A New Diterpenoid Alkaloid from *Aconitum episcopale*

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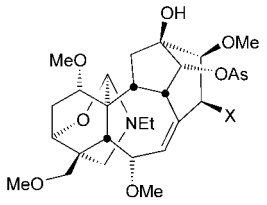
A new diterpenoid alkaloid with an epoxy ring between C(3) and C(17) was isolated from *Aconitum episcopale*. The structure was elucidated on the basis of spectral and chemical evidence.

Introduction. – *Aconitum episcopale* is a plant belonging to the family Ranunculaceae. It is used as a folk medicine to treat fever, rheumatism, and fracture in Tibet and among the Naxi people of Lijiang, Yunnan, China [1]. In our previous papers [2], we reported many new diterpenoid alkaloids from *Aconitum sp.* plants. In subsequent studies of chemical constitutes of *A. episcopale*, a new diterpenoid alkaloid, named secoyunaconitine (**1**) was isolated. The new alkaloid is a novel diterpenoid alkaloid having an epoxy ring between C(3) and C(17)[1]). Upon the treatment of this epoxide with acetic acid, it could be converted to the normal C_{19} aconitine-type alkaloid, yunaconitine (**2**).

Secojesaconitine (**3**), which was isolated in 1988 [3], was the first known example of a C_{19} diterpenoid alkaloid having an epoxy ring between C(3) and C(17). Herein, we report the isolation and characterization of the new alkaloid **1**.

Results and Discussion. – Secoyunaconitine (**1**) was isolated as colorless needles. Its molecular formula, determined to be $C_{33}H_{45}NO_9$, was derived from HR-FAB-MS (*m/z* 600.3195; calc. 600.30943) and ^{13}C-NMR data (*Table*). NMR Assignments were made on the basis of 2D experiments. The ^{1}H-, ^{13}C-NMR, and mass spectra showed that **1** is a C_{19}-type diterpenoid alkaloid with an *N*-ethyl group, five MeO groups, and an anisoyl (=4-methoxybenzoyl; As) group. Its spectral characteristics were similar to those of the known compound secojesaconitine (**3**) except for the OH group at C(15) in compound (**3**). The ^{13}C-NMR signals at δ 131.5 and 130.1 indicated that a trisubstituted double bond was located at C(7) and C(8). The ^{1}H-NMR signal at δ 5.42 ($J = 4.2$ Hz) also confirmed this deduction. Cross-peaks between H–C(5) and H–C(6) (δ 4.36, *m*), H–C(6) and H–C(7) were observed in the ^{1}H,^{1}H-COSY spectrum. The ^{13}C-NMR signals at δ 74.6 and 87.5 indicated that C(3) and C(17) were connected to an O-atom. In the COLO spectrum, ^{1}H,^{13}C-long-range correlations between C(17) and H–C(3) suggested an epoxy ring between C(3) and C(17), which was confirmed by comparison

[1]) Trivial numbering based on aconitine skeleton; see *Exper. Part* for systematic name.



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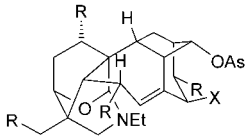
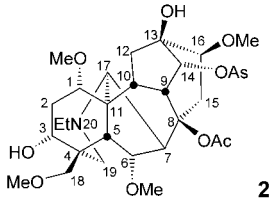


1 X = H
3 X = OH

R = MeO



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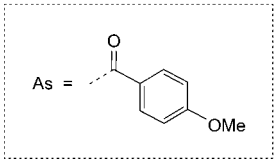

Table. *^{13}C-NMR* ($CDCl_3$) *Data*[1]) *of Secoyunaconitine* (**1**) *and Yunaconitine* (**2**). δ in ppm.

Position	**1**	**2**	Position	**1**	**2**
C(1)	86.3 (*d*)	83.1 (*d*)	C(14)	81.3 (*d*)	78.5 (*d*)
C(2)	29.6 (*t*)	33.4 (*t*)	C(15)	35.1 (*t*)	39.6 (*t*)
C(3)	74.6 (*d*)	71.6 (*d*)	C(16)	85.5	83.5
C(4)	42.2 (*s*)	43.2 (*s*)	C(17)	87.5	61.7
C(5)	38.6 (*d*)	47.5 (*d*)	C(18)	76.1 (*t*)	76.3 (*t*)
C(6)	78.9 (*d*)	82.2 (*d*)	C(19)	50.6 (*t*)	48.7 (*t*)
C(7)	131.5 (*d*)	44.7 (*d*)	N–Et	49.1 (*t*), 13.1 (*q*)	47.3 (*t*), 13.2 (*q*)
C(8)	130.1 (*s*)	85.5 (*s*)	MeO–C(1)	57.9 (*q*)	55.8 (*q*)
C(9)	44.9 (*d*)	48.8 (*d*)	MeO–C(6)	59.2 (*q*)	58.8 (*q*)
C(10)	38.5 (*d*)	40.8 (*d*)	MeO–(16)	58.9 (*q*)	57.3 (*q*)
C(11)	45.9 (*s*)	50.2 (*s*)	MeO–C(18)	57.3 (*q*)	59.1 (*q*)
C(12)	40.7 (*t*)	35.2 (*t*)	AcO–C(8)	–	169.9 (*s*), 21.6 (*q*)
C(13)	76.6 (*s*)	74.7 (*s*)	AsO–C(14)	166.5, 163.4, 131.8, 122.7, 113.6, 55.3	166.0, 163.5, 131.7, 122.6, 113.8, 55.4

of the spectra with those of literature compound [3]. The ^{1}H,^{13}C-COLO spectrum revealed correlations between C(17) and H–C(1), H–C(3), and H–C(19), C(7) and H–C(5), H–C(9), and H–C(15), C(13) and H–C(9), H–C(10), H–C(12), and H–C(14), as well as the carbonyl group at the methoxybenzoyl moiety (δ 166.5) and H–C(14), thereby defining the NMR assignments for **1**[1]). Therefore, the structure of secoyunaconitine (**1**) was assigned as (4*R*,5*S*,9*R*,10*S*,11a*S*,12*S*)-1,2,3,4,4a,5,7,8,9,10,10a,11-dodecahydro-hydroxy-5,8,12-trimethoxy-4-(methoxymethyl)-2-ethyl-9,11-methano-1,4,10b-(epoxy[1,1,3]propanetriyl)-11a*H*-benzo[5,6]cyclohepta[1,2-*c*]pyridin-10-yl-4-methoxybenzoate, which was further supported by the chemical transformation of **1** into the known alkaloid yunaconitine (**2**) by treatment with acetic acid. The mechanism of transformation of **1** into the normal aconitine-type skeleton was

suggested to involve the lone-pair electrons of the N-atom, considering the reactivity at C(8) in aconitine-type alkaloids [3].

Experimental Part

General: Column chromatography (CC): silica gel (200–300 mesh). TLC: silica gel GF_{254} (*Qingdao Marine Chemical Factory*, Qingdao, People's Republic of China). M.p.: *XT-4* apparatus, uncorrected. Optical rotation: *SEPA-300* polarimeter (*Horiba*, Tokyo, Japan). IR Spectra: *Bio-Win FT135* spectrophotometer; KBr pellets; ν in cm^{-1}. ^1H-, ^{13}C-, and 2D-NMR: *Bruker DRX AV-400* spectrometer; in $CDCl_3$; δ in ppm, J in Hz. MS: *Autospec-3000* spectrometer, at 70 eV for EI; m/z (rel. int.).

Plant Material: Roots of *A. episcopale* were collected in Lijiang, Yunnan Province, P.R. China. Voucher specimens are deposited at the Department of Chemistry of Yunnan University.

Extraction and Isolation: Dried and powdered roots of *A. episcopale* (5.0 kg) were extracted three times with 80% EtOH (10 l) at r.t. After the solvent was removed under vacuum, the ethanolic extract was washed with 5% aq. HCl soln. and adjusted with aq. NH_4OH to pH 10, then extracted with $CHCl_3$ to give a crude alkaloid fraction (30 g). The crude alkaloid was separated by CC eluted with a gradient of petroleum ether/AcOEt to give fractions *A* (15.0 g), *B* (3.5 g), *C* (2.8 g), *D* (1.7 g), and *E* (4.0 g).

Secoyunaconitine (=*4*R,*5*S,*9*R,*10*S,*11a*S,*12*S*)-1,2,3,4,4a,5,7,8,9,10,10a,11-Dodecahydro-9-hydroxy-5,8,12-trimethoxy-4-(methoxymethyl)-2-ethyl-9,11-methano-1,4,10b-(epoxy[1,1,3]propanetriyl)-11a*H*-benzo[5,6]cyclohepta[1,2-c]pyridin-10-yl 4-Methoxybenzoate*; **1**). *Fr. D* was dissolved in CH_2Cl_2 and adsorbed on 50 g of silica gel (100–200 mesh) and subjected to CC (petroleum ether/AcOEt/Et_3N 5:1:0.3, decreasing the proportion of petroleum ether gradually). After repeated CC, 103 mg of **1** was obtained. Colorless needles ($CHCl_3$/acetone): M.p. 180–182°. $[\alpha]_D^{13.9}$ + 56.1 (c = 0.77; $CHCl_3$). IR: 3450 (OH), 1710, 1600, 1250, 1130, 1090, 830. ^1H-NMR[1]): 0.97 (t, J = 7.2, NCH_2Me); 1.53, 2.12 (m, CH_2(12)); 1.76, 1.93 (m, CH_2(2)); 1.85 (d, J = 7.0, H–C(5)); 2.12 (q, J = 7.2, NCH_2Me); 2.75 (m, CH_2(15)); 2.92 (d, J = 8.8, H–C(19)); 3.28 (m, H–C(10)); 3.16, 3.16, 3.18, 3.38, 3.84 (5*s*, 5 MeO); 3.93 (d, J = 5.0, H–C(3)); 4.10 (s, H–C(17)); 4.36 (m, H–C(6)); 5.00 (d, J = 3.4, H–C(14)); 5.42 (d, J = 5.2, H–C(7)); 6.87, 7.99 (2d, J = 8.8, A_2B_2 system, 4 arom. H). ^{13}C-NMR: see the *Table*. HR-FAB-MS 600.3195 (M^+) (calc. $C_{33}H_{45}NO_9$). FAB-MS: (Glycine matrix): 600 (100, $[M+1]^+$), 584 (10, $[M+1-Me]^+$), 568 (12, $[M+1-OMe]^+$), 266 (18), 135 (45).

Yunaconitine (= *(1α,3α,6α,16β)-8-(Acetoxy)-20-ethyl-3,13-dihydroxy-1,6,16-trimethoxy-4-(methoxymethyl)-aconitan-14-yl 4-Methoxybenzoate*; **2**). To the CH_2Cl_2 soln. of **1** (30 mg in 10 ml), 1 ml of AcOH was added. The mixture was stirred at r.t. for 12 h. The reaction was quenched by removing the solvents, and the residue was purified by CC to give **2** (23 mg), which was identical to the natural product yunaconitine in terms of the IR, NMR, and MS data [4]. Colorless needles ($CHCl_3$/acetone). M.p. 141–143°. IR: 3450, 1730, 1252, 1608, 1515, 1440, 850; ^1H-NMR: 1.10 (t, J = 7.0, NCH_2Me); 1.34 (s, AcO); 3.16, 3.25, 3.30, 3.55, 3.87 (5*s*, 5 MeO); 6.93, 8.01 (2d, J = 8.8, 4 arom. H); 4.84 (d, J = 4.5, H–C(14)). ^{13}C-NMR: see the *Table*. MS: 659 (M^+, $C_{35}H_{49}NO_{11}$).

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