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A NEW DITERPENE ALKALOID FROM *ACONITUM BRUNNEUM* HAND-MAZZ

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Abstract – A new diterpenoid alkaloid, 3α-hydroxy-12-*epi*-napelline (**1**), was isolated from the ethanol extract of the roots of *Aconitum brunneum* Hand-Mazz along with six known alkaloinds, *i.e*., 12-*epi*-napelline (**2**), liangshanine (**3**), 12-*epi*-dehydronapelline (**4**), songorine (**5**), aconitine (**6**) and benzoylaconine (**7**). The structure of the new compound was elucidated by spectroscopic methods.

Aconitum brunneum is a perennial herb distributed around an altitude of 4000 meters in the Northwest of China. Its roots have been used in Chinese folk medicines as anti-inflammatory and analgesic drugs for long time.[1] Although the chemical constituents of plants of genus *Acotinum* have been extensively studied,[2,3] the chemical constituents and biological activities of *Aconitum brunneum* have not been reported previously. In our effort to find biologically active components from Chinese medicinal plants,[4] we found from the ethanol extract of the roots of *Aconitum brunneum* a new diterpenoid alkaloid, 3α-hydroxy-12-*epi*-napelline (**1**), as well as six known alkaloids, *i.e.,* 12-*epi*-napelline (**2**),[5,6] liangshanine (**3**),[6] 12-*epi*-dehydronapelline (**4**),[7] songorine (**5**),[7] aconitine (**6**)[2,3] and benzoylaconine (**7**).[3,8] We report herein the isolation and structural elucidation of the new compound.

The crushed roots of *Aconitum brunneum,* which were collected from Gannan Forest Farm, Gansu, China in the autumn of 2002 and identified by Professor Yifeng Wang at Department of Botany, Northwest Normal University, were extracted with ethanol followed by silica gel column chromatogrphic separation to give compounds (**1 – 7**).

Compound (**1**) was obtained as colorless crystals, mp 201-202 ^{o}C (uncorrected), $[\alpha]_D^{27}$ -11.6° (c 0.5,

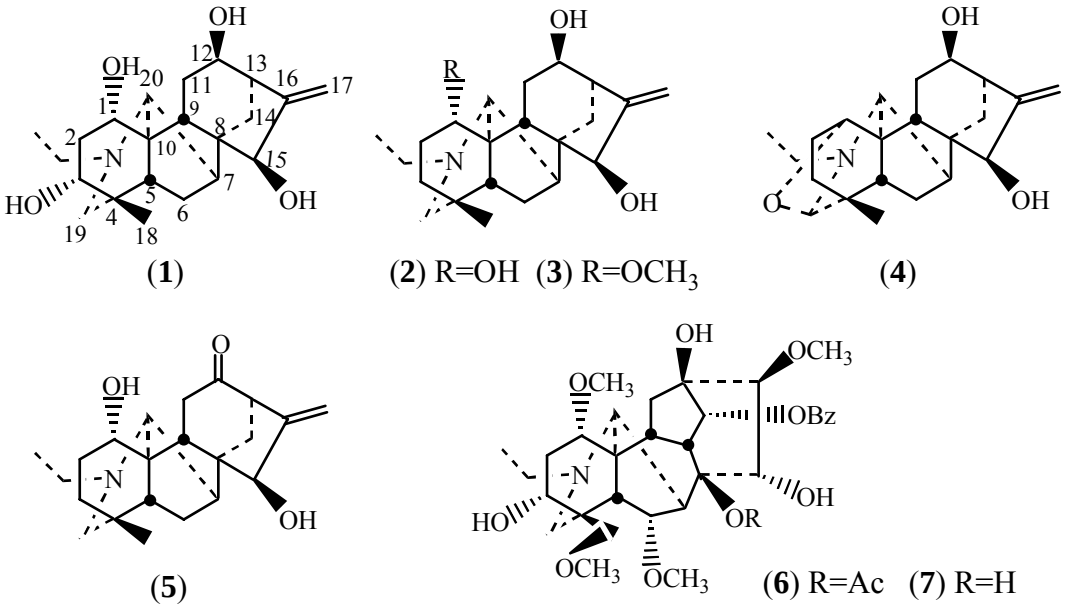

Figure 1.

$CHCl_3$). The HR-EI-MS spectrum exhibits a molecular ion peak at *m/z* 375.2410 corresponding to a molecular formula of $C_{22}H_{33}NO_4$ (calcd for M^+: 375.2404). Its IR spectrum shows absorption bands for hydroxyl groups (3384 cm^{-1}) and exomethylene (1677 and 917 cm^{-1}) functionalities. The ^{13}C NMR and DEPT spectra of **1** exhibit 22 carbon signals (2×CH_3, 7×CH_2, 9×CH, 4×C). In conjunction with its ^1H NMR spectrum it is clearly seen that compound (**1**) possesses a methyl group (δ_C 25.5 and δ_H 0.76), an *N*-ethyl group [δ_C 12.6 and 50.3; δ_H 1.17 (3H, t, *J* = 7.2 Hz), 2.42 (1H, m) and 2.60 (1H, m)], an exomethylene moiety [δ_C 111.6 and 154.6; δ_H 5.10 (1H, br) and 5.29 (1H, br)], as well as four hydroxymethine moieties (δ_C 67.5, 68.0, 70.9 and 76.9, δ_H 4.18, 3.78, 3.76 and 4.25, respectively). The molecular formula and these spectral data suggested that **1** is a C_{20}-diterpenoid alkaloid.[2] Comparison of its ^1H and ^{13}C NMR spectra with those of napellines[5] demonstrated that **1** might possess a napelline skeleton since the coupling constants for H-13 (δ 2.81, 1H, dd, *J* = 8.8, 3.2 Hz), H-14β (δ 1.81, 1H, d, *J* = 12.0 Hz) and H-14α (δ 1.13, 1H, dd, *J* = 12.0, 3.2 Hz) suggest that the dihedral angle between C-13/H-13 and C-14/H-14β or C-14/H-14e is close to 90°, hence the ring C of **1** exists in boat conformation. This is characteristic for napelline-type alkaloids as reported previously.[5] As a matter of fact, the NMR spectral data of **1** (Table 1) are almost identical to those of 12-*epi*-napelline[5,6] except of one additional hydroxymethine moiety (δ_C 68.0 and δ_H 3.78, br) in lieu of the methylene moiety in 12-*epi*-napelline (δ_C 36.3).[6] The connection of this new hydroxyl group to C-3 is confirmed by the HMBC spectrum which shows clear correlations of H-3/C-1 and H-3/C-19, and by the H-H COSY spectrum which shows clear correlations of H-3/H-2α and H-3/H-2β. The NOESY correlations between H-3/H-1β and H-3/H-5β indicate that the H-3 must be β-oriented and in the axial position. Therefore, the 3-hydroxyl must be α-oriented and compound (**1**) is assigned as 3α-hydroxyl-12-*epi*-napelline. Other connections and

conformations are also confirmed by H-H COSY, HMBC and NOESY spectra and the significant correlations are listed in Table 1. Compounds (**2**-**7**) were identified by their m.p., IR, MS, ^{1}H and ^{13}C NMR data and compared with those reported in literatures as 12-*epi*-napelline (**2**),[5,6] liangshanine (**3**),[6] 12-*epi*-dehydronapelline (**4**),[7] songorine (**5**),[7] aconitine (**6**)[2,3] and benzoylaconine (**7**)[3,8] respectively.

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) chemical shifts and 2D correlations of compound (**1**)[a]

No	δ_H (*J*, Hz)	δ_C	H-H COSY	HMBC (H⟶C)	NOESY
1	3.76, br	70.9	H-2α,β	H-2α,β, H-3	H-2α,β, H-3β, H-5β
2	1.52 (β), m	44.2	H-1, H-2α	H-5	
	1.86 (α), m		H-1, H-2β		
3	3.78 br	68.0	H-2α,β	H-2α, H-18	H-2α,β, H-5β
4		33.0		H-2α,β, H-6β, H-18, H-19α,β	
5	1.32 (β), m	49.7	H-6α,β	H-7, H-9β, H-18, H-19α,β, H-20	H-9β, 18-CH_3
6	1.29 (β), m	23.1	H-6α, H-5, H-7,	H-20	
	2.40 (α) d (8.0)		H-6β, H-5, H-7,		
7	2.11 m	43.8	H-6α,β, H-20	H-5, H-6β	
8		51.2		H-6β, H-11β, H-13, H-14α,β	
9	2.07 (β), m	38.4	H-11α,β	H-5, H-11α,β, H-12, H-15	
10		54.2		H-1, H-2α,β, H-6α, H-9, H-11α, H-20	
11	1.67 (β), ddd (14.4, 6.8, 2.0)	30.1	H-9, H-11α, H-12	H-20	
	2.40 (α), m		H-9, H-11β, H-12		
12	4.18 dt (8.8, 2.0)	67.5	H-13, H-11α	H-11β, H-14α	H-13, H-15α, H-17
13	2.81 dd (8.8, 3.2)	44.6	H-12, H-14β	H-11β, H-14β, H-17	H-12α, H-15α, H-17
14	1.13 (α), dd (12.0, 3.2)	33.2	H-14α, H-13	H-9	
	1.81 (β), d (12.0)		H-14β	H-7, H-9	
15	4.25 br	76.9		H-13, H-14α, H-17	H-13,H-7,H-17
16		154.6		H-12, H-13, H-15, H-17	
17	5.10, br,	111.6	H-17	H-15	H-13
	5.29, br		H-17		H-12α,H-15α,H-17
18	0.76 s	25.5		H-5, H-19	
19	2.15 (β) m	55.6	H-19α	H-3, H-5, H-18, H-20, N-CH_2	
	2.42 (α) m		H-19β	H-5	
20	3.24 br	63.5	H-7	H-1, H-5, H-9, H-19α, N-CH_2	H-11β, H-7, *N*-CH_2, H-14α
N-CH_2	2.48, m	50.3	*N*-CH_2, -CH_3	-CH_3	
	2.58, m		*N*-CH_2, -CH_3	-CH_3	
-CH_3	1.17 t (7.2)	12.6	*N*-CH_2	*N*-CH_2	

[a] Determined in $CDCl_3$.

EXPERIMENTAL

General Experimental procedures

Optical rotation was measured on a Perkin-Elmer 241 polarimeter. The IR spectra were taken with KBr pellets on a Nicolet NEXUS 670 IR spectrometer. ^{1}H, ^{13}C and 2D NMR spectra were recorded on a Bruker AM 400 NMR spectrometer in $CDCl_3$ with TMS as internal standard. HR-ESI-MS and EI-MS spectra were obtained on a Bruker APEX II FT-MS and HP 5988 MS spectrometers respectively.

Plant material

The roots of *Aconitum brunneum* were collected from Gannan Forest Farm, Gansu, China in the autumn of 2002 and identified by Professor Yifeng Wang at Department of Botany, Northwest Normal University. A Voucher specimen was deposited in the Department of Botany, Northwest Normal University).

Extraction and isolation procedures

The air-dried ground roots of *Aconitum brunneum* (1.5 kg) were extracted with 90 % EtOH (5 L) at rt for 5 days. The EtOH extract was treated with 5 % HCl, then the acidic solution was basified with 25 % NH_4OH to pH 11 followed by extraction with $CHCl_3$ to give crude alkaloids (48 g) after removing the solvent. This residue was separated by silica gel (100-200 mesh) column chromatography (CC) with gradient elution of PE-Me_2CO-Et_2NH, giving, in order of the increasing polarity, **1** (20 mg), **2** (38 mg), **3** (15 mg), **4** (23 mg), **5** (36 mg), **6** (30 mg) and **7** (45 mg).

3α-Hydroxy-12-*epi*-napelline (**1**). white crystals. mp 201-202 ^oC (uncor.), $[\alpha]_D^{27}$ -11.6° (c 0.5, $CHCl_3$). IR (KBr, cm^{-1}): 3384, 2932, 1167, 1456, 1093, 1055, 917, 931. HR-EIMS: m/z 375.2410 $[M]^+$, Calcd for $C_{22}H_{33}NO_4$: 375.2404. EIMS m/z (rel. int): 375 $[M]^+$ (100), 358 $[M\text{-}OH]^+$ (25), 346 (25), 300 (29), 186 (16), 152 (24), 122 (25), 84 (68), 41 (65). For NMR data see Table 1.

The structures of compounds (**2-7**) were characterized by their mp, IR, MS, ^1H and ^{13}C NMR spectral data and compared with those reported in literatures.[2-8]

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REFERENCES

1. The Flora Committee of Chinese Academy of Sciences, 'The Chinese Flora', Vol. 27, Science Press, Beijing, 1979, pp. 201-202.
2. S. W. Pelletier and N. V. Mody, 'The Alkaloids', Vol. 17, ed. by R. H. F. Manske and R. G. A. Rodorigo, Academic Press, New York, 1981, Chapt. 2, pp. 99.
3. S. W. Pelletier, N. V. Mody, B. S. Joshi, and L. C. Schramm, in 'Alkaloids, Chemical and Biological Perspective', Vol. 2, ed. by S. W. Pelletier, Wiley, New York, 1983, pp. 206-462; G. Hang, Y. Chen, and X. Liang, 'The Alkaloids' ed. by A. Brossi, vol. 32, Chapter 3, Academic Press, San Diego, 1988, p. 241.
4. J. Q. Dai, C. Y. Zhao, Y. L. Wang, Q. Zhang, Z. L. Liu, R. L. Zheng, and L. Yang, *J. Chem. Res. (S),* 2001, 74; J. Q. Dai, C. Y. Zhao, Q. Zhang, Z. L. Liu, R. L. Zheng, and L. Yang, *Phytochem*., 2001, **58**, 1107; J. Q. Dai, Z. L. Liu, and L. Yang, *Phytochem*., 2002, **59**, 537.
5. Z. G. Chen, A. N. Lao, H. C. Wang, and S. H. Hong, *Heterocycles*, 1987, **26**, 1455.
6. H. Takayama, F. E. Wu, H. Eda, K. Oda, N. Aimi, and S. I. Sakai, *Chem. Pharm. Bull*., 1991, **39**, 1644.
7. G. Fuente, M. Reina, E. Valencia, and A. R. Ojede, *Heterocycles*, 1988, **27**, 1109.
8. H. Wang, A. Lao, Y. Fujimoto, K. Kobayashi, T. Sakurai, and T. Tatsuno., *Heterocycles,* 1988, **27**, 1615.