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STENOCARPINE, A DITERPENOID ALKALOID FROM *ACONITELLA STENOCARPA*

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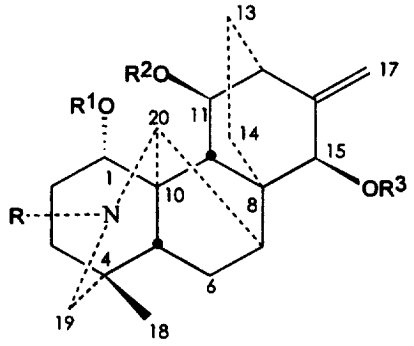
Key Word Index—*Aconitella stenocarpa*; *Consolida stenocarpa*; Ranunculaceae; diterpenoid alkaloids; lycoctonine; stenocarpine; ajaconine.

Abstract—A new diterpenoid alkaloid stenocarpine has been isolated from the epigeal parts of *Aconitella stenocarpa* along with the known alkaloids lycoctonine and ajaconine. The structure of the new alkaloid has been determined mainly by 2D NMR spectroscopy. © 1997 Elsevier Science Ltd

INTRODUCTION

Aconitella stenocarpa (Hossain and P. H. Davis) Sojak, syn. *Consolida stenocarpa* (Hossain and P. H. Davis) P. H. Davis, *Delphinium stenocarpum* Hossian and P. H. Davis, is an annual plant distributed in Central Anatolia [1]. In continuation of our work on the alkaloids of genera *Delphinium* and *Consolida* from Turkey [2–4], we report in this paper the structure determination of the diterpenoid alkaloid stenocarpine (**1**), isolated from the above-mentioned plant together with the alkaloids lycoctonine and ajaconine.



1 R = Me, R1 = R2 = R3 = H
2 R = Me, R1 = R2 = R3 = Ac
3 R = Et, R1 = R2 = R3 = H

RESULTS AND DISCUSSION

The molecular formula $C_{21}H_{31}NO_3$ of stenocarpine (**1**) was derived from its HR-mass spectrum. The ^{13}C NMR spectrum resembles that of gomandonine [5], lassiocarpine [6], 11-acetyl-1,19-epoxydenudatine [7], dictyzine [8], lepenine (**5**) and denudatine [9], suggesting that the compound is a diterpenoid alkaloid of the denudatine subtype. The NMR spectra (Tables 1 and 3) contained signals at δ_H 0.61 (3H, *s*) and δ_C 25.5 *q* for a tertiary methyl group, δ_H 2.24 (3H, *s*) and δ_C 43.2 *q* for an *N*-methyl group, δ_H 4.88 (1H, *t*, J = 1.7 Hz) and 5.10 (1H, *t*, J = 1.6 Hz) and δ_C 109.2 *t* and 153.0 *s* for an exocyclic double bond, and δ_H 3.99 (1H, *dd*, J = 11.6 and 6.6 Hz), 41.2 (1H, *br s*) and 4.25 (1H, *d*, J = 9.2 Hz) and δ_C 69.5 *d*, 72.3 *d*, and 77.3 *d* for three secondary hydroxyl groups. Acetylation of stenocarpine afforded a triacetate (**2**), which gave rise to signals at δ_H 1.96, 2.00 and 2.16 (3H each, *s*), and 4.92 *m*, 5.20 (1H, *dd*, J = 11.9 and 6.3 Hz) and 5.40 (1H, *t*, J = 2.3 Hz).

In the HMBC spectrum of stenocarpine (**1**) (Table 1) [10] the three-proton singlets at δ_H 0.61 of the angular methyl group, and 2.24 of the *N*-methyl group gave three-bond connectivities with the carbon resonances at δ_C 51.1 *d* and 58.9 *t*, and δ_C 58.9 *t* and 68.7 *d*, respectively, allowing these carbon resonances to be assigned to C-5, C-19 and C-20, respectively. The one-proton resonances at δ_H 1.21 (*d*, J = 9.2 Hz), 2.21 and 2.47 (*d* each, J = 11.7 Hz), and 3.54 *s* were later ascribed to H-5, H-19β and H-19α, and H-20, respectively, since in the HMQC spectrum (Table 1) [11] they showed one-bond correlation with the C-5, C-19 and C-20 resonances, respectively.

The one-proton signal at δ_H 3.99 (*dd*, J = 11.6 and

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Table 1. ^{1}H, HMQC and HMBC NMR data of stenocarpine (**1**)

Proton		HMQC	Correlated C-atom HMBC					
1β	3.99 *dd* (11.6, 6.6)	69.5 *d*	C-2	C-5	C-9	C-20		
2α	2.07 *m*	30.0 *t*	C-1	C-3				
2β	1.72 *m*	30.0 *t*	C-1	C-3	C-4	C-10		
3α	1.48 *ddd* (13.4, 4.4, 2.6)	37.9 *t*	C-1	C-2	C-4	C-5		
3β	1.15 *m*	37.9 *t*	C-2	C-5				
5	1.21 *d* (9.2)	51.1 *d*	C-7	C-9	C-18	C-19	C-20	
6α	1.15 *m*	22.6 *t*	C-4	C-7	C-8	C-10		
6β	2.69 *dd* (14.1, 7.8)	22.6 *t*	C-4	C-7	C-8	C-10	C-20	
7	2.15 *d* (5.4)	41.3 *d*	C-5	C-8	C-9	C-10	C-14	
9	1.21 *d* (9.2)	53.3 *d*	C-5	C-7	C-8	C-14	C-15	C-20
11α	4.25 *d* (9.2)	72.3 *d*	C-9	C-10	C-13	C-16		
12	2.04 *br s*	46.5 *d*	C-9	C-11	C-14	C-15	C-17	
13α	1.60 *ddd* (15.0, 2.8, 2.8)	24.2 *t*	C-12	C-14				
13β	1.34 *m*	24.2 *t*	C-11	C-12	C-14			
14α	1.79 *m*	27.1 *t*	C-8	C-13	C-15			
14β	0.99 *t* (11.8)	27.1 *t*	C-8	C-9	C-13	C-15		
15α	4.12 *br s*	77.3 *d*	C-9	C-14	C-16			
17e	5.10 *t* (1.6)	109.2 *t*	C-12	C-15	C-16	C-17		
17z	4.88 *t* (1.7)	109.2 *t*	C-12	C-15	C-17			
18	0.61 *s*	25.5 *q*	C-3	C-4	C-5	C-19		
19α	2.47 *d* (11.7)	58.9 *t*	C-3	C-4	C-5	C-20		
19β	2.21 *d* (11.7)	58.9 *t*	C-3	C-4	C-18	C-21		
20	3.54 *s*	68.7 *d*	C-5	C-6	C-19			
21	2.24 *s*	43.2 *q*	C-19	C-20				

* Chemical shifts in ppm rel. to TMS. Coupling constants (*J*) in Hz. C-Multiplicities were determined by DEPT data.

Table 2. Scalar and Spatial correlations of the protons of stenocarpine (**1**)

Proton	COSY	ROESY
1β	H-2α, H-2β	H-2α, H-2β, H-5, H-9
2α	H-1β, H-2β, H-3α, H-3β	H-2β, H-3α
2β	H-1β, H-2α, H-3α, H-3β	H-1β, H-2α
3α	H-2α, H-2β, H-3β	H-2α, H-3β, H-18
3β	H-2α, H-2β, H-3α	H-3α, H-18
5	H-6β	H-1β, H-6β, H-18
6α	H-6β, H-7	H-6β, H-18, H-19β
6β	H-5, H-6α	H-5, H-6α
7	H-6α	H-6α, H-14β, H-15α, H-20
9	H-11α	H-1β, H-6β
11α	H-9	H-1β, H-12, H-13α, H-14α
12	H-13α	H-11α, H-13α, H-13β, H-17e, H-17z
13α	H-12, H-13β, H-14α, H-14β	H-11α, H-12, H-13β
13β	H-13α, H-14α, H-14β	H-12, H-13α, H-14β, H-15α
14α	H-13α, H-13β, H-14β	H-14β, H-20
14β	H-13α, H-13β, H-14α	H-7, H-13α, H-13β, H-14α, H-15α
15α	H-17e, H-17z	H-7, H-13β, H-14β, H-17e, H-17z
17e	H-15α, H-17z	H-12, H-17z
17z	H-15α, H-17e	H-12, H-17e
18		H-3α, H-5, H-6α, H-19β
19α	H-19β	H-19β, H-21
19β	H-19α	H-6α, H-18, H-19α
20		H-7, H-14α

Table 3. ^{13}C NMR chemical shift assignments for compounds **1**, **2** and **3***

Carbon	1	2	3		Carbon	1	2	3	
1	69.5 *d*	73.6 *d*	68.7†	70.6‡	12	46.5 *d*	42.6 *d*	46.4†	46.8‡
2	30.0 *t*	25.9 *t*	30.6	31.1	13	24.2 *t*	23.7 *t*	24.1	34.6
3	37.9 *t*	37.8 *t*	38.5	38.6	14	27.1 *t*	26.9 *t*	27.1	27.3
4	33.7 *s*	33.4 *s*	33.0	33.7	15	77.3 *d*	77.6 *d*	76.3	77.8
5	51.1 *d*	49.5 *d*	51.9	53.8	16	153.0 *s*	146.6 *s*	153.9	154.3
6	22.6 *t*	22.1 *t*	22.8	23.0	17	109.2 *t*	109.8 *t*	107.9	109.4
7	41.3 *d*	40.9 *d*	41.4	42.9	18	25.5 *q*	25.6 *q*	26.0	25.9
8	43.6 *s*	42.9 *s*	43.0	43.6	19	58.9 *t*	58.9 *t*	56.8	57.0
9	53.3 *d*	51.9 *d*	52.8	52.3	20	68.7 *d*	69.4 *d*	66.8	67.7
10	51.1 *s*	48.1 *s*	50.3	50.9	21	43.2 *q*	43.7 *q*	50.1	50.7
11	72.3 *d*	75.1 *d*	71.7	72.9	22			13.3	13.5

* Chemical shifts in ppm rel. to TMS. Carbon multiplicities were determined by DEPT experiments. Resonances for the acetate group in **2**: 170.7 *s*, 171.0 *s*, 171.2 *s*, 21.1 *q*, 21.4 *q* and 21.6 *q*.
† DMSO-d_6 [9].
‡ $CDCl_3$ [16].

6.6 Hz) (HMQC δ_C 69.5 *d*) in the ^1H NMR spectrum of stenocarpine (**1**) which moved down-field to δ 5.02 (*dd*, J = 11.9 and 6.3 Hz) in the spectrum of the tri-acetate **2**, could be assigned to C-1βH, αOH in this class of alkaloids (Ring A chair) [5, 6]. The existence of a secondary hydroxyl group at C-1α was corroborated by the three-bond connectivities between the H-1β signal and the C-5 and C-20 carbon resonances given in the HMBC spectrum, and the spatial correlation between H-1β and H-5 observed in the ROESY spectrum of senocarpine (Table 2) [12].

The one-proton signal at δ_H 2.69 (*dd*, J = 14.1 and 7.8 Hz) was coupled with the signal at δ_H 1.21 (*d*) for H-5 in the ^1H COSY spectrum of stenocarpine (Table 2), and as the dihedral angle between H-5 and H-6α, and H-5 and H-6β are $\approx$90 and 15°, respectively, when measured on a Dreiding molecular model, the signal at δ_H 2.69 (*dd*) was assigned to H-6β. This signal also showed coupling with the one-proton signal at δ_H 1.15 (*m*), and both presented one-bond connectivity with the methylene carbon resonance at δ_C 22.6, in the HMQC spectrum. This allowed the signal at δ_H 1.15 (*m*) to be assigned to H-6α. The one-proton signal at δ_H 2.15 (*d*, J = 5.4 Hz) was then ascribed to H-7 (HMQC δ_C 41.3 *d*) because its coupling with the H-6α signal ($\approx$25° dihedral angle between H-6α and H-7, and $\approx$90° between H-6β and H-7).

In the HMBC experiment as the methylene double bond signals at δ_H 4.88 (*t*, J = 1.7 Hz) and 5.10 (*t*, J = 1.6 Hz) gave three-bond correlations with the methine carbon resonances at δ_C 46.5 and 77.3 (HMQC δ_H 4.12 *br s*), they were assigned to C-12 and C-15, respectively, which indicated that a secondary hydroxyl group is located at C-15. Inasmuch as the H-15 signal at δ_H 4.12 showed NOE, among others, with H-7 in the ROESY spectrum, H-15 must be in ψ-equatorial (α) configuration, with C-15βOH.

The following long-range connectivities were observed in the HMBC spectrum of stenocarpine: δ_H 1.72 (*m*) (H-2β from ^1H COSY and HMQC) with δ_C 33.7 *s* and 51.1 *s*, H-7 with δ_C 43.6 *s* and 51.1 *s*, and H-6α and H-6β with δ_C 33.7 *s*, 43.6 *s* and 51.1 *s*, permitted these carbon singlet resonances to be assigned to C-4, C-8 and C-10, respectively. Also the one-proton signal at δ_H 1.21 (*d*, J = 9.2 Hz) was attributed to C-9 from its three-bond correlations with the C-5, C-7, C-15, and C-20 carbon resonances. Hence the third secondary hydroxyl group was located at C-11, since in the HMBC spectrum the one-proton signal at δ_H 4.25 (*d*, J = 9.2 Hz) exhibited three-bond connectivities with the carbon singlets at δ_C 55.1 (C-10) and 153.0 (C-16). Furthermore, in the ^1H COSY spectrum the H-11 signal gave only scalar correlation with the H-9 signal, and as the dihedral angles between H-9 and H-11α, and H-9 and H-11β, are $\approx$150° and $\approx$30° respectively, H-11 must be a ψ-equatorial (α) configuration, with the C-11βOH group. The ^{13}C NMR spectrum of stenocarpine (**1**) almost matched that of lepenine (**3**) [9, 16], but in accordance with the NMR data of this work the methine carbon resonances at δ_C 41.4 and 46.4 reported for lepenine [9] must be reassigned to C-7 and C-12, respectively.

EXPERIMENTAL

General. Mps: uncorr.; OR: $CHCl_3$; EIMS and exact mass measurements: Hewlett Packard-5995 and VG-Micromass-ZAB-2F instruments, respectively, 70 eV; NMR: Bruker WP-200 SY and AMX-400 spectrometers, TMS and solvent as int. standard; DEPT, ^1HCOSY, HMQC, HMBC (J = 7 Hz) and ROESY (spin lock 700 ms): standard pulse sequences given by Bruker; CC: Alumina neutral (Merck Art. 1077) and Sephadex LH-20 Pharmacia Code 17-0090-01; TLC: Polygram Alox N (Macherey-Nagel Art. 802023). Spots on chromatograms were detected under UV light (254 nm) and with Dragendorff's reagent.

Plant material. Plants were collected between Har-

din and Kiralan, Denizli, Turkey, at an altitude of 950 m, and authenticated by Professors J. Molero and C. Blanché, Botany Laboratory, Faculty of Pharmacy, University of Barcelona, where a voucher specimen, BCF 37797, has been deposited.

Extraction and isolation. The aerial parts of plants were air-dried and ground (2.8 kg), and extracted with EtOH at room temp. After removal of EtOH under red. pres., the extract was treated with 0.5 M H_2SO_4 and filtered. The acid soln was extracted with $CHCl_3$ and then basified with 20% NaOH to pH 12 and extracted with $CHCl_3$ to give a crude alkaloidal material (5.52 g). This material was chromatographed over Al_2O_3 and eluted with EtOAc, EtOAc–MeOH (19:1) and EtOAc–MeOH (9:1) to give two main frs F_1 (310 mg) and F_2 (37 mg). CC of F_1 on Al_2O_3 using EtOAc–MeOH (49:1) led to the isolation of lycoctonine (62.3 mg) [3, 13] and stenocarpine (**1**) (77.8 mg). CC of F_2 on Sephadex LH-20 eluting with EtOAc–MeOH (9:1) gave the alkaloid ajaconine (20.1 mg) [14, 15]. The known alkaloids were identified by comparison with authentic samples (mp, IR, MS, ^1H and ^{13}C NMR).

Stenocarpine (**1**). Crystalline, mp 179–182° from hexane–EtOAc. $[\alpha]_D$ −44.3° (*c* 0.16). $[M]^+$ *m/z* 345.2297 for $C_{21}H_{31}NO_3$ (calcd 345.2304). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3525, 3490, 2995, 2990, 1650, 1600, 1490, 1460, 1405, 1375, 1280, 1175, 1100, 1065, 1050, 1030, 1005, 980, 950, 910, 860; EIMS *m/z* (rel. int.): 345 (60) $[M]^+$, 344 (33), 330 (8), 328 (43), 327 (100), 326 (13), 316 (16), 314 (5), 312 (5), 310 (11), 300 (14), 298 (16), 286 (29), 284 (9), 270 (21), 232 (10), 216 (8), 186 (7), 180 (6), 174 (8), 170 (5), 166 (5), 44 (8), 43 (11), 42 (7); ^1H (400 MHz) and ^{13}C (100 MHz) NMR ($CDCl_3$–CD_3OD, 4:1): Tables 1 and 3.

Triacetylstenocarpine (**2**). A mixt. of stenocarpine (**1**) (10 mg), pyridine (0.5 ml) and Ac_2O (0.5 ml) was kept at room temp. for 24 hr. Toluene (5 ml) was added, the solvent removed under vacuum and the reaction product was chromatographed on a short Al_2O_3 column with hexane–EtOAc (3:1) to give triacetylstenocarpine (**2**) (9.6 mg, 70%), crystallised from hexane–EtOAc, mp 98–102°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2900, 1720, 1650, 1455, 1355, 1315, 1180, 1130, 1080, 1060, 1025, 950, 900, 865; ^1H NMR (200 MHz, $CDCl_3$) δ 0.70 (3H, *s*, H-18), 1.96, 2.00, 2.16 (3H each, *s*, 3xOAc), 2.28 (3H, *s*, H-21), 2.42 (1H, *d*, 11.3 Hz, H-19α), 3.64 (1H, *s*, H-20), 4.92 (3H, *m*, H-17 and H-11α), 5.02 (1H, *dd*, J = 11.9 and 6.3 Hz, H-1β), 5.40 (1H, *t*, J = 2.3 Hz, H-15α); EIMS *m/z* (rel. int.): 471 (23) $[M]^+$, 470 (7) 429 (11), 428 (49), 414 (12), 413 (69), 412 (100), 411 (11), 384 (8), 369 (10), 368 (26), 352 (21), 324 (5), 268 (5), 252 (2), 222 (2), 210 (3), 181 (4), 167 (4) 136 (4), 108 (6), 91 (7), 71 (10), 57 (20), 55 (14), 44 (20), 43 (54); ^{13}C NMR (50 MHz, $CDCl_3$): Table 3.

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