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Two new diterpenoid alkaloids from *Aconitum cochleare*

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Two new diterpenoid alkaloids, cochleareine (**1**) and acoleareine (**2**) together with the known alkaloids 14-acetyltalatisamine (**3**) and talatisamine (**4**) have been isolated from the aerial parts of *Aconitum cochleare* Woroschin growing wild in Eastern Turkey (Van).

1. Introduction

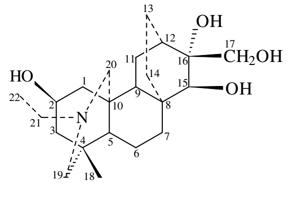
There are four *Aconitum* species (Ranunculaceae) growing wild in Turkey, *A. orientale* Mill., *A. nasutum* Fisch. et Reichb., *A. anthora* L. and *A. cochleare* Woroschin. The first three plants together with a plant from Pakistan *A. leave* Royle have been studied by our group (Ulubelen et al. 1996; Meriçli et al. 1996a, 1996b; Meriçli et al. 2000a, 2000b; Ulubelen et al. 2002). In the present study we investigated the last Turkish species namely *A. cochleare*. The plant was collected from Eastern Turkey (Van). A literature survey has revealed that *Aconitum* preparations have been used as cardiotonics, febrifugics, sedatives and anodynes for many centuries (Benn et al. 1983; Bisset 1981). However, in Turkey they are not included in folk medicine and are used only as pain relievers under physicians' control (Baytop 1984).

2. Investigations, results and discussion

The powdered plant material (526 g) was macerated with EtOH, filtered and evaporated to dryness. The crude extract was processed to obtain a crude alkaloidal mixture. This mixture was separated by centrifugally accelerated radial TLC (Chromatotron) using Al_2O_3 plates (Merck Art.1092) (Desai et al. 1985). Here we report the isolation and identification of three norditerpene and one diterpene alkaloids in the order 14-acetyltalatisamine (**3**), talatisamine (**4**) (Konno et al. 1982), acoleareine (**2**) and cochleareine (**1**).

The structures of the compounds were deduced by extensive ^1H and ^{13}C NMR studies. The unambiguous chemical shift assignments of **1** and **2** were achieved through a study of their ^1H detected by 2D NMR experiments ^1H-^1H COSY, HMQC, HMBC, and NOESY.

The first new compound, cochleareine (**1**), has a rather unusual structure. Its HRMS indicated the molecular formula $C_{22}H_{37}NO_4$ m/z 379.2658. The lack of methoxy group(s) suggested a C_{20} diterpenoid alkaloid, but the absence of typical exo-methylene signals at C-16 in its ^1H NMR and the presence of an ethyl moiety attached to the nitrogen atom were at first confusing. The ^{13}C NMR (DEPT, APT) findings showed the presence of two methyl quartets, eleven methylene triplets, five methine doublets and four quaternary singlets correlated with the presence of 22 C atoms in the molecule. The ^1H NMR spectrum was quite indicative of the structure of **1**, as δ 0.79 (3 H, s, Me-18), 1.19 (3 H, t, J = 7.0 Hz, Me-22), 4.02 (1 H, d, J = 11.5 Hz, H-17), 3.63 (1 H, d, J = 11.5 Hz, H-17′) indicated the presence of an hydroxymethylene group (CH_2OH) instead of the typical exo-methylene group at C-16, and the ^{13}C NMR signal at δ 66.56 corresponds to the C atom of CH_2OH group. There are only a few examples having a CH_2OH group at C-16, in the compounds dictysine (Joshi et al. 1987) and



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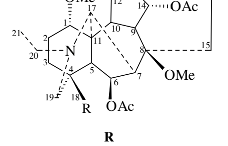
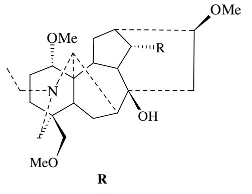


	R
2	**CH_2OMe**
5	**CH_3**



	R
3	**OAc**
4	**OH**

Table 1: NMR data of cochleareine (1) (in CD_3OD, J values (Hz) in parentheses)

	^{13}C	^{1}H	COSY	HMBC	NOESY
1	33.82 t	1.70 m	H-2α		
2α	67.38 d	3.89 dd (6.6, 11.1)	H-1α, H-1β	C-10, C-4, C-5	H-22α, H-3α
3	41.88 t	1.95 m	H-2α		H-22α, H-6α
4	37.74 s	–			
5β	35.42 d	1.70 m	H-6α, H-6β	C-18, C-6, C-9	H-11β, H-12β
6	30.70 t	1.30 m	H-5β		
7	27.27 t	1.25 m	H-6α, H-6β		
8	41.76 d	–			
9	42.98 d	2.20 d (4.9)	H-11α, H-11β		
10	52.00 s	–			
11	21.30 t	1.35 dd (8.0,17.3)	H-9, H-12		H-21β
12	41.67 d	2.06 m	H-11α, H-11β		
13	23.19 t	1.22 m 1.34 m	H-14		
14	23.79 t	2.30 m	H-13	C-12	
15α	85.01 d	3.93 s			
16	79.40 s	–			
17	66.56 t	4.02 d (11.5)			H-17′
17′		3.63 d (11.5)			H-17
18	24.96 q	0.79 s			
19	51.32 t	1.3 m			
20	57.05 t	2.50 d (12.0)		C-2, C-6	H-20′
20′		2.80 d (12.0)			H-20
21	51.94 t	2.75 dd (7.5,13.8)			H-22, H-11α
21′		2.96 dd (7.5,13.8)			
22	11.42 q	1.19 t (7.0)			

Table 2: NMR data of acoleareine (2) (in $CDCl_3$, J values (Hz) in parentheses)

	^{13}C	^{1}H	COSY	HMBC
1β	84.0 d	3.20 dd (10.0, 7.0)	H-2α, H-2β	C-6, C-17
2	27.3 t		H-1β, H-3α, H-3β	
3	37.5t		H-2α, H-2β	
4	34.1 s			
5β	56.4 d	1.40 brs	H-6α	
6α	73.4 d	5.27 t (7.0)	H-5β,H-7β	
7β	42.0 d	2.62 d (7.2)	H-6α	
8	78.5 s			
9	40.9 d			C-10
10	45.9 d			C-9, C-12
11	48.4 s			
12	27.9 t			C-9, C-13
13	39.0 d			C-12
14β	76.4 d	4.80 t (4.5)	H-13	C-16, C-10
15	35.6 t			C-16
16α	83.9 d			C-15
17	63.8 d	2.92 d (2.0)		
18	80.1 t	3.65 d (10.0)		
18′		3.50 d (10.0)		
19	57.4 t	3.04 d (12.7)		
19′		2.59 d (12.7)		
20	48.5 t	2.25 m		
21	13.4 q	0.83 t (7.0)		
OMe-1	56.0 q	3.15 s		
OMe-8	47.9 q	3.29 s		
OMe-16	56.4 q	3.19 s		
OMe-18	59.2 q	3.29 s		
OAc-6	171.0 s 22.0 q	1.99 s		
OAc-14	172.1 s 21.5 q	2.10 s		

macrocentrine (Benn et al. 1987). Other ^{1}H NMR signals were at δ 3.93 (1 H, s, H-15α), 3.89 (1 H, dd, J = 6.6 and 11.1 Hz, H-2α) indicating the presence of hydroxy groups at C-15 and C-2, and at δ 2.75 (1 H, dd, J = 7.5 and 13.8 Hz, H-21), 2.96 (1 H, dd, J = 7.5 and 13.8 Hz, H-21′), 2.50 (1 H, d, J = 12.0 Hz, H-20) and 2.80 (1 H, d, J = 12.0 Hz, H-20′). ^{13}C NMR signals showed the presence of three oxygen carrying C atoms in addition to the CH_2OH group, at δ 85.01 (d), 79.40 (s) and 67.38 (d) and these were assigned to C-15, C-16 and C-2 respectively by studying HMQC and HMBC spectral data (Table 1) as well as by comparing the chemical shifts of dictysine and macrocentrine. The stereochemistry at C-2, C-15 and C-16 was decided by NOESY studies. $^{1}H-^{1}H$ primary connectivities in the COSY spectrum and the $^{1}H-^{13}C$ correlations in the HMQC spectrum gave the structure of compound **1**. Long range $^{1}H-^{13}C$ coupling in the HMBC spectrum and $^{1}H-^{1}H$ NOE connectivities confirmed the structure of compound **1**.

The second new alkaloid, acoleareine (**2**), had a molecular formula $C_{29}H_{45}NO_8$ m/z 535.1045 correlated with the ^{13}C NMR spectrum having seven methyl quartets, seven methylene triplets, ten methine doublets and five quaternary carbon singlets. The ^{1}H NMR spectrum showed a methyl triplet at δ 0.83 (3 H, t, J = 7.0 Hz, Me-21), two acetyl methyl signals at δ 1.99 (3 H, s), 2.10 (3 H, s) and four methoxy groups at δ 3.15 (3 H, s), 3.19 (3 H, s), 3.29 (6 H, s). The acetyl groups were situated at C-6 and at C-14 as followed from ^{1}H NMR signals at δ 5.27 (1 H, t, J = 7.0 Hz, H-6α), 4.80 (1 H, t, J = 4.5 Hz, H-14β). Compound **2** resembles 14-acetylperegrine (De la Fuente et al. 1995) with the exception of the presence of a methoxymethylene group instead of a methyl group attached to C-4. The ^{1}H and ^{13}C NMR findings were quite similar to those of 14-acetylperegrine (**5**), except for the C-18 signals (see Table 2). MS degradation also showed the presence of two acetyl groups in the molecule. HMBC experiments indicated the placement of the acetyl groups at C-6 and C-14, and the ^{1}H NMR shifts were also indicative of these positions as observed in a number of examples in the literature (Kulanthaivel et al. 1986; Aiyar et al. 1986).

3. Experimental

3.1. Equipment

IR spectra were recorded on a Perkin-Elmer Model 983 spectrometer. Optical rotations were determined in an Opt. Act. Ltd. AA-5 polarimeter. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded on a Varian Mercury-Vx instrument. EIMS and HRMS were recorded on a JMS 600H spectrometer.

3.2. Plant material

The aerial parts of *A. cochleare* Woroschin were collected from Eastern Turkey (Van, Güzeldere Pass) at an elevation of 2100 m in July 2002 and identified by one of us (F.Ö.). A voucher specimen is deposited in the Herbarium of Faculty of Science and Letter, Balıkesir University FS 10947.

3.3. Extraction and isolation

The powdered plant material (526 g) was macerated with EtOH, left to stand for 48 h and filtered, this procedure being repeated 3 times. The extract was evaporated under vacuum and processed as given by Pelletier et al. (1985). A crude alkaloidal mixture (0.9 g) was obtained. Using a Chromatotron apparatus this mixture was fractioned on Al_2O_3 60 GF_{254} neutral (Typ E) radial plates (Merck Art. 1092) (Desai et al. 1985) and eluted with a gradient of petroleum ether, chloroform and methanol. By using TLC plates (Merck Art. 5554) cochleareine (**1**, 12 mg), acoleareine (**2**, 7 mg), 14-acetyltalatisamine (**3**, 25 mg) and talatisamine (**4**, 20 mg) were isolated. The known compounds were identified by comparing their ^{1}H and ^{13}C NMR data to those of authentic samples and by co-TLC behavior with standards.

3.4. Cochleareine (1)

$[\alpha]_D = -25$ ° (c 0.4, MeOH); IR ν_{max}: 3412, 2928, 2355, 1643, 1573, 1453, 1409, 1261, 1218, 1051, 802, 763. ^{1}H NMR (CD_3OD) (see Table 1), ^{13}C NMR (CD_3OD) (see Table 1), EIMS (rel. int.) at m/z 379 $[M]^+$ (3), 348 $[M\text{-}CH_2OH]^+$ (70), 319 $[348\text{-}C_2H_5]^+$ (68), 286 (72), 258 (23), 228 (17), 186 (100), 158 (12), 122 (16). HRMS: 379.2658 $C_{22}H_{37}NO_4$ (calc. 379.2654).

3.5. Acoleareine (2)

$[\alpha]_D = -42$ ° (c 0.2, MeOH); IR ν_{max}: 2930, 2854, 1730, 1461, 1377, 1259, 1160, 1110, 1093, 992, 979, 870. ^{1}H NMR ($CDCl_3$) (see Table 2), ^{13}C NMR ($CDCl_3$) (see Table 2), EIMS (rel. int.) at m/z 535 $[M]^+$ (4), 491 $[M\text{-}CH_2OCH_3 + H]^+$ (5), 462 $[491\text{-}C_2H_5]^+$ (4), 404 $[462\text{-}OAc + H]^+$ (75), 345 $[404\text{-}OAc]^+$ (10), 314 $[345\text{-}OCH_3]^+$ (15), 283 $[314\text{-}OCH_3]^+$ (11), 251 $[283\text{-}OCH_3\text{-}H]^+$ (19), 233 (21), 207 (24), 167 (28), 141 (35), 129 (51), 115 (67), 91 (100), 77 (70), 67 (65). HRMS: 535.1045 $C_{29}H_{45}NO_8$ (calc. 535.1044)

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