Acovulparine, a New Norditerpene Alkaloid from *Aconitum vulparia*

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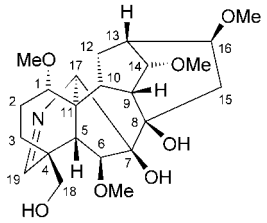
Acovulparine (=1α,6β,7*R*,8β,14α,16β)-4-(hydroxymethyl)-1,6,14,16-tetramethoxyaconit-19-ene-7,8-diol; **1**), a new norditerpene alkaloid, was isolated from the MeOH extract of the whole plants of *Aconitum vulparia* REICHENB., together with the known compounds lycoctonine (**2**) and delcosine (**3**). The structures were established by HR-EI-MS and advanced 2D-NMR, including ^{1}H-NMR, JMOD, ^{1}H,^{1}H-COSY, HSQC, and HMBC experiments. Acovulparine was found to contain the rare C(19)=N azomethine group.

Introduction. – *Aconitum* species accumulate highly toxic diterpene and norditerpene alkaloids that have attracted considerable interest because of their complex structures, interesting chemistry, and noteworthy physiological effects [1]. Previous pharmacological studies of these compounds have focused mainly on their powerful anti-arrhythmic, anti-epileptic, and antinociceptive activities, which are due to their interactions with voltage-gated Na^+ channels [2]. Different types of norditerpene alkaloids have been reported either as agonists (*e.g.*, aconitine and mesaconitine) or antagonists (*e.g.*, lappaconitine 6-benzoyllappaconitine) of Na^+ channels [3]. Moreover, recent investigations of some norditerpene alkaloids have revealed that they exert selective antagonistic activity in nanomolar concentration on the neuronal nicotinic acetylcholine (nACh) receptor. Such compounds, *e.g.*, methyllycaconitine, are valuable neurobiological tools to study the nACh receptor. Also, methyllycaconitine is a lead compound for the treatment of *Alzheimer*'s disease [4].

As a continuation of our ongoing studies on biologically active compounds from Hungarian Ranunculaceae species, the alkaloidal constituents of *Aconitum vulparia* REICHENB. were studied. *A. vulparia* is a perennial plant with stout leafy stems and yellowish flowers that occurs in many European countries [5]. The present paper reports the isolation and structure elucidation of a new norditerpene alkaloid, named acovulparine (**1**), together with the known compounds lycoctonine (**2**) and delcosine (**3**). The structures were determined spectroscopically, and previously unpublished NMR spectral data on lycoctonine are also presented.

Results and Discussion. – Dried whole plants of *A. vulparia* were extracted with MeOH. The extract was subjected to solvent partitioning and then to multiple chromatographic separations, affording compounds **1**–**3**.

Compound **1** was isolated as an amorphous solid, with $[\alpha]_D^{29} = +25.3$ ($c = 2$, $CHCl_3$). HR-EI-MS established the molecular formula $C_{23}H_{35}NO_7$, with the M^+ signal at m/z 437.2396 (calc. 437.2414). From the ^{1}H-NMR and JMOD (*J*-modulated spin-echo



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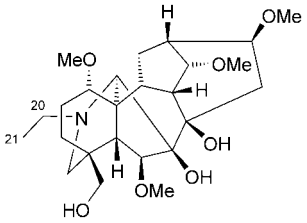

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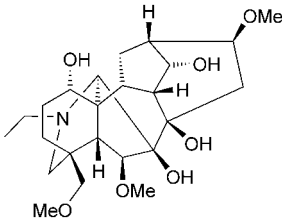

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experiment) spectra of **1**, four MeO groups were identified (δ(H) 3.16, 3.36, 3.42, 3.44 (4*s*); δ(C) 56.1, 56.2, 57.7, 58.4). The signals at δ(H) 7.46 (*s*) and δ(C) 166.9 suggested the presence of an azomethine group instead of the *N*-Et or *N*-Me group characteristic of many norditerpenoid alkaloids [6][7]. The HSQC spectrum of **1** revealed a C_{19} norditerpene skeleton composed of five CH_2, four oxygenated and five alkyl-substituted CH groups, and two oxygenated and two alkyl-substituted quaternary C-atoms (*Table*).

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

Position	^{1}H	^{13}C	HMBC (C → H)	^{1}H,^{1}H-COSY[a])	NOESY[a])
1	3.24 (*t*, *J* = 3.8)	81.6	1-MeO, 2α, 3α, 3β	2α, 2β	2β, 10, 1-MeO
2α	1.71 (*m*)	20.9	3β	1, 2β, 3α, 3β	–
2β	1.42 (*m*)			1, 2α, 3β	1, 5
3α	1.73 (*m*)	24.2	1, 2α, 2β, 18a, 18b	2α, 3β	–
3β	1.64 (*m*)			2α, 2β, 3α	5
4	–	48.2	2α, 3α, 3β, 6, 18a, 18b, 19	–	–
5	1.80 (*s*)	45.4	1, 6, 18a, 18b, 19	6, 17[b]), 19[b])	2β, 3β, 6-MeO
6	3.88 (br. *s*)	90.8	5, 17, 6-MeO	5	5, 18a, 6-MeO
7	–	86.5	5, 6, 14[c]), 15α, 15β, 17	–	–
8	–	77.2	6, 9, 10, 14, 15α, 15β, 17	–	–
9	2.85 (*t*, *J* = 5.5)	42.9	10, 13, 14, 15α	10, 13[b]), 14	10, 14, 16-MeO
10	1.99 (*m*)	43.4	1, 9, 12α, 12β, 13	9, 12α, 12β	1, 9
11	–	50.4	1, 5, 6, 10, 12α	–	–
12β	2.03 (*m*)	30.2	9, 10, 13, 16	10, 12α, 13	12α, 13
12α	1.48 (*dd*, *J* = 13.0, 4.4)			10, 12β	12β, 16, 17
13	2.41 (*dd*, *J* = 6.5, 4.4)	37.9	9, 12α, 12β, 14, 15α	9[b]), 12β, 14	12β, 14, 16-MeO
14	3.66 (*t*, *J* = 4.4)	84.1	9, 12α, 13, 16, 14-MeO	9, 13, 16[b])	9, 13, 16-MeO
15α	2.80 (*dd*, *J* = 15.0, 8.5)	33.1	9, 13	15β, 16	15β, 16
15β	1.70 (*m*)			15α, 16	15α
16	3.27 (*t*, *J* = 8.5)	82.5	12α, 12β, 13, 14, 15α, 15β, 16-MeO	14[b]), 15α, 15β	12α, 15α
17	3.76 (br. *s*)	64.4	1, 5, 10	5[b])	12α
18a	3.81 (*d*, *J* = 10.8)	64.3	5	18b	6
18b	3.77 (*d*, *J* = 10.8)			18a, 19[b])	–
19	7.46 (br. *s*)	166.9	3α, 5, 17, 18	5[b]), 18b[b])	–
1-MeO	3.16 (*s*)	56.1	1	–	1
6-MeO	3.44 (*s*)	58.4	6	–	5, 6
14-MeO	3.42 (*s*)	57.7	14	–	–
16-MeO	3.36 (*s*)	56.2	16	–	9, 13, 14

[a]) Position of H-atoms. [b]) ^4J(H,H) coupling. [c]) ^4J(C,H) coupling.

The ^{1}H,^{1}H-COSY correlations between δ(H) 3.24 (H–C(1)) and 1.71/1.42 (CH_2(2)) and between δ(H) 1.42 and 1.64 (H_b–C(2) and H_b–C(3)) indicated a CHR–CH_2–CH_2 structural element (fragment A; see *Figure* below). This fragment was identified as the C(1)–C(3) part of the molecule, because correlations were observed between H–C(1) and C(11), between H_a–C(2) and C(4), and between CH_2(3) and C(4) in the HMBC spectrum of **1**. Further, HMBC correlations were detected between C(4) and the azomethine H-atom (H–C(19)), confirming the position of the C(19)=N– azomethine group.

The correlation between the signals at δ(H) 1.80 and 3.88 in the ^{1}H,^{1}H-COSY spectrum established the presence of a CH–CH structural moiety (fragment B) in **1**. For identification of this fragment, W-type ^4J(H,H) correlations between H–C(5) and H–C(19), and between H–C(5) and H–C(17) in the ^{1}H,^{1}H-COSY spectrum, and HMBC cross-peaks between C(4) and H–C(6), C(11) and H–C(5), and C(11) and H–C(6), were informative, revealing that fragment B corresponds to the C(5)–C(6) part of the norditerpene core. An isolated CH_2 group was detected at δ(H) 3.81 and 3.77, and assigned as CH_2(18), since these signals demonstrated long-range ^{1}H,^{13}C correlations with C(3), C(4) and C(5), respectively. An isolated CH group at δ(H) 3.76 (br. *s*) was identified as H–C(17) through its long-range couplings with C(6), C(19), and the quaternary C-atoms C(7) and C(8). The HMBC cross-peak of H–C(5) and C(7) (δ(C) 86.5) allowed distinction of C(7) and C(8).

The structure of **1** was further elucidated with the aid of the ^{1}H,^{1}H-COSY spectrum, from which a five-membered ring, *i.e.*, a CH–CH–CH_2–CH–CHOR (R = H or Me) sequence was derived (fragment C). This unit was assigned as the C(9)–C(10)–C(12)–C(13)–C(14) part of compound **1** on the basis of HMBC correlations of C(8) with H–C(9), H–C(10) and H–C(14), and the cross-peaks of C(11) with H–C(10) and H_β–C(12). The fourth structural element identified from the ^{1}H,^{1}H-COSY spectrum was a CH_2–CH unit (fragment D), which was identified as CH_2(15)–CH(16) from the ^4J(H,H) couplings of H–C(14) and H–C(16), and from the heteronuclear long-range correlations between C(8) and H–C(15), C(12) and H–C(16), and C(14) and H–C(16) (*Figure*).

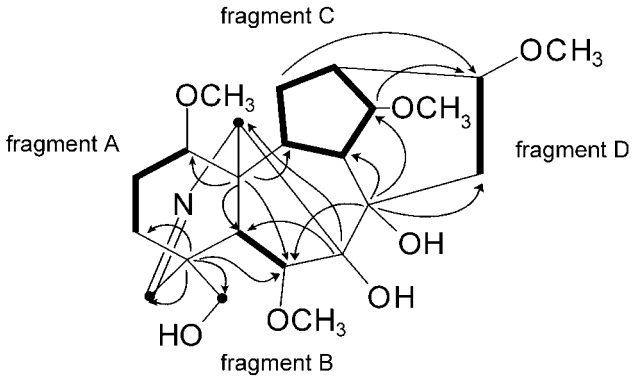

Figure. *Selected ^{1}H,^{1}H-COSY* (▬) *and HMBC* (C → H) *correlations for acovulparine* (**1**)

The four MeO groups were located at C(1), C(6), C(14), and C(16), respectively, as determined from the HMBC cross-peaks between the MeO C-atoms (δ(C) 56.1, 58.4, 57.7, 56.2) and H–C(1), H–C(6), H–C(14), and H–C(16), respectively.

The relative configurations of the stereogenic centers were assigned by means of nuclear-*Overhauser*-enhancement spectroscopy (NOESY). In the NOESY spectrum of **1**, correlations were detected between H–C(1) and H–C(10), H–C(10) and H–C(9), H–C(9) and H–C(14), H–C(14) and H–C(13), H–C(1) and H_β–C(2), and H_β–C(2) and H–C(5), all indicating β-oriented H-atoms. Further, the NOESY correlations of MeO–C(16) with H–C(9), H–C(13), and H–C(14), respectively, also demonstrated the β-position of these H-atoms and the MeO group. A J(5,6) value of 0 Hz dictated the α-orientation of H–C(6) [7–9]. Further important NOEs were observed between H_α–C(12) and H–C(17), leading to the conclusion that the CH=N–CH bridge (C(19)=N–C(17)) is oriented below the plane of rings *A* and *B*. NOESY Cross-peaks between H_β–C(12) and H–C(13), H_α–C(12) and H–C(16), H_β–C(3) and H–C(6), and H_α–C(15) and H–C(16) led to the steric differentiation of CH_2(12), CH_2(3), and CH_2(15), as listed in the *Table*. All of the above data led to the structural assignment of acovulparine (**1**) as (1α,6β,7*R*,8β,14α,16β)-4-(hydroxymethyl)-1,6,14,16-tetramethoxyaconit-19-ene-7,8-diol.

The known alkaloids lycoctonine (**2**) and delcosine (**3**) were also isolated from *A. vulparia*, and identified by means of NMR. As a result of ^{1}H-NMR, JMOD, ^{1}H,^{1}H-COSY, NOESY, HSQC, and HMBC experiments, complete chemical-shift assignments for all ^{1}H- and ^{13}C-NMR resonances of **2** were achieved, and the previously published NMR data [10] were supplemented (see the *Exper. Part*). Delcosine (**3**) was identified by comparing its spectral data with those reported in the literature, and by TLC co-chromatography with an authentic sample [6][11].

Conclusions. – From *A. vulparia*, a new aconitene alkaloid named acovulparine (**1**) was identified, together with lycoctonine (**2**) and delcosine (**3**). The isolated compounds are biogenetically related: **1** and **2** differ only with respect to the N-function (azomethine vs. *N*-Et group, respectively). Delcosine (**3**) is also a compound of the lycoctonine type, but with a dissimilar methylation pattern. Lycoctonine (**2**) had been isolated earlier from the seeds and roots of *A. vulparia* (syn. *A. lycoctonum* ssp. *lycoctonum*) [12], but this is the first report of delcosine (**3**) from this plant species.

Experimental Part

General. For vacuum liquid chromatography (VLC), silica gel (*Kieselgel GF$_{254}$*, 15 μm; *Merck*) and Al_2O_3 (*Aluminiumoxid G (Typ E)*; *Merck*) were used. For gel chromatography, *Sephadex LH-20* (*Pharmacia LKB*) was applied. Prep. TLC was carried out on *Kieselgel 60F$_{254}$* (*Merck*) and on *Aluminiumoxid 60F$_{254}$* (*Merck*) plates. Chromatographic fractions were monitored by TLC on silica-gel plates (*Merck 5715*), visualized by spraying with *Dragendorff* reagent or conc. H_2SO_4, followed by heating. Optical rotations: *Perkin-Elmer 341* polarimeter. NMR spectra: in $CDCl_3$ on a *Bruker Avance DRX-500* spectrometer at 500 MHz (^{1}H) or 125 MHz (^{13}C); chemical shifts δ in ppm rel. to residual solvent signals ($CHCl_3$), J in Hz. 2D-NMR Data were acquired and processed with standard *Bruker* software. In the ^{1}H,^{1}H-COSY, HSQC, and HMBC experiments, gradient-enhanced versions were used. HR-EI-MS: *Finnigan MAT 95 S*; in *m*/*z* (rel. %).

Plant Material. *Aconitum vulparia* REICHENB. was collected from wild stock growing in the region of Nagyvázsony, Hungary, in September 2002. The plant was identified by *András Mészáros*, Balaton Uplands

National Park, Veszprém, Hungary. A voucher specimen (No. 617) has been deposited in the Herbarium of the Department of Pharmacognosy, University of Szeged, Szeged, Hungary.

Extraction and Isolation. Dried, ground whole plants of *A. vulparia* (250 g) were crushed in a blender and then extracted exhaustively with MeOH (12 l). After evaporation, the crude extract was dissolved in 4% aq. H_2SO_4 (250 ml) and extracted exhaustively with $CHCl_3$. The org. layer was extracted with 2% aq. H_2SO_4. The aq. phase was then rendered alkaline with 5% aq. NaOH soln., and extracted with $CHCl_3$. The $CHCl_3$ phase was evaporated under reduced pressure, and separated by VLC (Al_2O_3; hexane/AcOEt/MeOH 50:50:0.5, 50:50:1, 50:50:1.5, 50:50:2, 50:50:3, 50:50:4, 50:50:5, 50:50:10, and 1:1:1). The fractions obtained by eluting with the above solvent systems 50:50:3 and 50:50:4 were further fractionated by VLC (SiO_2; cyclohexane/$CHCl_3$/MeOH mixtures of increasing polarity). The subfractions eluted with the systems 50:50:6 and 50:50:8 were repeatedly purified by VLC (Al_2O_3; cyclohexane/$CHCl_3$/MeOH 50:50:2, 50:50:4, 50:50:7, 50:50:10, and 50:50:20). The fraction eluted with the solvent system 50:50:3 was subjected to gel chromatography (*Sephadex LH-20*; MeOH): 27 1-ml fractions (Fr.), from which *Fr. 16–18* afforded compound **1** (36 mg). The fraction obtained from the first VLC (Al_2O_3; cyclohexane/$CHCl_3$/MeOH 50:50:1) was purified first by prep. TLC (Al_2O_3; cyclohexane/$CHCl_3$/MeOH 50:50:10), and then by gel chromatography (*Sephadex LH-20*; MeOH). *Fr. 12* and *13* from gel chromatography were further purified by prep. TLC (Al_2O_3; hexane/AcOEt/MeOH 50:50:4), which yielded compound **2** (10 mg). Compound **3** was isolated from the $CHCl_3$ phase obtained from the alkaline soln. of the crude MeOH extract. The $CHCl_3$ phase was subjected first to VLC (Al_2O_3; gradient of hexane/AcOEt/MeOH 50:50:1, 50:50:1.5, 50:50:2, 50:50:3, 50:50:4, 50:50:5, and 50:50:10), and then purified by prep. TLC (SiO_2; toluene/acetone/EtOH/NH_3 70:50:35:6), which furnished compound **3** (20 mg).

*(1α,6β,7*R*,8β,14α,16β)-4-(Hydroxymethyl)-1,6,14,16-tetramethoxyaconit-19-ene-7,8-diol* (=*Acovulparine*; **1**). Amorphous solid. $[\alpha]_D^{29} = +25.3$ ($c = 2$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: see the *Table*. HR-EI-MS: 437.23958 (36, M^+, $C_{23}H_{35}NO_7^+$; calc. 437.24135), 422 (90, $[M-Me]^+$), 406 (100, $[M-MeO]^+$).

Lycoctonine (**2**). Colorless crystals. M.p. 110 – 113°. $[\alpha]_D^{29} = +56$ ($c = 0.05$, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): 4.04 (*s*, 8-OH); 3.85 (*s*, H–C(6)); 3.64 (*d*, $J = 10.6$, H_a–C(18)); 3.60 (*t*, $J = 5.0$, H–C(14)); 3.44 (*s*, 6-MeO); 3.41 (*s*, 14-MeO); 3.36 (*d*, $J = 10.6$, H_b–C(18)); 3.34 (*s*, 16-MeO); 3.25 (*s*, 1-MeO); 3.21 (*t*, $J = 8.5$, 7.7, H–C(16)); 3.07 (*t*, $J = 5.9$, 5.0, H–C(9)); 2.94 (*m*, H–C(1)); 2.93 (*m*, H_a–C(20)); 2.91 (*s*, H–C(17)); 2.79 (*dq*, $J = 12.9$, 7.0, H_b–C(20)); 2.59 (*d*, $J = 12.0$, H_a–C(19)); 2.58 (*dd*, $J = 15.3$, 8.5, H_a–C(15)); 2.44 (*dd*, $J = 14.3$, 4.8, H_a–C(12)); 2.33 (*dd*, $J = 6.9$, 4.8, H–C(13)); 2.28 (*d*, $J = 12.0$, H_b–C(19)); 2.15 (*m*, H_β–C(2)); 2.08 (*m*, H_α–C(2)); 1.90 (*m*, H–C(10)); 1.84 (*m*, H_b–C(12)); 1.69 (*s*, H–C(5)); 1.66 (*m*, H_b–C(15)); 1.66 (*m*, H_a–C(3)); 1.49 (*m*, H_b–C(3)); 1.04 (*t*, $J = 7.0$, Me(21)). ^{13}C-NMR (125 MHz, $CDCl_3$): 90.8 (C(6)); 88.5 (C(7)); 84.3 (C(1)); 84.0 (C(14)); 82.7 (C(16)); 77.5 (C(8)); 68.0 (C(18)); 64.8 (C(17)); 57.9 (6-MeO); 57.8 (14-MeO); 56.2 (16-MeO); 55.7 (1-MeO); 52.7 (C(19)); 51.1 (C(20)); 49.8 (C(5)); 49.0 (C(11)); 46.3 (C(10)); 43.4 (C(9)); 38.6 (C(4)); 38.2 (C(13)); 33.7 (C(15)); 31.7 (C(3)); 28.8 (C(12)); 26.2 (C(2)); 14.1 (C(21)).

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