Diterpenoid Alkaloids of *Aconitum vulparia* Rchb.

Sezin Kurtoğlu[a], Burcu Şen[a], Gülay Melikoğlu[a], Hans Becker[b], Josef Zapp[c], Alexandra K. Kiemer[c], Filiz Meriçli[a], and Ali H. Meriçli[a,*]

[a] Istanbul University, Faculty of Pharmacy, Department of Pharmacognosy, 34116 Beyazıt-Istanbul, Turkey. Fax: +902124400252. E-mail: alimer@istanbul.edu.tr
[b] Amselweg 3, D-66386 St. Ingbert, Germany
[c] Saarland University, Institute of Pharmaceutical Biology, P. O. Box 151150, D-66041 Saarbrücken, Germany

* Author for correspondence and reprint requests

Z. Naturforsch. **67c**, 103–107 (2012); received April 4/November 2, 2011

From the roots of *Aconitum vulparia* Rchb., collected in Prüm (Germany), a new norditerpenoid alkaloid, named alexhumboldtine, has been isolated along with the known norditerpenoid alkaloids lappaconitine, anthranoyllycoctonine, lycoctonine, puberaconitine, ajacine, and septentriodine. The structure of alexhumboldtine was established on the basis of ^{1}H, ^{13}C, DEPT, homonuclear ^{1}H COSY, NOESY, HSQC, and HMBC NMR studies. From the aerial parts of the plant another norditerpenoid alkaloid, aconorine, has been isolated.

Key words: Diterpenoid Alkaloids, *Aconitum vulparia*, Alexhumboldtine

Introduction

Aconitum (wolfsbane) species are very toxic plants due to thei diterpenoid alkaloid content. 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 pharmaceutical products (Bisset, 1981; Benn and Jacyno, 1983; Meriçli A. H. *et al.*, 2004). In continuation of our investigations on *Aconitum* species (Meriçli A. H. *et al.*, 1996a, b, 2000a, b, 2006a, b; Ulubelen *et al.*, 1996) we now report the alkaloid contents of *Aconitum vulparia* Rchb. (syn. *A. lycoctonum* L., *A. septentrionale* Koellee). This plant has extensively been investigated for its diterpenoid alkaloid constituents by a number of workers (Joshi *et al.*, 1988, 1994; Csupor *et al.*, 2004, 2007; De la Fuente and Ruiz-Mesia, 1994; Khairitdinova *et al.*, 2008; Zinurova *et al.*, 2000; Yunusov *et al.*, 2000; Chen and Katz, 1999; Usmanova *et al.*, 1996; Ross *et al.*, 1993; Sayed *et al.*, 1992; Villar del Fresno and Cabo, 1973). A new norditerpenoid alkaloid, alexhumboldtine (**1**), has been isolated along with the already known norditerpenoid alkaloids lappaconitine (**2**), anthranoyllycoctonine (**3**), lycoctonine (**4**), puberaconitine (**5**), ajacine (**6**), and septentriodine (**7**) from the roots of *A. vulparia.* The aerial parts contain another norditerpenoid alkaloid, aconorine (**8**) (Pelletier *et al.*, 1979, 1980, 1981, 1988; Dequan and Das, 1983; Tel'nov *et al.*, 1975). Alexhumboldtine (**1**) is dedicated to the Alexander von Humboldt Foundation, Germany, for giving A. H. M. the opportunity to do research several times in Germany.

Material and Methods

General

Optical rotations were measured on a Perkin Elmer Model 241 polarimeter (Foster City, CA, USA). NMR spectra were recorded on a Bruker 500 MHz spectrometer (Bremen, Germany). LC-mass spectra were determined on a Finnigan MAT 90 spectrometer (San Jose, CA, USA). Vacuum liquid chromatography (VLC) was carried out with Al_2O_3 (EM 1085) and SiO_2 60 G (7731) from Merck (Darmstadt, Germany). Chromatographic separations on a chromatotron were carried out on rotors coated with an 1 mm thick layer of Al_2O_3 60 GF-254 (1092) or SiO_2 60 PF-254 (7749) from Merck. Thin layer chromatograms were run using the solvent systems toluene/EtOAc/diethylamine (7:2:1, 7:4:1, or 7:4:2, v/v/v) and $CHCl_3$/MeOH/NH_4OH (5:3:1, v/v/v).

Plant material

The roots (270 g) and aerial parts (520 g) of *Aconitum vulparia* Rchb. were collected and identified by one of us (H. B.) in Prüm (Germany) at an elevation of 500 m, in July 2006. A voucher specimen (No. ISTE 85264) has been deposited in the herbarium of the Faculty of Pharmacy, Istanbul University, Istanbul, Turkey.

Extraction and isolation

The crude alkaloidal extract (10.75 g) obtained from 270 g roots was first separated by VLC on a neutral Al_2O_3 column with petroleum ether/$CHCl_3$/MeOH mixtures. VLC fractions 37–44 ($CHCl_3$/MeOH, 96:4 to 90:10, v/v) (622 mg) were combined and chromatographed on a SiO_2 rotor with petroleum ether/$CHCl_3$/MeOH mixtures. Fractions 43–45 from this rotor were combined (417 mg) and chromatographed on a Al_2O_3 rotor with petroleum ether/$CHCl_3$/MeOH mixtures. Fractions 41–42 from the Al_2O_3 rotor were combined (25 mg) and chromatographed on a Sephadex LH-20 column with MeOH, and alexhumboldtine (**1**, 17 mg) was obtained. Fractions 5–12 were combined (90 mg) and purified by preparative TLC on SiO_2 with toluene/EtOAc/diethylamine (7:2:1, v/v/v), and a mixture of lappaconitine (**2**) and anthranoyllycoctonine (**3**) (28 mg), lycoctonine (**4**, 22 mg), puberaconitine (**5**, 10 mg), and ajacine (**6**, 11 mg) was obtained. Lappaconitine (17 mg) and anthranoyllycoctonine (11 mg) were purified on a Sephadex LH-20 column with MeOH. From fraction 4 of the Al_2O_3 rotor, septentriodine (**7**, 5 mg) was obtained.

The crude alkaloidal extract (0.625 g) obtained from 520 g aerial parts of *A. vulparia* was first separated by VLC on a SiO_2 column with petroleum ether/$CHCl_3$/MeOH mixtures. VLC fraction 39 ($CHCl_3$/MeOH, 95:5, v/v) (103 mg) was separated on a Sephadex LH-20 column with MeOH, and aconorine (**8**, 23 mg) was obtained.

All known compounds were identified by comparison of their ^{1}H and ^{13}C NMR data and Co-TLC behaviour with those of authentic samples.

Results and Discussion

A novel norditerpenoid alkaloid, designated alexhumboldtine (**1**), from the roots of *A. vulparia*, collected at an altitude of 500 m in Prüm (Germany), has been isolated exhibiting $[\alpha]_D^{20}$ +21.25° (0.16, $CHCl_3$). The molecular formula, $C_{25}H_{40}NO_7$ (LC-MS, *m/z* 466 $[M+H]^+$), was derived for the alkaloid by HRMS *m/z* 466.27865 $[M+H]^+$ (calcd. 466.25122) and confirmed by ^{1}H,^{13}C NMR spectral and DEPT data. The IR spectrum showed hydroxy and aldehyde group absorptions at 3400 and 1730 cm^{-1}, but no aromatic absorptions. A completely decoupled ^{13}C NMR spectrum confirmed 25 carbon atoms of the molecule. The ^{13}C NMR and DEPT spectra showed three signals for quaternary carbon atoms at δ_C 76.7, 51.9, and 37.7 ppm; eleven signals for methine groups at δ_C 179.8, 87.6, 83.7, 82.6, 82.0, 69.7, 52.6, 45.4, 42.5, 42.1, and 37.7 ppm; seven signals for methylene groups at δ_C 65.7, 56.4, 56.4, 33.0, 30.9, 29.6, and 26.6 ppm; and four signals for methyl groups at δ_C 57.9, 56.4, 56.3, and 56.1 ppm (Table I).

Table I. ^{1}H and ^{13}C NMR data of alexhumboldtine (**1**) and ^{13}C NMR data of aconitilearine (**9**) for comparison [δ in ppm (*J* in Hz)].

Position		**1**		**9**
		δ_H	δ_C	δ_C
1	1β	3.38 dd (9, 6)	87.6 d	82.6 d
2	2α	1.79 m	29.6 t	28.6 t
	2β	1.47 m		
3	3α	1.82 m	30.9 t	33.6 t
	3β	2.42 m		
4		-	37.7 s	38.5 s
5		1.87 m	42.5 d	43.2 d
6		4.15 dd (1, 6)	82.0 d	83.3 d
7		2.14 d (1)	52.6 d	52.3 d
8		-	76.7 s	73.4 s
9		1.80 m	45.4 d	43.2 d
10		1.46 m	42.1 d	44.6 d
11		-	51.9 s	48.9 s
12	12a	2.42 m	26.6 t	28.8 t
	12b	1.50 m		
13		2.42 m	37.7 d	38.0 d
14		3.66 t (5)	83.7 d	84.0 d
15	15a	1.73 m	33.0 t	33.8 t
	15b	2.63 dd (12, 14)		
16		3.57 dd (7, 12)	82.6 d	83.8 d
17		2.87 s	69.7 d	64.7 d
18	18a	3.31 d (10)	65.7 t	67.7 t
	18b	3.66 d (10)		
19	19a	1.79 m	56.4 t	90.5 d
	19b	3.31 m		
20	20a	2.86 m	56.4 t	51.1 t
	20b	2.86 m		
21		9.22 s	179.8 d	13.0 q
OCH_3-1		3.38 s	56.1 q	55.8 q
OCH_3-6		3.34 s	57.9 q	57.9 q
OCH_3-14		3.34 s	56.4 q	57.9 q
OCH_3-16		3.30 s	56.3 q	56.3 q

Diterpenoid alkaloids usually belong to one of two main groups, those with a C_{19} lycoctonine/aconitine-type skeleton with characteristic methoxy groups and those derived from a C_{20} atisine-type skeleton with an exocyclic methylene group (Joshi and Pelletier, 1999). The ^{1}H NMR spectrum of alexhumboldtine (**1**) proved the presence of four methoxy groups, therefore it should be a C_{19} norditerpenoid alkaloid. The most important point is the presence of an aldehyde group in the molecule. Aldehyde signals at δ_H 9.22 ppm, s and δ_C 179.8 ppm, d indicate the presence of a N-CH_2-CHO structure in the molecule as in the case of the diterpenoid alkaloids chellespontine

Table II. Summary of COSY, NOESY, and HMBC correlation data of alexhumboldtine (**1**).

Position	COSY	NOESY	HMBC
H-1β	H-2α, H-2β	H-2α, H-2β, H-10, H-12b, H-21	C-3, C-10, OCH_3-1
H-2α	H-1β, H-2β, H-3α, H-3β	H-1β, H-3β	C-4, C-5, C-10
H-3α	H-2α, H-2β, H-3β	H-19a	-
H-3β	H-2α, H-2β, H-3α	H-2α, H-18b	C-1, C-2, C-19
H-5	H-6	H-2β, H-6, H-9, C-18a, C-19b	C-17, C-18, C-19
H-6b	H-5	H-5, H-7, H-18a, H-18b, H-19b, OCH_3-6	OCH_3-6
H-7	H-6, H-17	H-6, H-15b, H-17, H-19b, OCH_3-6	C-9, C-17
H-9	H-10, H-14	H-5, H-10, H-12a, H-14	C-7, C-12, C-13, C-14, C-16
H-10	H-9, H-12b	H-1β, H-9, H-12a, H-14, OCH_3-16	-
H-12a	H-12b, H-13	H-12b, H-13, H-14	-
H-12b	H-10, H-12a	H-1β, H-12a, H-13, H-16, H-17	C-14, C-16
H-13	H-12a, H-14	H-12a, H-12b, H-14, H-16, OCH_3-16	C-14, C-15, C-16
H-14	H-9, H-13	H-9, H-10, H-12a, H-13	C-16, OCH_3-14
H15a	H-15b	H-16, OCH_3-14	C-7, C-16
H-15b	H-15a, H-16	-	C-7, C-16
H-16	H-15b	H-7, H-12b, H-13, H-15a, OCH_3-16, H-17	C-12, C-14, OCH_3-16
H-17	H-5	H-7, H-12b, H-16, H_2-20, H-21	C-5, C-6, C-10, C-19
H-18a	H-18b	H-5, H-6, H-18b, H-19a, OCH_3-6	C-3, C-19
H-18b	H-18a	H-3β, H-6, H-18a, H-19a, H-19b	C-3, C-5, C-19
H-19a	H_2-20	H-3α, H-18a, H-18b, H_2-20	C-3, C-18, C-20
H-19b	H_2-20	H-6b, H-7, H-18a, H-18b, H_2-20	C-3, C5, C-17
H_2-20	H-19a, H-19b, H-21	H-17, H-19a, H-19b, H-21	C-17, C-19, C-21
H-21	H_2-20	H-1, H-17, H-20	C-11, C-20
OCH_3-1	-	H-17, H-21	C-1
OCH_3-6	-	H-18a	C-6
OCH_3-14	-	H-15a	C-14
OCH_3-16	-	H-10, H-13, H-16	C-16

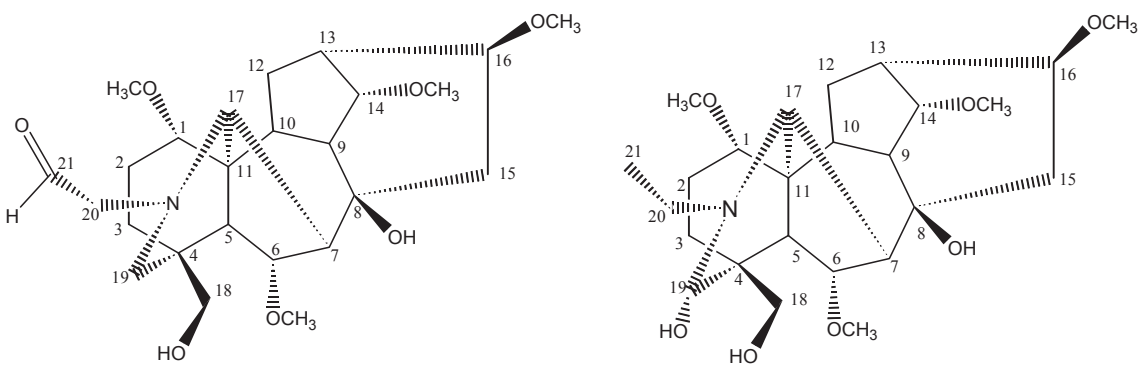

Fig. 1. Chemical structures of alexhumboldtine (**1**) and aconitilearine (**9**).

and consorientealine (Desai *et al.*, 1993; Meriçli F. *et al.*, 2001). Except for the aldehyde group, there were six O-bearing C atoms present as shown by the signals at δ_C 87.6, 83.7, 82.6, 82.0, 76.7, and 65.7 ppm, four of them carrying MeO groups, the other two, therefore, should have OH groups. Four methoxy groups (δ_H 3.30 ppm, 3H s; 3.34 ppm, 6H s; 3.38 ppm, 3H s; δ_C 56.3 ppm, q; 56.4 ppm, q; 57.9 ppm, q; 56.1 ppm, q) could be assigned at C-16 (δ_H 3.57 ppm, dd, J = 7 and 12 Hz; δ_C 82.6 ppm, d), C-14 (δ_H 3.66 ppm, t, J = 5 Hz; δ_C 83.7 ppm, d), C-6 (δ_H 4.15 ppm, dd, J = 1 and 6 Hz; δ_C 82.0 ppm, d), and C-1 (δ_H 3.38 ppm, dd, J = 9 and 6 Hz; δ_C 87.6 ppm, d), respectively. The two OH groups should be located on C-18 (δ_H 3.31 ppm, d, J = 10 Hz; δ_C 65.7 ppm, t) and C-8 (δ_C 76.7 ppm, s). Alexhumboldtine (**1**) is very similar to the C_{19} norditerpenoid alkaloid aconitilearine (**9**), the only differences being that **9** does not contain any aldehyde group and has an OH group on C-19 (Meriçli A. H. *et al.*, 2006a). The presence of an aldehyde substituent at C-21 is significantly confirmed by interactions of C-20, C-17, and C-1 in the 2D-NMR analysis as shown in Table II.

The presence of aconorine has been shown here for the first time in *Aconitum vulparia.*

The NMR data of alexhumboldtine (**1**) are given in the Tables I and II.

The chemical structures of alexhumboldtine (**1**) and aconitilearine (**9**) are presented in Fig. 1.

Acknowledgement

A. H. M. thanks the Alexander von Humboldt Foundation for a fellowship.

Benn M. H. and Jacyno J. M. (1983), The toxicology and pharmacology of diterpenoid alkaloids. In: Alkaloids: Chemical and Biological Perspectives, Vol. 1 (Pelletier S. W., ed.). John Wiley & Sons, New York, pp. 153–210.

Bisset N. G. (1981), Arrow poisons in China part II. *Aconitum* – botany, chemistry and pharmacology. J. Ethnopharmacol. **4**, 247–336.

Chen Y. and Katz A. (1999), Isolation of norditerpenoid alkaloids from flowers of *Aconitum lycoctonum*. J. Nat. Prod. **62**, 798–799.

Csupor D., Forgo P., Mathe I., and Hohmann J. (2004), Acovulparine, a new norditerpene alkaloid from *Aconitum vulparia.* Helv. Chim. Acta **87**, 2125–2130.

Csupor D., Forgo P., Zupko I., Szabo P., and Hohmann J. (2007), Anthranoyl-substituted norditerpene alkaloids from *Aconitum vulparia* Rchb. and their cytotoxic activities. Z. Naturforsch. **62b**, 135–141.

De la Fuente G. and Ruiz-Mesia L. (1994), Norditerpenoid alkaloids from *Aconitum vulparia neapolitanum.* Phytochemistry **37**, 271–274.

Dequan Y. and Das B. C. (1983), Alkaloids of *Aconitum barbatum*. Planta Med. **49**, 85–89.

Desai H. K., Joshi B. S., Pelletier S. W., Şener B., Bingöl F., and Baykal T. (1993), New alkaloids from *Consolida hellespontica*. Heterocycles **36**, 1081–1089.

Joshi B. S. and Pelletier S. W. (1999), Recent developments in the chemistry of norditerpenoid and diterpenoid alkaloids. In: Alkaloids: Chemical and Biological Perspectives, Vol. 13 (Pelletier S. W., ed.). Pergamon, New York, pp. 289–293.

Joshi B. S., Desai H. K., Pelletier S. W., and Aasen A. J. (1988), Septentriosine, a new C_{20} diterpenoid alkaloid from *Aconitum septentrionale.* J. Nat. Prod. **51**, 265–271.

Joshi B. S., Sayed H. M., Ross S. A., Desai H. K., and Pelletier S. W. (1994), Septatisine, a novel diterpenoid alkaloid from *Aconitum septentrionale* Koelle. Can. J. Chem. **72**, 100–104.

Khairitdinova E. D., Tsyrlina E. M., Sprikhin L. V., Balandina A. A., Latypov S. K., and Yunusov M. S. (2008), Norditerpenoid alkaloids from *Aconitum septentrionale.* Russ. J. Org. Chem. **44**, 536–541.

Meriçli A. H., Meriçli F., Becker H., İlarslan R., and Ulubelen A. (1996a), 3-Hydroxytalatisamine from *Aconitum nasutum.* Phytochemistry **42**, 909–911.

Meriçli A. H., Meriçli F., Becker H., and Ulubelen A. (1996b), A new prodelphinine type alkaloid from *Aconitum nasutum.* Turk. J. Chem. **20**, 164–167.

Meriçli A. H., Meriçli F., Ulubelen A., Bahar M., İlarslan R., Algül G., Desai H. K., Teng Q., and Pelletier S. W. (2000a), Diterpenoid alkaloids from the aerial parts of *Aconitum anthora.* Pharmazie **55**, 696–698.

Meriçli A. H., Meriçli F., Desai H. K., Joshi B. S., Teng Q., Bhattacharrya K., Melikoğlu G., Küçükislamoğlu M., Ulubelen A., and Pelletier S. W. (2000b), Norditerpenoid and diterpenoid alkaloids from the roots of *Aconitum nasutum* Fisch. ex Reichb. Heterocycles **53**, 1987–1996.

Meriçli A. H., Meriçli F., and Ulubelen A. (2004), Türkiye'de yetişen *Aconitum* türleri üzerinde araştırmalar. In: Turhan Baytop Anma Kitabı, No: 81 (Farmakognozi A. D., ed.). Istanbul Üniversitesi Eczacilik Fakültesi Yayinlari, Istanbul, pp. 29–39.

Meriçli A. H., Pırıldar S., Süzgeç S., Bitiş L., Meriçli F., Özçelik H., Zapp J., and Becker H. (2006a), Norditerpenoid alkaloids from the aerial parts of *Aconitum cochleare* Woroshin. Helv. Chim. Acta **89**, 210–217.

Meriçli A. H., Süzgeç S., Bitiş L., Meriçli F., Özçelik H., Zapp J., and Becker H. (2006b), Diterpenoid

alkaloids from the roots of *Aconitum cochleare.* Pharmazie **61**, 483–485.

Meriçli F., Meriçli A. H., Ulubelen A., Desai H. K., and Pelletier S. W. (2001), Norditerpenoid and diterpenoid alkaloids from Turkish *Consolida orientalis*. J. Nat. Prod. **64**, 787–789.

Pelletier S. W., Mody N. V., and Sawnhey R. S. (1979), Carbon-13 nuclear magnetic resonance spectra of some C_{19}-diterpenoid alkaloids and their derivatives. Can. J. Chem. **57**, 1652–1655.

Pelletier S. W., Sawnhey R. S., Desai H. K., and Mody N. V. (1980), The diterpenoid alkaloids of *Consolida ambigua*. J. Nat. Prod. **43**, 395 – 406.

Pelletier S. W., Dailey O. D., Mody N. V., and Olsen J. D. (1981), Isolation and structure elucidation of the alkaloids of *Delphinium glaucescens* Rybd. J. Org. Chem. **46**, 3284–3293.

Pelletier S. W., Panu A. M., Kulanthaivel P., and Olsen J. D. (1988), New alkaloids from *Delphinium andersonii* Gray. Heterocycles **27**, 2387–2393.

Ross S. A., Joshi B. S., Pelletier S. W., Newton M. G., and Aasen A. J. (1993), 2-Acetylseptentriosine, a new diterpenoid alkaloid from *Aconitum septentrionale.* J. Nat. Prod. **56**, 424–429.

Sayed H. M., Desai H. K., Ross S. A., Pelletier S. W., and Aasen A. J. (1992), New diterpenoid alkaloids from the roots of *Aconitum septentrionale*: Isolation by ion exchance method. J. Nat. Prod. **55**, 1595 – 1606.

Tel'nov V. A., Yunusov M. S., Yunusov S. Y., and Ibragimov B. S. (1975), Structure of aconorine. Khim. Prir. Soedin. **11**, 814–815.

Ulubelen A., Meriçli A. H., Meriçli F., and Yılmaz F. (1996), Diterpenoid alkaloids from *Aconitum orientale.* Phytochemistry **41**, 957–961.

Usmanova S. K., Bessonova I. A., and Milgram E. G. (1996), Septentrine and septephine, novel alkaloids from *Aconitum septentrionale.* Khim. Prir. Soedin. **32**, 225–228.

Villar del Fresno A. and Cabo T. J. (1973), Phytochemical study of Sierra Nevada *Aconitum lycoctonum* I. Quantitative determination of its alkaloid content. Boll. Chim. Farm. **112**, 839–842.

Yunusov M. S., Tsyrlina E. M., Khairitdinova E. D., Sprikhin L. V., Kovalevsky A. Y., and Antipin M. Y. (2000), Anhydroaconitine, a new diterpene alkaloid from *Aconitum septentrionale* K. Russ. Chem. Bull. **49**, 1629–1633.

Zinurova E. G., Khakimova T. V., and Yunusov M. S. (2000), Alkaloids of *Aconitum septentrionale* seeds. Chem. Nat. Compd. **36**, 387–389.