Institute for Natural Products Research[1] and the Department of Chemistry,[2] Chemical Sciences NMR Facilities,[3] The University of Georgia, Athens, Georgia, USA., Department of Pharmacognosy,[4] Department of General Chemistry,[5] Faculty of Pharmacy, University of Istanbul, Istanbul, Turkey. Department of Biology, Faculty of Sciences, University of Ankara,[6] Ankara, Turkey

Diterpenoid alkaloids from the aerial parts of *Aconitum anthora* L.

A. H. Meriçli[1,4], F. Meriçli[1,4], A. Ulubelen[5], M. Bahar[4], R. Ilarslan[6], G. Algül[6], H. K. Desai[1], Q. Teng[3], and S. W. Pelletier[1,2]

Isolation and identification of six diterpenoid alkaloids, from *Aconitum anthora* L. are described. All proton detected 2D NMR techniques have been used for unambiguous ^{1}H and ^{13}C chemical shift assignments of guan-fu-base-Y (**6**). This is the first investigation of the diterpenoid alkaloids of this plant species.

1. Introduction

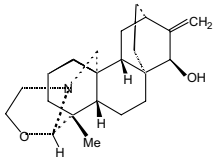
The medicinal uses of *Aconitum* and *Delphinium* species (Ranunculaceae) span many centuries. *Aconitum* preparations have been used as cardiotonics, febrifugies, sedatives, and anodynes [1, 2]. *Delphinium* extracts have been employed in analgesic balms and also as sedatives, emetics and anthelmintics [1, 2]. In our continuing work in isolating new diterpenoid alkaloids from *Aconitum* and *Delphinium* species, we have developed various methods for rapid isolation of pure alkaloids [3–6]. Testing of the pure diterpenoid alkaloids and their derivatives for the cardiovascular (hypotensive and bradycardic) action has also been conducted [7, 8].

2. Investigations, results and discussion

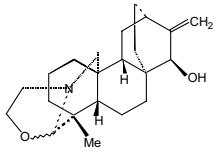
In the present work we have investigated the diterpenoid alkaloids from the aerial parts of *Aconitum anthora* L. (Ranunculaceae), collected in Ardahan-Çildir, Turkey, at an altitude of 2000 m. This is the first report of the investigation of the chemistry of alkaloids from *A. anthora* L. Dried and powdered aerial parts of *A. anthora* (400 g) were extracted with ethanol and the concentrated extract was processed [9] to give 1.14 g of the crude alkaloidal mixture. By a combination of vacuum liquid chromatography (VLC) [4], centrifugally accelerated radial TLC (Chromatotron) [5], and PTLC, we report the isolation and identification of six diterpenoid alkaloids: isoatisine (**1**), 19-*epi*-isoatisine (**2**), a mixture of 20_{α} and 20_{β}-atisines (**3**), hetisine (**4**), isotalatizidine (**5**) and guan-fu-base-Y (**6**). These alkaloids were identified through their NMR spectra (^{1}H, ^{13}C, DEPT experiments), ESI MS and comparison of data with those reported in the literature.

The unambiguous chemical shift assignments for **6** were carried out through a study of its all ^{1}H detected 2D NMR experiments (^{1}H-^{1}H COSY, TOCSY, ROESY, HMQC, HMBC and HMQC-TOCSY) (Tables 1 and 2) and are in agreement with structure **6.** Guan-fu-base-Y (**6**) is a rare diterpenoid alkaloid and so far, has been reported in only two other *Aconitum* species [10, 11].

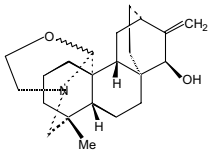
Assignments of ^{13}C and ^{1}H chemical shifts (Tables 1 and 2) were accomplished using the two-stage method [12]. In the first stage, the segments were established by the spin systems identified on the basis of scalar coupling in TOCSY (total correlation spectroscopy) [13] and HMQC-TOCSY [14] spectra. The sequence-specific assignments of ^{1}H and ^{13}C within a segment were obtained by the ^{1}H-^{1}H primary connectivities in the COSY [15] spectrum and the ^{1}H-^{13}C correlations in the HMQC [16] spectrum. Then, the overall structure was obtained by the long-range ^{1}H-^{13}C coupling between the segments in the HMBC [17]



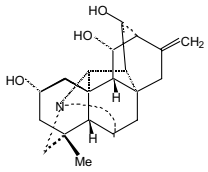
1 ISOATISINE



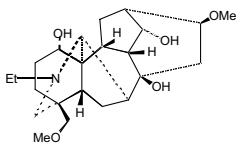
2 19-*EPI* -ISOATISINE



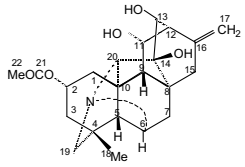
3 ATISINE



4 HETISINE



5 ISOTALATIZIDINE



6 GUAN-FU-BASE-Y

Table 1: ^{13}C ($CDCl_3$), Chemical shift assignments and their HMBC correlation of 6

C #	$\delta^{13}C$	HMBC
1	30.96 (t)	H-2, H-3_{α}, H-3_{β}, H-20, H-5, H-9
2	69.33 (d)	H-1_{α}, H-1_{β}, H-3_{α}, H-3_{β}
3	36.30 (t)	H-2, H-1_{α}, H-1_{β}, H-19a, b, H-18
4	36.91 (s)	H-2, H-5, H-18, H-19
5	59.05 (d)	H-7, H-3_{α}, H-3_{β}, H-9, H-18, H-19a, b
6	63.48 (d)	H-5, H-7, H-9, H-18, H-19a,b, H-20
7	31.39 (t)	H-9, H-$15_{\alpha,\beta}$
8	44.06 (s)	H-6, H-7, H-9, H-$15_{\alpha,\beta}$, H-20
9	53.40 (d)	H-5, H-7, H-11, H-12
10	46.38 (s)	H-1_{α}, H-1_{β}, H-2, H-6, H-9, H-11
11	75.59 (d)	H-12, H-13, H-$15_{\alpha,\beta}$
12	51.71 (d)	H-9, H-11, H-13, H-$15_{\alpha,\beta}$, H-$17_{a,b}$
13	80.18 (d)	H-9, H-11, H-12, H-$17_{a,b}$
14	80.20 (s)	H-7, H-9, H-12, H-$15_{\alpha,\beta}$, H-20
15	30.74 (t)	H-7, H-9, H-12, H-$17_{a,b}$
16	144.14(s)	H-11, H-12, H-13, H-$15_{\alpha,\beta}$, H-$17_{a,b}$
17	108.32 (t)	H-12, H-$15_{\alpha,\beta}$
18	29.54 (q)	H-3, H-5, H-19a, b,
19	62.00 (t)	H-3_{α}, H-3_{β}, H-5, H-20
20	69.33 (d)	H-1_{α}, H-1_{β}, H-5, H-6, H-13, H-19a, b
21	170.48(s)	H-2, H-22
22	21.68 (q)	–

Chemical shifts in ppm downfield from TMS.
s = singlet (quaternary carbon), d = doublet (CH), t = triplet (CH_2), q = quartet (CH_3).

Table 2: ^{1}H NMR Chemical shift[a] assignments of 6 and their COSY and ROESY correlations

Obs.^{1}H	(δ_H) (J, Hz)	COSY	ROESY
H-1$_\alpha$	2.95 d (15.5)	H-1$_\beta$, H-2	H-1$_\beta$, H-2, H-3, H-20
H-1$_\beta$	1.99 m	H-1$_\alpha$, H-2	H-1$_\beta$, H-5
(overlap H-3$_\alpha$)			
H-2	5.14 br s	H-1$_\alpha$ H-1$_\beta$ H-3$_\alpha$, H-3$_\beta$	H-1$_\beta$, H-3a, H-5
H-3$_\alpha$	1.87 m	H-2, H-3$_\beta$	H-2, H-3$_\beta$, H-18, H-19
H-3$_\beta$	1.58 dd (15.5, 4.5)	H-2, H-3$_\alpha$	H-3$_\alpha$, H-5, H-18
H-5	1.67 s	H-6	H-1$_\beta$, H-2, H-3$_\beta$, H-6, H-9, H-18
H-6	3.39 br m	H-5, H-7$_{\alpha b}$, H-20	H-5, H-7, H-9, H-18, H-19$_b$
H-7$_\alpha$	1.87 m	H-6, H-7$_\beta$	H-7$_\beta$
H-7$_\beta$	1.43 dd (14.0,2.3)	H-6, H-7$_\alpha$	H-6, H-7$_\alpha$, H-9
H-9	2.02 m	H-11	H-6, H-7$_\beta$
H-11	4.21 d (9)	H-9, H-12	H-12, H-15$_\alpha$
H-12	2.51 br m	H-11, H-13	H-11, H-13, H-17$_b$
H-13	4.10 br m	H-12	H-12, H-15$_\alpha$
H-15$_\alpha$	2.03 m	H-17$_a$, H-17$_b$	H-11, H-12, H-13
H-15$_\beta$	1.98 m	H-17$_a$, H-17$_b$	–
H-17$_a$	4.88 br s	H-15$_{\alpha,\beta}$, H-17$_b$	H-12, H-15$_\beta$, H-17$_b$
H-17$_b$	4.69 br s	H-15$_{\alpha,\beta}$, H-17$_\alpha$	H-15$_{\alpha,\beta}$, H-17$_b$
H-18	1.06 s	–	H-6, H-19$_b$
H-19$_a$	3.19 d (12.0)	H-19$_b$	H-3$_\alpha$, H-19$_b$, H-20
H-19$_b$	2.73 d (12.0)	H-19$_a$	H-6, H-18, H-19$_a$
H-20	3.87 s	H-6	H-1$_\alpha$, H-19$_a$
H-22	2.07 br s	–	–

[a] Chemical shifts of overlapping peaks estimated from HMQC spectrum.

spectrum and the inter-segment ^{1}H-^{1}H NOE connectivities in the ROESY [18] spectrum, summarized in Table 2. HMQC data were acquired with GARP [19] decoupling during acquisition. Quadrature detection in the indirectly observed dimensions was obtained using TPPI [20] (time proportional phase increment).

Twenty-two carbons were observed using the 1D ^{1}H-decoupled ^{13}C, DEPT and 2D HMQC spectra, which were determined by their characteristic ^{1}H and ^{13}C chemical shift patterns as two methyl, six methylene, eight methine and six quaternary carbons. Inspection of the HMQC-TOCSY spectrum, shown (Fig.) reveals six segments, one isolated methine, methylene and a methyl group. Further inspection of the TOCSY spectrum locates a pair of weak cross peaks along the resolved proton at 4.21 ppm, which connects segments 3 and 4. The primary ^{1}H-^{1}H and ^{1}H-^{13}C correlations within the above four spin systems were obtained using the COSY and HMQC spectra, re-

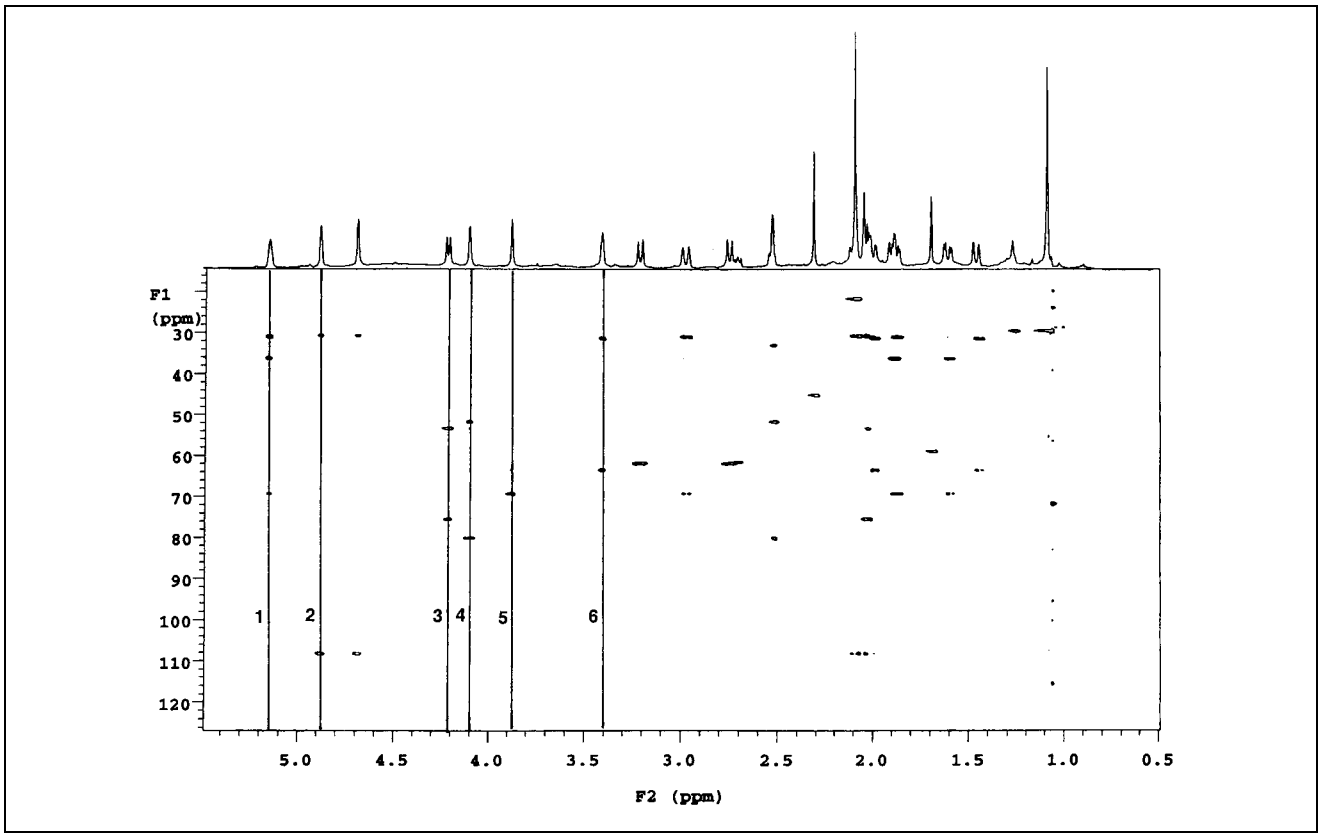


Fig.: HMQC-clean-TOCSY spectrum of **6**. The identified spin systems are labeled by vertical lines.

spectively. The assignments for ^1H signals were initiated at the resolved ^1H resonances such as CH protons of 5.14, 4.21, 4.10 and 3.39 ppm, CH_2 protons of 4.88 and 4.69 ppm.

Starting at the CH resonance frequency of 5.14 ppm, the TOCSY and HMQC-TOCSY spectra showed that this methine scalar-coupled to two pair of CH_2 protons at 2.95, 1.99 ppm and 1.87, 1.58 ppm (segment 1 in the Fig.). Thus, the three carbons were assigned to C-2, C-1 and C-3 (69.34, 30.96 and 36.30 ppm), respectively. The H-3 proton at 1.87 ppm showed ROESY connectivity to one of the isolated CH_2 protons (3.21 ppm) which was assigned as H-19. H-2 has ^1H-^{13}C couplings in the HMBC spectrum to quaternary carbons at 170.5 and 36.9 ppm which were assigned to C-21 and C-4, respectively. C-4 also has long range ^1H-^{13}C couplings to H-2, H-3 and to all four protons in segment 6 which were assigned to H-5, H-6, and H-7α, H-7β.

H-6 has long range ^1H-^{13}C couplings to two quaternary carbons at 46.38 and 44.06 ppm. The quaternary carbon at 46.38 ppm was assigned as C-10 because it showed long-range ^1H-^{13}C couplings to H-1, H-2, H-3 protons. The carbon with resonance at 44.06 ppm was assigned to C-8. This carbon gives long-range ^1H-^{13}C couplings with the protons in segment 2, which were assigned as H-15 (2.03, 1.98 ppm) and H-17 (4.88, 4.69 ppm), respectively. C-16 was located by the ^1H-^{13}C couplings from H-17 and H-15 protons, which also shows HMBC couplings to protons of segments 3 and 4. The protons in position 9 to 13 were assigned based on COSY connectivities within the segments and ^1H-^{13}C couplings from these protons to C-16, C-10 carbons. C-14 quaternary carbon was determined according to the ^1H-^{13}C couplings from this carbon to H-17a,b, H-12, H-13, H-15, to H-17a,b, and H-20.

3. Experimental

Melting points are corrected and were determined on a Thomas-Koffler hot stage equipped with a microscope and a polarizer. HRMS were determined on a Fisons Auto Spec ETOFFPD FAB^+ mass spectrometer. 1D NMR spectra including DEPT were recorded on a Bruker AC-300 spectrometer. All ^1H detected 2D NMR data were acquired at 25° C on a Varian Inova 500 spectrometer (499.8 MHz ^1H). Chromatographic separations on a Chromatotron [5] were carried out on rotors coated with 1 mm layers of Merck Al_2O_3 60 PF 254, 365 (EM 1104) or 1 mm layers of SiO_2 (EM 7749).

Aerial parts (400 g dry wt.) of *Aconitum anthora* L. were collected by G. Akgül in Ardahan-Çildir, Turkey, at an elevation of 2000 m, in August 1998. The plant was identified by R. Ilarslan and a voucher specimen is deposited in the Herbarium of the Faculty of Sciences, Ankara University (No. Ilarslan 1749), Ankara, Turkey.

Extraction of the powdered plant material with EtOH and processing the concentrated extract for the isolation of alkaloids [9] yielded 1.14 g of the crude alkaloidal mixture. This mixture was first fractionated on a VLC [4] column (90 g, Al_2O_3 EM 1085) eluting with a solvent gradient of hexane-$CHCl_3$ and MeOH. Twenty five fractions (100 mL each) were collected and the fractions thus obtained were combined on the basis of their TLC similarity. Further fractionations, of the combined fractions, were carried out on Chromatotron [5] rotors. VLC fractions 5–11 (200 mg, eluted with hexane- $CHCl_3$, 60 : 40 to 100% $CHCl_3$) on further fractionation on a basic alumina rotor of a Chromatotron, eluting with a gradient of hexane, $CHCl_3$ and MeOH gave isoatisine (**1**, 25 mg). Similarly VLC fractions 12–15 (80 mg, $CHCl_3$/MeOH, 0.5 : 99.5 to 3 : 97%, SiO_2 rotor); 16–18 (250 mg, $CHCl_3$/MeOH, 4 : 96 to 10 : 90%, SiO_2 rotor); and 19–21 (146 mg, $CHCl_3$/MeOH, 20; 80 to 30 : 70%, SiO_2 rotor) gave respectively, 19-*epi*-isoatisine (**2**, 9 mg), 20_α and 20_β-atisine mixture (**3**, 54 mg), hetisine (**4**, 15 mg), isotalatizidine (**5**, 22 mg) and guan-fu-base-Y (**6,** 43 mg).

Compound **6** was obtained as colorless crystals (acetone) mp 217–219 °C. HRFABMS m/z 388.21239 $[M+H]^+$ for $C_{22}H_{29}NO_5$. All ^1H detected 2D NMR experiments were carried out on this sample (15 mg in $CDCl_3$).

Acknowledgment: The authors thank NATO for a Collaborative Research Grant (CRG 931261). The Turkish authors also thank the University of Istanbul Research Fund for the Grant No. UP-2-150197.

References

1 Benn, M. H.; Jacyno, J. M.; Pelletier, S. W. (Ed.): Alkaloids: Chemical and Biological Perspectives, vol. 1, p. 153, John Wiley & Sons, New York 1983
2 Bisset, N. G.: J. Ethnopharmacol. **4**, 247 (1981)
3 Pelletier, S. W.: Pure & Appl. Chem. **69**(1), 119 (1997)
4 Pelletier, S. W.; Chokshi, H. P.; Desai, H. K.: J. Nat. Prod. **49**, 892 (1986)
5 Desai, H. K.; Trumbull, E. R.; Pelletier, S. W.: J. Chromatogr. **366**, 439 (1986)
6 Srivastava, S. K.; Desai, H. K.; Vobalaboina, V.; Pelletier, S. W.: J. Liq. Chrom. & Rel. Techno. **22**, 1687 (1999)
7 Chiao, H.; Pelletier, S. W.; Desai, H. K.; Rabagay, N. R.; Caldwell, R. W.: Eur. J. Pharmacol. **283**, 103 (1995)
8 Desai, H. K.; Hart, B. P.; Caldwell, R. W.; Huang, J.; Pelletier, S. W.: J. Nat. Prod. **61**, 743 (1998)
9 Pelletier, S. W.; Joshi, B. S.; Desai, H. K.: Vlietinck, A. J.; Dommisse, R. A. (Ed): in Advances in Medicinal Plant Research, p. 153, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart 1985
10 Reinecke, M. G.; Minter, D. E.; Chen, D. C.; Yan, W. M. Tetrahedron, **42**, 6621 (1986)
11 Bessonova, I. A.; Yunusov, M. S.; Kondratev, V. G.; Shreter, A. S. Khim. Prir. Soedin., 690 (1987)
12 Meriçli, A. H.; Meriçli, F.; Seyhan, V.; Ulubelen, A.; Desai, H. K.; Joshi, B. S., Teng, Q.; Pelletier, S. W.: Heterocycles **45**, 1955 (1997)
13 Bax, A.; Davis, D. G.: J. Magn. Reson. **65**, 355 (1985)
14 Lerner, L.; Bax, A.: J. Magn. Reson. **69**, 375 (1986)
15 Bax, A.; Subramanian. S.: J. Magn. Reson. **67**, 565 (1986)
16 Bax, A.; Griffey, R. H. and Hawkins, B. L.: J. Magn. Reson. **55**, 301 (1983)
17 Bax, A.; Summers, M. F.: J. Am. Chem. Soc. **108**, 2093 (1986)
18 Bax, A.; Davis, D. G.: J. Magn. Reson. **63**, 207 (1985)
19 Shaka, A. J.; Barker. P. B.; Freeman, R.: J. Magn. Reson. **64**, 547 (1985)
20 Marion, D.; Wüthrich, K.: Biochem. Biophys. Res. Comm. **113**, 967 (1983)

Received November 12, 1999
Accepted February 12, 2000

Prof. Dr. S. W. Pelletier
Institute for Natural Products Research
Chemistry Building
The University of Georgia
Athens, GA 30602–2556
USA