<sub>3</sub> rotor to afford lycoc-tonine (190 mg) and gigactonine (25 mg). The alkaloids were identified by comparison of the TLC, <sup>1</sup>H and <sup>13</sup>C NMR spectra with those of authentic samples.

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