A New Diterpenoid Alkaloid from the Roots of a White-Flowering *Aconitum orientale* Sample

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From the roots of a white-flowering *Aconitum orientale* MILLER sample, collected in Artvin-Şavşat, Turkey, a new diterpenoid alkaloid named aconitorientaline (**1**) was isolated, along with the known diterpenoid alkaloids septentiriodine, lappaconitine, finaconitine, ranaconitine, puberanidine and delstaphinine (*Fig.*). The structure of **1** was established on the basis of ^{1}H- and ^{13}C-NMR, DEPT, ^{1}H,^{1}H-COSY, NOESY, HSQC, and HMBC studies. All the known compounds were identified by comparison of their ^{1}H- and ^{13}C-NMR data and co-TLC behavior with those of authentic samples.

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, febrifuges, sedatives, and anodynes. Today, *Aconitum* is very popular in homoeopathy and is included in many pharmaka [1–3]. In continuation of our investigations on Turkish *Aconitum* species [4–10], we now report the presence of a new norditerpenoid alkaloid isolated from the roots of a white-flowering *Aconitum orientale* sample. There are two *Aconitum orientale* samples with either white or pale-purple flowers that grow wild in Turkey. Some authors believe that they are different species [11].

The chemical investigation of the roots of *A. orientale* led to the isolation of a new diterpenoid alkaloid, aconitorientaline (**1**), together with septentriodine (**2**), lappaconitine (**3**), finaconitine (**4**), ranaconitine (**5**), puberanidine (**6**), and delstaphisinine (**7**) (*Fig.*) [12–17]. Among these alkaloids lappaconitine (**3**), ranaconitine (**5**), and puberanidine (**6**) have been isolated several times before from *A. orientale* samples [18–21].

Results and Discussion. – A novel diterpenoid alkaloid, designated as aconitorientaline (**1**) was isolated from the roots of *A. orientale* collected at an altitude of 2400 m on Yalnızçam Mountain, Artvin-Şavşat, Turkey. The molecular formula of **1** was determined to be $C_{25}H_{39}NO_7$ by the quasi-molecular-ion peak at m/z 466.27903 ($[M+H]^+$) in the HR-ESI-MS and confirmed by the ^{1}H- and ^{13}C-NMR and DEPT

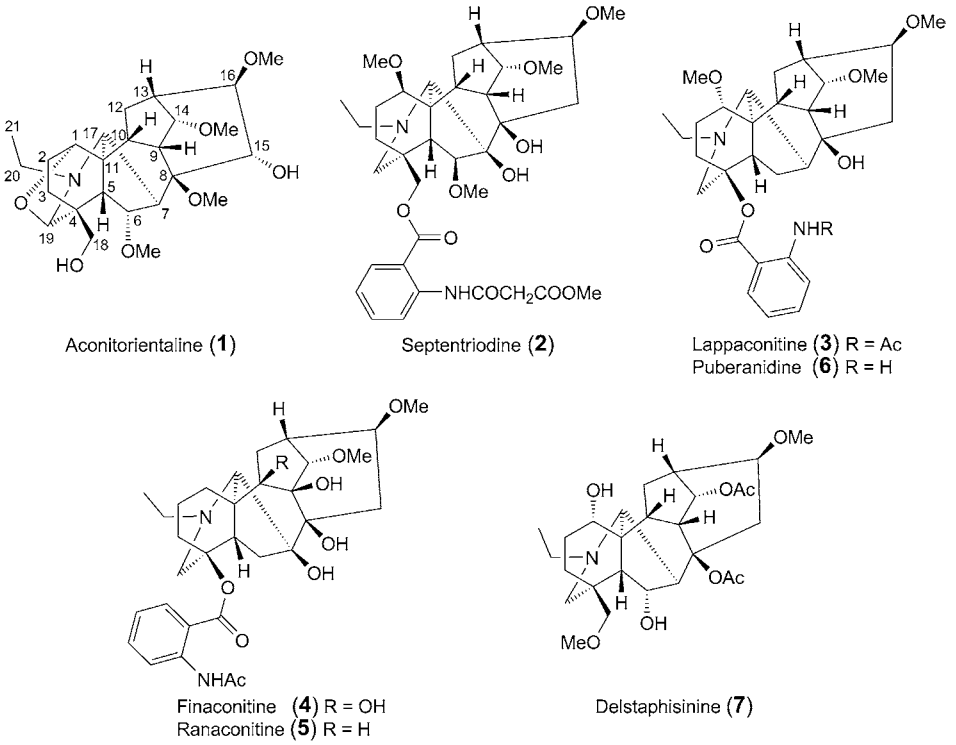

Figure. *Compounds isolated from* Aconitum orientale MILLER

data (*Table 1*). The IR spectrum showed an OH-group absorption at 3365 cm^{-1} but no aromatic absorptions. A ^{13}C-NMR spectrum confirmed the 25 C-atoms of the molecule. The ^{13}C-NMR and DEPT spectra established the presence of three quaternary C-atoms at δ(C) 79.1, 48.1, and 39.2, twelve CH groups at δ(C) 86.4, 85.4, 82.4, 81.8, 79.8, 68.9, 68.3, 46.9, 44.5, 41.8, 41.5, and 40.0, five CH_2 groups at δ(C) 65.8, 48.2, 32.0, 29.9, and 29.0, and five Me groups at δ(C) 57.9, 57.0, 55.7, 55.5, and 12.2. Diterpenoid alkaloids usually belong to two main groups, those with a C_{19} lycoctonine/aconitine-type skeleton with characteristic MeO groups and those derived from a C_{20} atisine-type skeleton with an exocyclic CH_2 group [22]. The ^{1}H-NMR spectrum of aconitorientaline (**1**) suggested the presence of four MeO groups, and according to the NMR signals it should be an aconitin-type C_{19} norditerpenoid alkaloid carrying an Et group attached to the N-atom (*Me*CH_2N at δ(C) 12.2 (*q*) and δ(H) 1.12 (*t*, $J = 7$ Hz) and Me*C*H_2N at δ(C) 48.2 (*t*) and δ(H) 2.44–2.46 and 2.58–2.61 (2*m*)) [9]. There were eight O-bearing C-atoms present, four of them being MeO groups. The MeO groups (δ(H) 3.20, 3.32, 3.35, and 3.40 (each *s*); δ(C) 55.5, 55.7, 57.0, and 57.9 (each *q*)) could be positioned at C(16) (δ(H) 3.75 (*dd*, $J = 7, 12$ Hz); δ(C) 86.4 (*d*)), at C(14) (δ(H) 3.59 (*t*, $J = 5$ Hz); δ(C) 82.4 (*d*)), at C(6) in α-position (δ(H) 4.14 (*dd*, $J = 1, 6$ Hz); δ(C) 81.8 (*d*)), and at C(8) (δ(C) 79.1 (*s*)), respectively [15][23][24], however the chemical shift of C(16) was slightly more downfield than expected. The molecular mass at *m*/*z* 465 suggested that the other O-

bearing C-atoms should carry two OH groups and an epoxy group since the compound did not contain any acetyl group. If the C_{19} norditerpenoid alkaloids contain an epoxy group, it is generally located between C(1) and C(19) [17][25][26]. Compared to those compounds, the chemical shifts and HMBC cross-peaks of **1** (*Table 2*) suggested the same position of the epoxy group H–C(1) at δ(H) 3.71–3.69 (*m*) and δ(C) 85.4 (*d*)); H–C(19) at δ(H) 3.58 (*s*) and δ(C) 68.9 (*d*)). One of the remaining two OH groups was positioned at C(18) (δ(H) 3.52 and 3.32 (2*d*, each $J = 10$, CH_2(18)), δ(C) 65.8 (*t*)) [27]. The last OH group could be at C(2), C(3), C(12), or C(15). The positions C(2), C(3), and C(12) were excluded because of their chemical shifts, the better argument of attaching it to C(15) being the chemical shift of C(15) and its slight difference with that of C(16), compared to the other possible alternatives; thus the last OH group was in α-position at C(15) (δ(H) 4.35 (*d*, $J = 6$ Hz); δ(C) 79.8 (*d*)) [28]. The HMBC results also certified this interpretation.

Table 1. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; $CDCl_3$) *of Aconitorientaline* (**1**) *and ^{13}C-NMR Data* (125 MHz, $CDCl_3$) *of Delstaphisinine* (**7**). δ in ppm, *J* in Hz.

	1		**7**
	δ(H)	δ(C)	δ(C)
H–C(1)	3.69–3.71 (*m*)	85.4 (*d*)	71.9 (*d*)
CH_2(2)	1.72–1.74 (*m*, H_α), 1.65–1.67 (*m*, H_β)	29.9 (*t*)	27.8 (*t*)
CH_2(3)	1.74–1.77 (*m*, H_α), 2.43–2.46 (*m*, H_β)	32.0 (*t*)	30.7 (*d*)
C(4)	–	39.2 (*s*)	37.8 (*s*)
H–C(5)	1.90–1.92 (*m*)	44.5 (*d*)	45.4 (*d*)
H–C(6)	4.14 (*dd*, $J = 1, 6$)	81.8 (*d*)	73.2 (*d*)
H–C(7)	2.25 (*d*, $J = 1$)	41.8 (*d*)	44.7 (*d*)
C(8)	–	79.1 (*s*)	85.2 (*s*)
H–C(9)	1.79–1.82 (*m*)	46.9 (*d*)	45.1 (*d*)
H–C(10)	1.61–1.65 (*m*)	40.0 (*d*)	38.9 (*d*)
C(11)	–	48.1 (*s*)	49.2 (*s*)
CH_2(12)	2.29–2.31 (*m*, H_a), 1.61–1.65 (*m*, H_b)	29.0 (*t*)	29.7 (*t*)
H–C(13)	2.39–2.42 (*m*)	41.5 (*d*)	43.1 (*d*)
H–C(14)	3.59 (*t*, $J = 5$)	82.4 (*d*)	75.5 (*d*)
H–C(15)	4.35 (*d*, $J = 6$)	79.8 (*d*)	38.3 (*t*)
H–C(16)	3.75 (*dd*, $J = 7, 12$)	86.4 (*d*)	82.0 (*d*)
H–C(17)	2.87 (*s*)	68.3 (*d*)	65.2 (*d*)
CH_2(18)	3.52 (*d*, $J = 10$, H_a), 3.32 (*d*, $J = 10$, H_b)	65.8 (*t*)	79.8 (*d*)
H–C(19)	3.58 (*s*)	68.9 (*d*)	56.8 (*t*)
CH_2(20)	2.58–2.61 (*m*, H_a), 2.44–2.46 (*m*, H_b)	48.2 (*t*)	48.1 (*t*)
Me(21)	1.12 (*t*, $J = 7$)	12.2 (*q*)	13.1 (*q*)
*Me*O–C(6)	3.20 (*s*)	55.5 (*q*)	–
*Me*O–C(8)	3.40 (*s*)	57.9 (*q*)	–
*Me*O–C(14)	3.35 (*s*)	57.0 (*q*)	–
*Me*O–C(16)	3.32 (*s*)	55.7 (*q*)	56.5 (*q*)
*Me*O–C(18)	–	–	59.1 (*q*)
*Ac*O–C(8)	–	–	22.0 (*q*), 169.5 (*s*)
*Ac*O–C(14)	–	–	21.0 (*q*), 170.6 (*s*)

Table 2. *$^1H,^1H$-COSY, NOESY, and HMBC Features of Aconitorientaline* (**1**)

	COSY	NOESY	HMBC
H–C(1)	H_α–C(2), H_β–C(2)	H_α–C(2), H_β–C(2), H–C(10), H_b–C(12)	C(3), C(10)
H_α–C(2)	H–C(1), H_α–C(3), H_β–C(3)	H–C(1), H_β–C(3)	C(5), C(10)
H_β–C(2)	H_α–C((2)	H–C(1), H–C(5)	C(5), C(10)
H_α–C((3)	H_α–C(2), H_β–C(2), H_β–C(3)	–	–
H_β–C(3)	H_α–C(2), H_β–C(2), H_α–C(3)	H_α–C(2), H_b–C(18)	C(1), C(2), C(19)
H–C(5)	H–C(6)	H_β–C(2), H–C(6), H–C(9), H_a–C(18), H–C(19)	C(17), C(18), C(19)
H_β–C(6)	H–C(5)	H–C(5), H–C(7), H_a–C(18), H_b–C(18), *Me*O–C(6)	*Me*O–C(6)
H–C(7)	H–C(6), H–C(17)	H–C(6), H–C(15), H–C(17), H–C(19), *Me*O–C(6)	C(9), C(17)
H–C(9)	H–C(10), H–C(14)	H–C(5), H–C(10), H_a–C(12), H–C(14)	C(7), C(12), C(13), C(14), C(16)
H–C(10)	H–C(9), H_b–C(12)	H–C(1), H–C(9), H_b–C(12), H–C(14), *Me*O–C(16)	–
H_a–C(12)	H_b–C(12), H–C(13)	H_b–C(12), H–C(13), H–C(14)	–
H_b–C(12)	H–C(10), H_a–C(12)	H–C(1), H_a–C(12), H–C(13), H–C(16), H–C(17)	C(14), C(16)
H–C(13)	H_a–C(12), H–C(14)	H_a–C(12), H_b–C(12), H–C(14), H–C(16), *Me*O–C(16)	C(14), C(15), C(16)
H–C(14)	H–C(9), H–C(13)	H–C(9), H–C(10), H_a–C(12), H–C(13)	C(16), *Me*O–C(14)
H–C(15)	H–C(16)	–	C(7), C(16)
H–C(16)	H–C(15)	H–C(7), H_b–C(12), H–C(13), *Me*O–C(16), H–C(17)	C(12), C(14), *Me*O–C(16)
H–C(17)	H–C(7)	H–C(7), H_b–C(12), H–C(16), H_a–C(20), H_b–C(20), Me(21)	C(5), C(6), C(10), C(19)
H_a–C(18)	H_b–C(18)	H–C(5), H–C(6), H_b–C(18), *Me*O–C(6)	C(3), C(19)
H_b–C(18)	H_a–C(18)	H_β–C(3), H–C(6), H_a–C(18), H–C(19)	C(3), C(5), C(19)
H–C(19)	–	H–C(6), H–C(7), H_a–C(18), H_b–C(18), H_a–C(20), H_b–C(20)	C(3), C(5), C(17)
H_a–C(20)	H_b–C(20), Me(21)	H–C(17), H_b–C(20), Me(21)	C(17), C(19), C(21)
H_b–C(20)	H_a–C(20), Me(21)	H–C(17), H_a–C(20), Me(21)	C(17), C(19), C(21)
Me(21)	H_a–C(20), H_b–C(20)	H–C(17), H_a–C(20), H_b–C(20)	–
*Me*O–C(6)	–	H_a–C(18)	C(6)
*Me*O–C(8)	–	–	C(8)
*Me*O–C(14)	–	H–C(15)	C(14)
*Me*O–C(16)	–	H–C(13), H–C(10), H–C(16)	C(16)

The structural significance of aconitorientaline (**1**) is to carry an OH group at C(15) and an epoxy group between C(1) and C(19), as established by its NMR data (*Tables 1* and *2*) and comparison with the ^{13}C-NMR data of delstaphisinine (**7**).

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Experimental Part

General. Vacuum liquid chromatography (VLC): *Merck* Al_2O_3 (*EM 1085*) and SiO_2 *60 G* (*7731*). CC = Column chromatography. Chromatographic separations on a chromatotron were carried out on rotors coated with a 1 mm thick layer of *Merck* Al_2O_3 *60 GF*$_{254}$ (*1092*) or SiO_2 *60 PF*$_{254}$ (*7749*). TLC: toluene/AcOEt/Et_2NH 7:4:1 or 7:4:2, $CHCl_3$/MeOH/$NH_3 \cdot H_2O$ 5:3:1, and toluene/acetone/MeOH/$NH_3 \cdot H_2O$ 49.5:41.5:5.5:1.5. Optical rotations: *Perkin–Elmer-241* polarimeter. IR Spectra: *Perkin–Elmer-100* FT-IR spectrometer; in $CHCl_3$; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Varian-Unity-Inova* 500 MHz spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. MS: *Finnigan-MAT-90* spectrometer; in *m*/*z*.

Plant Material. The roots of *Aconitum orientale* MILLER (Ranunculaceae) were collected and identified by one of us (*H. Ö.*) on Yalnızçam Mountain, Artvin-Şavşat, Turkey, at an elevation of 2400 m, in July 2006. A voucher specimen has been deposited with the Herbarium of the Faculty of Science and Literature, Süleyman Demirel University (No. Özçelik 12565) Isparta, Turkey.

Extraction and Isolation. Dried and powdered roots (400 g) were extracted with 90% EtOH by percolation at r.t., and the extracts obtained were concentrated. The residues were treated with 0.5N H_2SO_4 and extracted with $CHCl_3$. NaOH soln. (5%) was then added to the aq. soln. (cooled in ice) to bring them to pH 10. The solns. were again extracted with $CHCl_3$. The $CHCl_3$ extracts were concentrated yielding the crude alkaloid extract. The latter (13.6 g) was first separated by VLC (neutral Al_2O_3, petroleum ether/$CHCl_3$/MeOH mixtures). VLC *Frs. 10* and *11* (petroleum ether/$CHCl_3$ 50:50; 806 mg) were combined and chromatographed (SiO_2 rotor, petroleum ether/$CHCl_3$/MeOH mixtures): *septentriodine* (**2**; 10 mg) and *lappaconitine* (**3**; 53 mg). VLC *Frs. 13–16* (petroleum ether/$CHCl_3$ 40:60 → 10:90; 896 mg) were combined and chromatographed (SiO_2 rotor, petroleum ether/$CHCl_3$/MeOH mixtures): finaconitine/ranaconitine (81 mg) and *puberanidine* (**6**; 10 mg). Purification by CC (*Sephadex LH-20*, $CHCl_3$) gave finaconitine (**4**; 30 mg) and *ranaconitine* (**5**; 10 mg). VLC *Frs. 21–24* ($CHCl_3$/MeOH 96:4 → 80:20; 782 mg) were combined and chromatographed (SiO_2 rotor, petroleum ether/$CHCl_3$/MeOH mixtures): *delstaphinine* (**7**; 25 mg) and *aconitorientaline* (**1**; 53 mg). Septentriodine (**2**) and lappaconitine (**3**) were purified by prep. TLC (SiO_2, toluene/AcOEt/Et_2NH 7:4:1). 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.

Aconitorientaline (= *(1α,6α,14α,15α,16β,19*R*)-1,19-Epoxy-20-ethyl-4-(hydroxymethyl)-6,8,14,16-tetramethoxyaconitan-15-ol*; **1**): Colorless oil. $[\alpha]_D^{20} = \pm 0$ ($c = 0.10$, $CHCl_3$). IR ($CHCl_3$): 3365, 1659, 1456, 1260, 1088, 1020. ^1H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 466.2790 ($[M+H]^+$, $C_{25}H_{40}NO_7^+$; calc. 466.2983).

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