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Diterpenoid alkaloids from the roots of *Aconitum cochleare*

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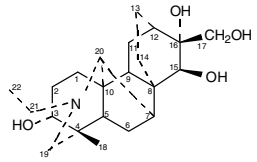
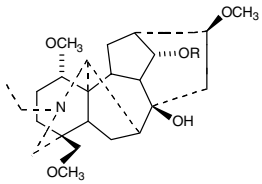
From the roots of *Aconitum cochleare* Woroschin, collected in Turkey, a new diterpenoid alkaloid named acochlearine has been isolated along with the known diterpenoid alkaloids talatisamine, 14-*O*-acetyltalatisamine, senbusine C and condelphine. The structure for acochlearine was established on the basis of ^{1}H, ^{13}C, DEPT, homonuclear ^{1}H COSY, NOESY, HSQC and HMBC NMR studies.

1. Introduction

Aconitum (wolfslayer) species are very toxic plants due to the diterpenoid alkaloid contents. These alkaloids are neurotoxic agents, causing bradycardy, muscle system spasms, hypotension and death by arrest of respiration. *Aconitum* preparations have been used in very diluted forms as cardiotonics, febrifugies, sedatives and anodynes. Today *Aconitum* is very popular in homoeopathy and is included in many pharmaka (Bisset 1981; Benn and Jacyno 1983; Meriçli et al. 2004). In continuation of our investigations on Turkish *Aconitum* species (Meriçli et al. 1996, 2000; Ulubelen et al. 1996) we now report the alkaloids contents of *Aconitum cochleare* Woroschin. No previous work investigated this species for its diterpenoid alkaloid contituents. The chemical investigation of the roots of *A. cochleare* has led to the isolation of a new diterpenoid alkaloid acochlearine **(1)**, together with talatisamine **(2)**, 14-*O*-acetyltalatisamine **(3)**, senbusine C **(4)** and condelphine **(5)** (Hikino et al. 1984; Pelletier et al. 1984; Aiyar et al. 1986).



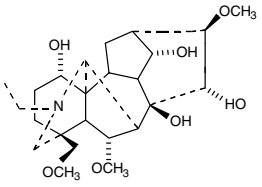
Acochlearine **(1)**



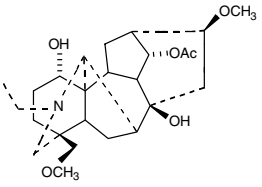
R=H Talatisamine **(2)**
R=Ac 14-O-acetyltalatisamine **(3)**

2. Investigations, results and discussion

A novel diterpenoid alkaloid designated as acochlearine from the roots of *A. cochleare* collected at an altitude 2800 m in Van-Güzeldere Pass, Turkey, has been isolated exhibiting $[\alpha]_D^{20}$ $-0.39°$ (0.13, $CHCl_3$). The molecular formula, $C_{22}H_{35}NO_4$ (El, MW $[M]^+$ m/z 377), was derived for the alkaloid by HRMS $[M]^+$ m/z 377.51670 (calc. 377.51764) and confirmed from the ^{1}H, ^{13}C NMR spectral and DEPT data. The IR spectrum showed hydroxyl absorption at 3380 cm^{-1}, but no carbonyl or aromatic absorptions. A completely decoupled ^{13}C NMR spectrum confirmed 22 carbon atoms of the molecule. The DEPT spectra showed four quaternary carbons at δ 78.8, 51.5, 42.2 and 33.9; seven signals for methines at δ 86.7, 70.0, 66.8, 52.3, 42.6, 40.8 and 36.5; nine signals for methylenes at δ 67.8, 56.9, 51.7, 38.0, 30.9, 26.9, 23.7, 23.5 and 21.3; and two signals for methyls at δ 24.1 and 12.4.



Senbusine C **(4)**



Condelphine **(5)**

Table 1: NMR Data of acochlearine (1)

Position		^{1}H (J, Hz)	^{13}C
1	1α	1.55 dt (13, 4)	38.0 t
	1β	1.25 m	
2	2α	2.13 m	30.9 t
	2β	1.93 m	
3	3β	3.80 dd (6.5, 10)	70.0 d
4	–		33.9 s
5	5	1.87 d (8.5)	40.8 d
6	6a	1.60 m	23.7 t
	6b	2.80 dd (7, 14)	
7	7	1.50 br s	36.5 d
8	–		42.2 s
9	9	1.34 dd (8, 9)	52.3 d
10	–		51.5 s
11	11a	1.92 m	21.3 t
	11b	1.62 m	
12	12	2.14 m	42.6 d
13	13a	1.23 m	23.5 t
	13b	1.93 m	
14	14a	1.12 m	26.9 t
	14b	2.12 m	
15	15	4.00 br s	86.7 d
16	–		78.8 s
17	17a	3.51 d (11)	67.8 t
	17b	4.15 d (11)	
18	CH_3-18	0.72 s	24.1 q
19	19a	2.69 d (11)	56.9 t
	19b	2.28 d (11)	
20	20	3.88 s	66.8 d
21	21a	2.54 m	51.7 t
	21b	2.60 m	
22	CH_3-22	1.23 t (7.3)	12.4 q

Diterpenoid alkaloids usually conform to two main groups, those with a C_{19} lycoctonine/aconitine-type skeleton with characteristic methoxyl groups and those derived from a C_{20} atisine-type one with an exocyclic methylene group (Joshi and Pelletier 1999). The ^{1}H NMR spectrum of acochlearine proved the absence of methoxyl groups, so it must be an C_{20} diterpenoid alkaloid.

On the other hand the ^{1}H NMR spectrum also proved the absence of an exocyclic methylene group, but there is a little group in C_{20} alkaloids which have a vic.-diol system in the position of exocyclic methylene group (Benn et al. 1987; Joshi et al. 1987). These alkaloids show the loss of a CH_2OH unit in the MS spectrum. Acochlearine also shows this fragmentation with m/z 346 ion peak and the NMR signals (δ_C 67.8 t; δ_H 4.15, 1 H, d, J = 11 Hz and 3.51, 1 H, d, J = 11 Hz) belong to C-17 in this system. Therefore acochlearine is a C_{20} diterpenoid alkaloid with a vic.-diol system. According to the NMR signals there is an ethyl group attached to the N-atom (δ_C 12.4 q, δ_H 3 H, t, J = 7.3 Hz, N–$CH_2\underline{CH_3}$ and δ_C 51.7 t, δ_H 2.54, 1 H, m and 2.60, 1 H, m N–$\underline{CH_2}$ CH_3). There two more hydroxyls attached to carbons, which were observed at δ_C 86.7 d and 70.0 d. One of them δ_H 4.00 (1 H, br s) δ_C 86.7 d, should be located at C-15 next to the vic.-diol groups according to the HMBC couplings to C(7), C(12), C(14) and C(16) carbons. From the NMR signals (δ_C 70.0 d, δ_H 3.80, 1 H, dd, J = 6.5 and 10.0 Hz), the second hydroxyl should be placed at C-3, the correlation of H-3 with the protons at C-2 (δ_C, 30.9 t, δ_H 2.13, 1 H, m H-2 α, 1.93 1 H, m H-2β) was observed in the COSY spectrum and the couplings to C(2), C(5), C(18) and C(19) were observed in the HMBC spectrum.

The NMR data of acochlearine **(1)** are given in the Tables 1 and 2.

3. Experimental

3.1. Equipment

Optical rotations were measured on a Perkin Elmer Model 241 polarimeter. NMR spectra were recorded on a Bruker, 500 MHz spectrometer. MS were determined on a Finnigan MAT 90 spectrometer. VLC was carried out with Merck A_2O_3 (EM 1085) and SiO_2 60 G (7731). Chromatographic separations

Table 2: Summary of COSY, NOESY and HMBC correlation data of acochlearine (1)

Position	COSY	NOESY	HMBC
H-1α	H-2β	H-11	C-2, C-3,C-5, C-10, C-20
H-1β	H-2α, H-2β	H-2β	C-2, C-3, C-5, C-10, C-11
H-2α	H-1α, H-1β, H-2β	H-1α, H-1β, H-2β	C-1, C-3
H-2β	H-1β, H-2α	H-20	C-3, C-10
H-3β	H-2α, H-2β	H-2β, H-18	C-2, C-5, C-18, C-19
H-5	H-6a	H-6a, H-18	C-4, C-7, C-9,C-18, C-19, C-20
H-6a	H-6b, H-7	H-6b, H-7	C-5, C-8, C-10, C-2
H-6b	H-5, H-6a	H-5, H-6a	C-4, C-7, C-8, C-20
H-7	H-6a, H-20	H-6a, H-6b	C-6, C-9, C-14
H-9	H-11	H-11	C-5, C-11, C-15, C-20
H-11a	H-9	H-9, H-12, H-13a,	C-9, C-13, C-16
H-11b	H-9	H-14a, H-20	C-8, C-13
H-12	H-11b, H-13a, H-13b	H-11b, H-13a, H-13b	C-11, C-14, C-15, C-17
H-13a	H-12, H-13b, H-14a, H-14b	H-11, H-12, H-13b, H-14a	C-16
H-13b	H-12, H-13a, H-14a, H-14b	H-12, H-13a, H-14b, H-20	C-11, C-12, C-14
H-14a	H-13a, H-13b ,H-14b	H-7, H-11, H-13a, H-14a	C-9, C-13, C-15
H-14b	H-13a, H-13b, H-14a	H-7, H-13b, H-14b,	C-8, C-13, C-15
H-15	H-17a, H-17b	H-12, H-13b, H-14b, H-17b	C-7, C-14, C-16
H-17a	H-15, H-17b	H-12, H-17a	C-12, C-15
H-17b	H-15, H-17a	H-15, H-17b	C-12, C-15, C-16
H-18		H-3β, H-5, H-19a, H-19b	C-3, C-4, C-5,C-19
H-19a	H-19b	H-18, H-19b	C-3, C-4, C-5, C-21
H-19b	H-19a	H-6a, H-18, H-19a	C-3, C-4, C-18, C-21
H-20	H-5, H-6a, H-7	H-7, H-11, H-14a, H-22	C-5, C-6, C-19
H-21a	H-21b, H-22	H-20, H-21b	C-19, C-20, C-22
H-21b	H-21a, H-22	H-21a	C-19, C-20, C-22
H-22	H-21a, H-21b	H-20, H-21a, H-21b	C-21

on a Chromatotron were carried out on rotors coated with 1 mm thick layer of Merck Al_2O_3 60 GF-254 (1092) or SiO_2 60 PF-254 (7749). Thin layer chromatograms were run using the solvent system toluene : EtOAC : DEA (9 : 2 : 1 or 7 : 2 : 1) and $CHCl_3$: MeOH : NH_4OH (5 : 3 : 1).

3.2. Plant material

The roots (1750 g) of *Aconitum cochleare* Woroschin were collected and identified by one of us (H.Ö.) in Van, Güzeldere Pass, Turkey at an elevation of 2800 m, in June 2000. A voucher specimen has been deposited in the Herbarium of Faculty of Science and Literature, Süleyman Demirel University (No. Ozcelik 9352) Isparta, Turkey.

3.3. Extraction and isolation

The crude alkaloidal extract (35 g) obtained from 1750 g of roots was first separated by VLC on a basic SiO_2 column with PE-$CHCl_3$–MeOH mixtures. VLC fractions 8–25 with PE-$CHCl_3$ (95 : 5 to 80 : 20) (800 mg) were combined and chromatographed on a SiO_2 rotor with PE-$CHCl_3$–MeOH mixtures to give condelphine (**5**, 10 mg), talatisamine (**2**, 99 mg) and 14-*O*-acetyltalatisamine (**3**, 74 mg). VLC fraction 37 (PE-$CHCl_3$ 50 : 50) (300 mg) was chromatographed on a Al_2O_3 rotor with PE-$CHCl_3$–MeOH mixtures to give acochlearine (**1**, 21 mg) and senbusine C (**4**, 2 mg). All the known compounds were identified by comparison of their ^1H and ^{13}C NMR data and CO-TLC behavior with those of authentic samples.

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