HETEROCYCLES, Vol. 106, No. 5, 2023, pp. 868 - 878. © 2023 The Japan Institute of Heterocyclic Chemistry
Received, 28th February, 2023, Accepted, 27th March, 2023, Published online, 3rd April, 2023
DOI: 10.3987/COM-23-14838

DITERPENOID ALKALOIDS FROM *ACONITUM ELLIOTII* LAUENER

Ziyu Zhang,[1,3] Yan Xiao,[1] Peixin Deng,[1,3] Lin Chen,[1] Shuai Huang,[1,3] Mengyi Deng,[1,3] and Xianli Zhou[1,2]*

School of Life Science and Engineering, Southwest Jiaotong University, Chengdu 610031, Sichuan, P.R. China. 2. The Affiliated Hospital of Southwest Jiaotong University, The Third People's Hospital of Chengdu, Chengdu, Sichuan, P. R. China. 3. Yibin Institute of Southwest Jiaotong University, Yibin 644000, China.

Abstract – Three new diterpenoid alkaloids (**1**–**3**), which were named elliotitine A (**1**), 8-*O*-ethylindaconitine (**2**) and 8-*O*-ethylbikhaconine (**3**), along with thirty-three known compounds, were isolated from the roots of *Aconitum elliotii* Lauener. Their structures were elucidated via HR-ESI-MS, 1D and 2D NMR, and X-ray data. Additionally, the anti-inflammation activities of all compounds were evaluated, no compound showed significant anti-inflammatory activity.

INTRODUCTION

There are about 350 species of *Aconitum* in Ranunculaceae in the world, more than half of which were found in China, widely distributed in temperate regions of the Northern Hemisphere, mainly distributed in Asia, Europe, and North America. The south of Hengduan Mountains in Southwest China (including West Sichuan, Northwest Yunnan and East Tibet) is an important distribution area of *Aconitum* in China.[1] *Aconitum* plants, as poisonous plants and medicinal plants, have been widely concerned for a long time. There are about 36 species of *Aconitum* plants available for medicine in China according to previous records.[2] The main characteristic components of *Aconitum* plants are diterpenoid alkaloids,[3] which have anti-inflammatory,[4-6] immunological,[7] analgesic,[8] cardiotonic,[9] antiarrhythmic,[10] anti-Alzheimer and other pharmacological activities.[11] Studies on diterpenoid alkaloids in *Aconitum* plants could contribute to the development of new natural medicines.[12-14] *Aconitum elliotii* Lauener, a herbaceous plant belonging to the family Ranunculaceae, is a species endemic to Motuo and Milin in eastern Tibet. At present, there is no relevant report on the chemical composition of *A. elliotii* Lauener. In our present investigation, three new alkaloids, elliotitine A (**1**), 8-*O*-ethylindaconitine (**2**) and 8-*O*-ethylbikhaconine (**3**), with 33 knowns 8-*O*-ethylforesticine (**4**), isoatisinium chloride (**5**),[15] talatisamine (**6**),[16] kongboendine (**7**),[17] crassicauline A

(**8**),[18] geniconitine (**9**),[19] 14-acetyltalatizamine (**10**),[20] indaconitine (**11**),[21] yunaconitine (**12**),[19] 6-epichasmanine (**13**),[22] condelphine (**14**),[16] isotalatisidine (**15**),[16] 14-*O*-anisoylneoline (**16**),[23] franchetine (**17**),[24] vilmorisine (**18**),[24] chasmaconitine (**19**),[25] acoforine(**20**),[26] sachaconitine (**21**),[27] 14-benzoylchasmanine (**22**),[28] kongboenine (**23**),[25] 8-acetyl-14-benzoylchasmanine (**24**),[18] columbidine (**25**),[20] 14-*O*-acetylsachaconitine (**26**),[29] 8-*O*-ethylyunaconitine (**27**),[30] austroconitine B (**28**),[31] 8-deacetyl-yunaconitine (**29**),[32] pseudaconine (**30**),[33] kongboentine B (**31**),[34] ludaconitine (**32**),[35] vilmorrianine C (**33**),[36] chellespontine (**34**),[37] pukeensine (**35**),[38] atidine (**36**)[39] were isolated from *A. elliotii* Lauener. Their structures were determined by spectral methods (HR-ESI-MS, 1D- and 2D-NMR) and X-ray spectroscopy. Here, the isolation and structure elucidation of those compounds and their anti-inflammatory activity are reported in this article.

RESULTS AND DISCUSSIONS

Compound **1** was obtained as a colorless amorphous powder. It's molecular formula was deduced as $C_{34}H_{45}NO_9$ on the basis of a protonated molecular ion peak at *m/z* 612.3188 $[M + H]^+$ in the HR-ESI-MS (calcd. for $C_{34}H_{45}NO_9$, 612.3173). The NMR spectrum revealed the presence of an *N*-ethyl group (δ_H 1.06, 3H, t, J = 7.2 Hz; δ_C 14.4 q, 46.0 t), three methoxy groups (δ_H 3.28, 3.29, 3.31, each 3H, s; δ_C 56.4 q, 56.3 q, 59.3 q), an anisoyl ester group (δ_H 6.91, 2H, d, J = 9.0 Hz; 8.05, 2H, d, J = 9.0 Hz; 3.85, 3H, s; δ_C 166.6 s, 123.2 s, 131.8 d × 2, 113.6 d × 2, 163.3 s, 55.6 q), an acetyl group (δ_H 1.44, 3H, s; δ_C 22.0 q, 169.7 s). The ^{13}C NMR spectra showed the presence of the other 19 carbon resonances, including eleven methines [δc 92.5, 86.3, 81.4, 78.7, 74.2, 62.5, 50.6, 50.2, 45.0, 41.8, 36.8], five methylenes [δc 79.9, 33.6, 27.8, 25.6, 24.9], and three quaternary carbons [δc 82.6, 46.9, 46.1]. Those spectroscopic data indicated compound **1** was an aconitine-type C_{19}-diterpenoid alkaloid.[40] In the HMBC spectrum, the long-range correlations between δ_H 3.31 (3H, s) and δ_C 86.3 (d), δ_H 3.29 (3H, s) and δ_C 81.4 (d), δ_H 3.28 (3H, s) and δ_C 79.9 (t) suggested three methoxy groups to be ascribed to C-1, C-16 and C-18 respectively. The anisoyl ester group could be assigned at C-14 due to the correlations from H-14 [δ_H 4.97 (1 H, t, J = 5.1 Hz)] to δ_C 166.6. The acetyl group was located at C-8 by the downfield chemical shift of carbon of C-8 (δ_C 82.6), with this observations supported by the HMBC correlations among H-7 (δ_H 3.41), H-9 (δ_H 2.37), H-14 (δ_H 4.97) to C-8.[41] By comparison of ^{13}C NMR data of **1** with those of the known compound transconitine D,[42] the similarity showed that compound **1** and transconitine D have similar structure, except the hydroxy group at C-8 in transconitine D was replaced by the acetyl group. The stereochemistry of compound **1** was deduced from the vicinal coupling constants and NOESY experiment (Figure 3). In the NOESY experiment, the cross-peak between H-1 and H-5, H-16 and H-17 proved the β-orientation of H-1 and 16-OMe. The H-6 was determined to have a β-orientation as well based on the correlation between H-6 and H-9 in the ^{1}H NOESY spectrum. H-14 at δ_H 4.97 (1H, t, J = 5.1 Hz) confirmed the β-position of H-14.[43] Up to now, the

absolute configuration of the skeleton of aconitine-type C_{19}-diterpenoid alkaloid has already been confirmed by X-ray crystallographic analysis.[44] Thus, the structure of compound **1** was elucidated as shown in Figure 2 and named elliotitine A.

Compound **2** was obtained as a white amorphous powder. It's molecular formula was deduced as $C_{34}H_{49}NO_9$, on the basis of a protonated molecular ion at *m/z* 616.3479 $[M+H]^+$ in the HR-EMI-MS (calcd. for $C_{34}H_{49}NO_9$, 616.3486). The NMR spectra revealed the presence of an *N*-ethyl group (δ_H 1.08, 3H, t, J = 6.9 Hz; δ_C 13.4 q, 48.6 t), four methoxy groups (δ_H 3.23, 3.26, 3.30, 3.54, each 3H, s; δ_C 55.9 q, 58.7 q, 59.1 q, 58.8 q), an benzoyl group (δ_H 7.44, 2H, t, J = 7.2 Hz; 8.07, 2H, d, J = 7.1 Hz; 7.53, 1H, t, J = 7.8 Hz; δ_C 166.4 s, 130.7 s, 129.7 d × 2, 128.2 d × 2, 132.7 d), an ethoxy group (δ_H 0.54, 3H, t, J = 6.8 Hz; δ_C 15.2 q, 55.9 t). Comparing the ^{13}C NMR data of **2** with those of ludaconitine,[35] a known compound, the signal of C-8 was shifted from δ_C 85.5 in indaconitine to δ_C 78.3 in **2**, which indicated the acetyl group was replaced by the ethoxy group.[42] The HMBC correlation of the ethoxy group (δ_H 3.35) with C-8 (δ_C 78.3) further confirmed the location of the ethoxy group. The relative configuration of compound **2** was deduced from a NOESY experiment (Figure 3). In the NOESY spectrum of **2**, the cross-peak between H-1 and H-5, H-3 and H-5, H-6 and H-9, H-10 and H-14 indicated that 1-OMe, 3-OH, 6-OMe and the benzoyl group were α-oriented. Moreover, H-17 was correlated to H-16 suggested 16-OMe was at β-position. Previous study has shown that the acetyl group at C-8 could be converted to ethoxy group by the substitution reaction under the condition of ethanol thermal reflux.[45] Accordingly, the structure of **2** was established as 8-*O*-ethylindaconitine (Figure 2).

Compound **3** was obtained as a white amorphous powder. It's molecular formula was deduced as $C_{27}H_{45}NO_7$, on the basis of a protonated molecular ion at *m/z* 496.3294 $[M+H]^+$ in the HR-EMI-MS (calcd. for $C_{27}H_{45}NO_7$, 496.3274). The NMR spectra revealed the presence of an *N*-ethyl group (δ_H 1.13, 3H, t, J = 7.3 Hz; δ_C 13.9 q, 49.3 t), four methoxy groups (δ_H 3.31, 3.25, 3.45, 3.33, each 3H, s; δ_C 59.1 q, 56.4 q, 58.8 q, 59.2 q), an ethoxy group (δ_H 1.18, 3H, t, J = 7.0 Hz; δ_C 16.1 q, 56.7 t). The four methoxy groups should be assigned to C-1, C-6, C-14 and C-18 based on the correlations between 1-OMe (δ_H 3.31) and C-1 (δ_C 85.5 d), 6-OMe (δ_H 3.25) and C-6 (δ_C 83.0 d), 16-OMe (δ_H 3.45) and C-16 (δ_C 84.0 d), 18-OMe (δ_H 3.33) and C-18 (δ_C 80.1 t). The NMR data of **3** were very similar to those of the known compound bikhaconine[46] except for the hydroxy group at C-8 was instead by the ethoxy group. The full assignment of **3** was based on the 1D and 2D NMR spectral data (Table 1). Accordingly, the structure of **3** was determined as shown (Figure 2), and it was named 8-*O*-ethylbikhaconine. Similar to compound **2**, compound **3** might also be an isolation artifact formed during the extraction.

Compound **4** was obtained as a white amorphous powder. Its molecular formula was deduced as $C_{26}H_{43}NO_6$. It was identified as the known compound 8-*O*-ethylforesticine[47] by 1D and 2D NMR. So far, no NMR data of this compound has been reported, and we report its ^{1}H and ^{13}C NMR data for the first time (Table 2).

Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **1-3**

(^{1}H: 400 MHz; ^{13}C: 100 MHz, $CDCl_3$)

Position	(**1**)		（**2**）		（**3**）	
	δ_C	δ_H(J = Hz)	δ_C	δ_H(J = Hz)	δ_C	δ_H(J = Hz)
1	86.3 d	3.24 s	82.7 d	3.12 m	85.5 d	3.05 *
2	24.9 t	1.85 m	33.4 t	1.93 m	29.8 t	1.28*
		2.38 m		2.35 m		1.42 d (7.5)
3	25.6 t	1.66 m	71.8 d	3.79 m	35.9 t	2.32 m
		2.10 m				
4	46.1 s		43.1 s		39.2 s	
5	50.6 d	2.00 s	48.7 d	2.35 s	49.2 d	2.41 s
6	78.7 d	4.47 m	83.2 d	4.04 d (5.8)	83.0 d	4.09 d (6.7)
7	50.2 d	3.41 s	46.0 d	2.09 s	49.5 d	2.31 s
8	82.6 s		78.3 s		78.9 s	
9	41.8 d	2.37 t (5.1)	45.9 d	2.68 t (6.2)	49.2 d	2.59 br.s
10	45.0 d	1.94 d (6.1)	41.3 d	2.06 m	41.5 d	1.94 *
11	46.9 s		50.7 s		50.7 s	
12	27.8 t	1.72 d (6.8)	35.7 t	2.09 m	36.6 t	1.98 *
		2.00 d (4.4)		2.52 m		
13	36.8 d	2.58 m	75.2 s		77.4 s	
14	74.2 d	4.97 t (5.1)	79.4 d	4.86 d (4.9)	78.6 d	3.88 d (4.2)
15	33.6 t	2.54 d (5.5)	37.6 t	2.32 d (4.8)	36.6 t	1.98 *
16	81.4 d	3.28 s	83.8 d	3.42 t (6.4)	84.0 d	3.35 s
17	62.5 d	3.31 *	61.0 d	2.72 s	62.1 d	2.93 s
18	79.9 t	2.94 d (9.0)	77.0 t	3.47 d (8.7)	80.1 t	3.08 d (8.5)
		3.19 d (8.7)		3.57 d (8.4)		3.68 d (7.3)
19	92.5 d	4.34 s	47.6 t	2.89 d (9.8)	53.6 t	2.62 *
21	45.9 t	2.62 d (7.3)	48.6 t	2.49 m	49.3 t	2.60 *
		2.85 d (7.0)				
22	14.4 q	1.06 t (7.0)	13.4 q	1.08 t (6.9)	13.9 q	1.13 t (7.3)
OMe-1	56.4 q	3.31 s	55.9 q	3.23 s	59.1 q	3.31 s
OMe-6			58.7 q	3.26 s	56.4 q	3.25 s
OMe-16	56.3 q	3.29 s	58.8 q	3.54 s	58.8 q	3.45 s
OMe-18	59.3 q	3.28 s	59.1 q	3.30 s	59.2 q	3.33 s
OEt-8			55.9 t	3.12 *	56.7 t	3.52 m
				3.35 d (7.0)		
			15.2 q	0.54 t (6.8)	16.1 q	1.18 t (7.0)
OAc-8	169.7 s					
	21.9 q	1.45 s				
OBz-14			166.4 s			
OAs-14	166.6 s					
1’	123.2 s		130.7 s			
2’, 6’	131.8 d	8.05 d (9.0)	129.7 d	8.07 d (7.1)		
3’, 5’	113.6 d	6.91 d (9.0)	128.2 d	7.44 t (7.2)		
4’	163.3 s		132.7 d	7.53 t (7.8)		
4’-OMe	55.6 q	3.85 s				

*means overlapped

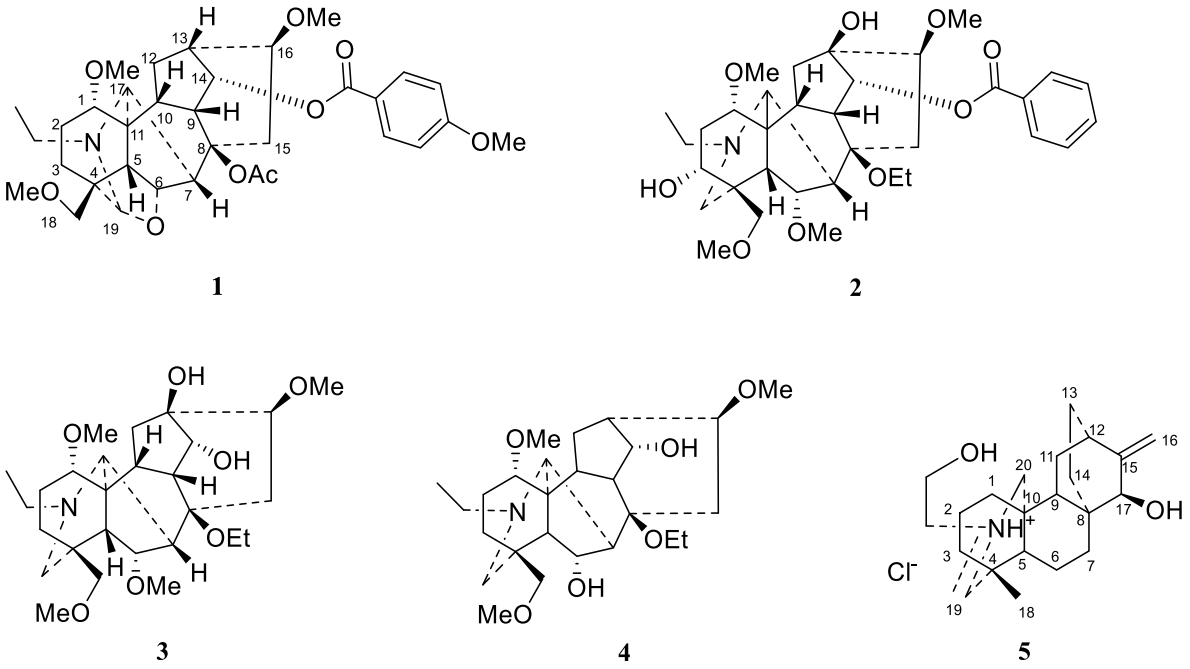

Figure 1. Structures of compounds **1-5**

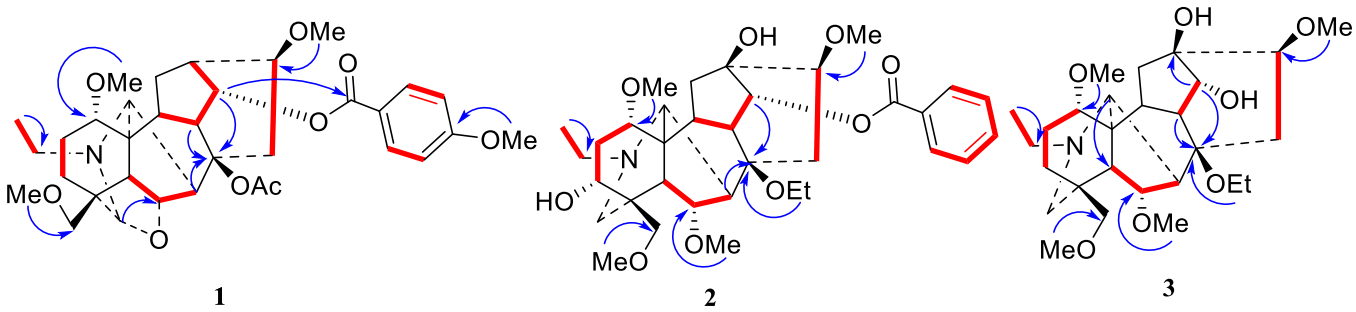

Figure 2. Key HMBC and ^{1}H-^{1}H COSY correlations for compounds **1**–**3**

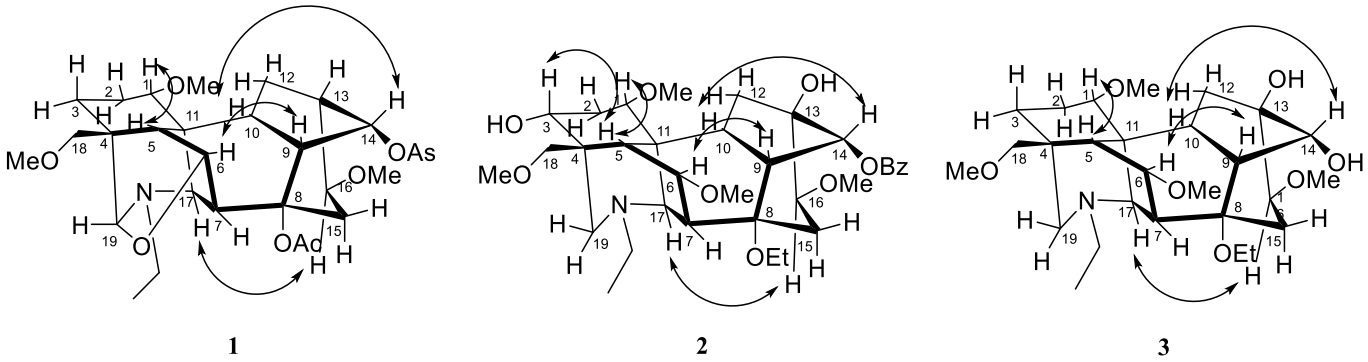

Figure 3. Key NOESY correlations of compounds **1-3**

Compound **5** was identifed as isoatisinium chloride[15] by compared NMR data with literature, and its absolute configuration has been unambiguously established by X–ray crystallographic analysis (Figure 4) for the first time.

Table 2. ^{1}H and ^{13}C NMR spectral data for compounds **4**
(^{1}H: 400 MHz; ^{13}C: 100 MHz, $CDCl_3$)

Position	δ_C	δ_H(J = Hz)	Position	δ_C	δ_H(J = Hz)
1	85.4 d	3.01 m	14	75.4 d	4.03 m
2	26.5 t	1.92 *	15	36.1 t	2.22 *
		2.33 *	16	82.8 d	3.30 s
3	36.7 t	1.62 m	17	62.2 d	2.85 s
4	38.9 s		18	82.1 t	3.23 d (8.1)
5	50.2 d	2.37 s			3.72 d (8.9)
6	72.2 d	4.77 d (7.1)	19	54.9 t	2.70 *
7	48.1 d	2.14 m	21	49.2 t	2.56 *
8	78.9 s		22	13.7 q	1.09 t (6.3)
9	51.7 d	2.01 d (5.2)	OMe-1	56.4 q	3.24 s
10	45.5 d	1.83 m	OMe-16	56.6 q	3.37 s
11	50.8 s		OMe-18	59.4 q	3.32 s
12	29.9 t	1.86 *	OEt-8	56.7 t	3.49 m
		2.28 m			3.58 m
13	39.8 d	2.28 m		16.3 q	1.15 t (7.2)

*means overlapped

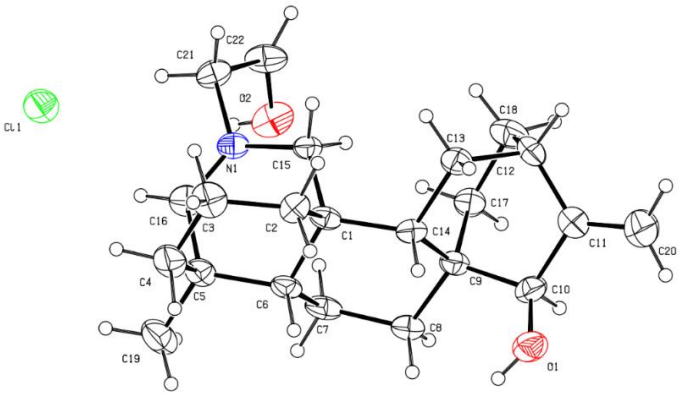

Figure 4. ORTEP drawing of **5**

The inhibitory activities on NO production stimulated by LPS in RAW264.7 macrophage cells of all compounds were determined by Griess method,[48] however, none of those compounds showed obvious activity.

EXPERIMENTAL

General experimental procedures.

Optical rotations were determined using a PerkinElmer polarimeter (Perkin Elmer Instruments (Shanghai) Co., Ltd., Waltham, America) with a sodium lamp operating at 598 nm and 20 ℃. The HR-ESI-MS data

were measured using a Q-TOF micro mass spectrometer (Waters, Milford, America). The 1D and 2D NMR spectra were recorded on a Bruker AV 400 NMR spectrometer (Brock Technology Co., LTD, Karlsruhe, Germany) with TMS as an internal standard. Thin-layer chromatography silica gel GF 254 plates and silica gel G and H (200−400 mesh) for column chromatography were purchased from Qingdao Ocean Chemical Plant (Qingdao, China). TLC plates were precoated with silica gel GF254 (Qingdao Haiyang Chemical Co., Ltd., China) and visualized under a UV lamp at 254 nm or by spraying the Dragendorff's reagent or by iodine. Unless otherwise specified, the reagents and solvents used in this work were all commercially available analytical or chemical grades and used directly without any purification.

Plant material.

Aconitum elliotii Lauener was collected from Bomê County, Nyingchi, Tibet, China, in August 2021 and were identifed by Professor Guo-Dong Li of the Yunnan University of Chinese Medicine. A voucher specimen (No. 3615HS20210805) was deposited in the School of Life Science and Engineering, Southwest Jiaotong University.

Extraction and Isolation.

Dried and powdered roots of *A. elliotii* Lauener (40.0 kg) were extracted with 95% EtOH (3 × 20 L) at room temperature for seven days. After removal of the solvent by evaporation, the EtOH extract (4.71 kg) was obtained. The extract was suspended in H_2O (5 L) and the pH was adjusted to 2.0 with 3% HCl solution, and extracted with petroleum ether (3 × 2 L) and EtOAc (3 × 2 L). Then the pH of aqueous layer was adjusted to 10.0 with 10% aq. NaOH solution and was extracted with CH_2Cl_2 (4 × 2 L). The CH_2Cl_2 extracts were concentrated to obtain the alkaloid extract (397.39 g). The alkaloid extract was chromatographed on a silica gel column, eluting with a PE-EtOAc-Et_2NH (50:1:0.05-0:1:0.05) gradient system, to give fractions (Fr.) A (15.23 g), B (55.86 g), C (31.68 g), D (29.8 g), E (2.27 g), F (32.82 g), G (41.34 g) and H (146.44 g) based on TLC analyses. Fr. A was further chromatographed on a silica gel column employing PE-EtOAc-Et_2NH (80:1:0.05-20:1:0.05) as eluent to afford Fr. A-1 (4.89 g), A-2 (1.06 g), A-3 (6.58 g) and A-4 (2.11 g). Fr. A-3 was separated on a silica gel colum (CH_2Cl_2-MeOH, 120:1-0:1) to obtain compounds **17** (177.6 mg), **18** (48.3 mg), **19** (49.6 mg) and **20** (92.4 mg). Separation of Fr. A-4 by silica gel column chromatography using CH_2Cl_2-MeOH (100:1-0:1) as eluent provided compound **5** (103.8 mg). Fr. C and D were loaded onto a silica gel column and eluted with CH_2Cl_2-MeOH (100:1-0:1) to obtain compound **33** (13.8 mg) and compound **36** (196.0 mg). Fr. E was chromatographed on a silica gel column using PE-EtOAc-Et_2NH (100:2:0.5-20:2:0.5) as eluent to give Fr. E-1 (155.6 mg), E-2 (286.7 mg), E-3 (625.3 mg), E-4 (284.9 mg), E-5 (42.1 mg), E-6 (229.8 mg) and E-7 (228.5 mg). Fr. E-1 were loaded onto a silica gel column, eluting with CH_2Cl_2-MeOH (100:1-0:1) to obtain compounds **6** (51.8 mg) and **9** (21.6 mg). Compounds **7** (38.1 mg), **8** (29.3 mg), **10** (12.8 mg) and **1** (6.8 mg) were obtained by purifying Fr. E-2 by silica gel column eluting with PE -Et_2NH (100:1-20:1). Fr. E-4 was isolated on a silica gel column eluted

with PE -Et_2NH (100:1-10:1) to yield compounds **11** (12.6 mg), **12** (19.5 mg), **13** (20.8 mg), **15** (23.9 mg) and **16** (12.4 mg). Fr. E-5 and E-6 were subjected to silica gel column and eluted with CH_2Cl_2-MeOH (90:1-0:1) to get compounds **14** (24.7 mg), **2** (34.6 mg) and **3** (20.5 mg). Chromatograph of Fr. E-7 over a silica gel column, using CH_2Cl_2-MeOH (90:1-0:1) as eluent provided compound **34** (68.2 mg). Fr. F and G were chromatographed together on a silica gel column with CH_2Cl_2-MeOH (80:1-0:1) as eluent to give Fr. G-1 (2.569 g), G-2 (12.460 g), G-3 (19.593 g), G-4 (14.926 g), G-5 (8.996 g), G-6 (10.302 g). Further purification of G-4 and G-5 by silica gel column chromatography using PE-EtOAc-Et_2NH (70:1:0.5-10:1:0.5) as eluent gave compounds **21** (11.4 mg), **22** (9.9 mg), **23** (25.3 mg), **24** (9.4 mg), **25** (17.8 mg), **26** (8.4 mg), **27** (336.1 mg), **35** (11.3 mg); repeated chromatography of Fr. G-4 and G-5 on a silica gel column (CH_2Cl_2-MeOH, 60:1–0:1) yielded compounds **28** (11.6 mg), **29** (6.8 mg), **30** (13.0 mg), **31** (24.8 mg), **32** (56.3 mg)and **4** (11.2 mg).

Elliotitine A (**1**)

White amorphous powder; $[\alpha]_D^{20}$ -40.6 (*c* 0.10, $CHCl_3$); HR-ESI-MS *m/z* 612.3188 $[M + H]^+$ (calcd. for $C_{34}H_{45}NO_9$, 612.3173); ^1H and ^{13}C NMR data see Table 1.

8-*O*-Ethylindaconitine (**2**)

White amorphous powder; $[\alpha]_D^{20}$ +18.0 (*c* 0.20, $CHCl_3$); HR-ESI-MS *m/z* 616.3479 $[M + H]^+$ (calcd. for $C_{34}H_{49}NO_9$, 616.3486); ^1H and ^{13}C NMR data see Table 1.

8-*O*-Ethylbikhaconine (**3**)

White amorphous powder; $[\alpha]_D^{20}$ +14.4 (*c* 0.20, $CHCl_3$); HR-ESI-MS *m/z* 496.3294 $[M + H]^+$ (calcd. for $C_{27}H_{45}NO_7$, 496.3274); ^1H and ^{13}C NMR data see Table 1.

8-*O*-Ethylforesticine (**4**)

White amorphous powder; $[\alpha]_D^{20}$ +8.8 (*c* 0.10, $CHCl_3$); HR-ESI-MS *m/z* 466.3180 $[M + H]^+$ (calcd. for $C_{26}H_{43}NO_6$, 466.3169); ^1H and ^{13}C NMR data see Table 2.

Isoatisinium chloride (**5**)

Colorless needle crystal; $[\alpha]_D^{20}$ +110.7 (*c* 0.40, CH_3OH); HR-ESI-MS *m/z* 344.2596 $[M + H]^+$ (calcd. for $C_{22}H_{34}NO_2$.Cl, 344.2584).

Single-crystal X-ray crystallography of 5.

The colorless needle crystal of **5** was obtained from dichloromethane at room temperature for several days. The crystal structure and absolute configuration of **5** were determined by the crystallography data collected

on a Super Nova, Dual, Cu at zero, and exposed to graphite – monochro mated Mo Kα irradiation. The crystal was kept at 293.15 K during data collection. Structure determination and refinement were performed by using SHELXL program. Crystallographic data of **5** was deposited into Cambridge Crystallographic Data Centre, and can be obtained free of charge from the CCDC Web site (www.ccdc.cam.ac.uk). CCDC NO. 2244373 contains the supplementary crystallographic data for this paper.

Crystal data of **5**: $C_{22}H_{34}NO_2.Cl$ (M = 378.94 g/mol), orthorhombic, space group $P2_12_12_1$, a = 7.7284(10) Å, b = 14.1348(19) Å, c = 18.518(3) Å, V = 2022.9(5) Å^3, Z = 4, T = 293.15 K, μ (MoKα) = 0.205 mm^{-1}, *Dcalc* = 1.244 g/cm^3, Flack parameter = 0.07(5), 6094 reflections measured (6.17° ≤ 2Θ ≤ 52.744°), 3692 unique (R_{int} = 0.0470, R_{sigma} = 0.0866) which were used in all calculations. The final R^1 was 0.0679 (I > 2σ(I)) and wR^2 was 0.1737, respectively.

Inhibitory activity of NO production

Inhibitory activities on NO production in RAW264.7 cells of all compounds isolated from *Aconitum elliotii* Lauener were assayed according to the procedure described in literature.[49] RAW264.7 macrophage cells were seeded in 96-well plates at a density of 8 × 10^3 cells/mL per well and coincubated with drugs (30 μM/mL) and LPS (1 μg/mL) for 24 h. The amount of NO was assessed by determining the nitrite concentration in the cultured RAW264.7 macrophages supernatants with Griess reagent. Aliquots of supernatants (100 μg/mL) were incubated, in sequence, with 50 μL 1% sulfanilamide and 50 μL 0.1% naphthyl ethylenediamine in 2.5% phosphoric acid solution. The absorbance at 570 nm was read using a microplate reader.

SUPPLEMENTARY DATA

Supplementary (synthesis of the starting azides, HPLC chromatograms, IR, ^{1}H and ^{13}C NMR, MS spectra, etc.) data associated with this article can be found, in the online version, at URL: https://www.heterocycles.jp/newlibrary/downloads/PDFsi/27905/106/5

ACKNOWLEDGEMENT

This work was financially supported by the National Natural Foundation of China (82073734) and the Fundamental Research Funds for Central Universities (2682022ZTPY078).

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

REFERENCES

1. P. G. Xiao and F. P. Wang, *J. Syst. Evol.*, 2006, **44**, 46.
2. H. L. Fu, *Zhong Yao Cai*, 2004, **27**, 149.
3. P. G. Xiao, F. P. Wang, F. Gao, L. P. Yan, D. L. Chen, and Y. Liu, *Acta Pharm. Sin.*, 2006, **44**, 46.
4. S. Verma, S. Ojha, and M. Raish, *J. Med. Plants Res.*, 2010, **4**, 1566.
5. Y. Shu, J. P. Wang, X. Y. Cai, X. L. Li, and Z. T. Ding, *Tetrahedron*, 2020, **76**, 131520.
6. L. L. Qin, F. Xing, L. Chen, and X. L. Zhou, *Heterocycles*, 2021, **102**, 1330.
7. Y. X. Wang and K. Xiao, *Chin. J. Inf. Tradit. Chin. Med.*, 1989, **5**, 40.
8. L. Y. Lin, Q. H. Chen, and F. P. Wang, *West. China J. Pharm. Sci.*, 2004, **19**, 200.
9. B. Y. Jiang, S. Lin, C. G. Zhu, S. J. Wang, and J. G. Shi, *J. Nat. Prod.*, 2012, **75**, 1145.
10. B. T. Salimov, K. K. Turgunjov, B. Tashkhodzhaev, and F. N. Dzhakhangirov, *Chem. Nat. Compd.*, 2004, **40**, 151.
11. M. Reina and A. González-Coloma, *Phytochem. Rev.*, 2007, **6**, 81.
12. X. Zhou, O. A. F. Firas, W. Xu, and L. Shan, *Heterocycles*, 2017, **94**.
13. W. L. Xu, L. Chen, F. Gao, and X. L. Zhou, *Heterocycles*, 2018, **96**, 1631.
14. J. F. Zhang, L. Chen, and X. L. Zhou, *Heterocycles*, 2018, **96**, 304.
15. Y. Han, J. H. Liu, and M. S. Wang, *Zhong Cao Yao*, 1994, **12**, 619.
16. S. W. Pelletier, L. H. Keith, and P. C. Parthasarathy, *Tetrahedron Lett.*, 1966, **7**, 4217.
17. P. A and D. L. Chen, *Nat. Prod. Res. Dev.*, 2002, **5**, 6.
18. F. P. Wang and Q. Fang, *Planta Med.*, 1981, **42**, 375.
19. S. Zheng, L. Gao, X. Hao, X. Wang, and X. Shen, *Phytochemistry*, 1997, **46**, 951.
20. S. W. Pelletier, S. K. Srivastava, B. S. Joshi, and J. D. Olsen, *Heterocycles*, 1985, **23**, 778.
21. R. E. Gilman and L. Marion, *Tetrahedron Lett.*, 1962, **3**, 923.
22. Z. B. Li and F. P. Wang, *Chin. Chem. Lett.*, 1996, **5**, 443.
23. H. S. Sang, S. K. Ju, and S. S. Kang, *Chem. Pharm. Bull.*, 2003, **51**, 999.
24. F. P. Wang, Z. B. Li, X. P. Dai, and C. S. Peng, *Phytochemistry*, 1997, **45**, 1539.
25. J. M. Yue, Y. Z. Chen, and Y. Z. Li, *Phytochemistry*, 1990, **29**, 2379.
26. S. W. Pelletier, B. S. Joshi, J. A. Glinski, H. P. Chokshi, and K. Go, *Heterocycles*, 1987, **25**.
27. J. Yang, W. Liu, X. D. Yang, J. F. Zhao, F. Liu, and L. Li, *J. Honghe Uni.*, 2009, **34**, 1927.
28. F. P. Wang and S. W. Pelletier, *J. Nat. Prod.*, 1987, **50**, 55.
29. V. N. Aiyar, P. Kulanthaivel, and M. Benn, *Phytochemistry*, 1986, **25**, 973.
30. H. Ji, Q. H. Chen, and F. P. Wang, *Chem. Pharm. Bull.*, 2009, **57**, 233.
31. Z. Jiang, S. Chen, and J. Zhou, *Acta Botan. Yunnan*, 1989, **4**, 461.
32. S. Y. Chen and Y. Q. Liu, *Acta Botan. Yunnan*, 1984, **6**, 338.

33. K. K. Purushothaman and S. Chandrasekharan, *Phytochemistry*, 1974, **13**, 1975.
34. P. A, Q. H. Chen, D. L. Chen, X. X. Jian, and F. P. Wang, *J. Asian Nat. Prod. Res.*, 2004, **6**, 154.
35. S. W. Pelletier, K. S. Khetwal, B. S. Joshi, and H. K. Desai, *Heterocycles*, 1992, **34**, 441.
36. C. R. Yang, X. J. Hao, D. Z. Wang, and J. Zhou, *Acta Chim. Sin.*, 1981, **39**, 147.
37. S. W. Pelletier, H. K. Desai, B. S. Joshi, B. Sener, F. Bingöl, and T. Baykal, *Heterocycles*, 1993, **36**, 1081.
38. L. S. Ding, F. E. Wu, and Y. Z. Chen, *Acta Pharm. Sin.*, 1992, **27**, 394.
39. H. Ahmad, S. Ahmad, S. A. A. Shah, A. Latif, M. Ali, F. A. Khan, M. N. Tahir, F. Shaheen, A. Wadood, and M. Ahmad, *Bioorg. Med. Chem.*, 2017, **25**, 3368.
40. F. P. Wang, Q. H. Chen, and X. Y. Liu, *Nat. Prod. Rep.*, 2010, **27**, 529.
41. S. Huang, Y. M. Feng, J. L. Ren, Z. L. Yang, L. Chen, and X. L. Zhou, *Chin. J. Org. Chem.*, 2022, **42**, 1856.
42. D. L. Chen, X. X. Jian, Q. H. Chen, and F. P. Wang, *Acta Chim. Sin.*, 2003, **6**, 901.
43. L. Shan, L. Chen, F. Gao, and X. Zhou, *Nat. Prod. Lett.*, 2019, **33**, 3254.
44. F. P. Wang and Q. H. Chen, *The Alkaloids: Chemistry and Biology*, 2010, **69**, 1.
45. J. Zou, J. M. Shi, C. L. He, X. H. Liu, and D. L. Chen, *West. China J. Pharm. Sci.*, 2020, **35**, 127.
46. J. B. Hanuman and A. Katz, *Phytochemistry*, 1994, **36**, 1527.
47. S. W. Pelletier, S. Y. Chen, B. S. Joshi, and H. K. Desai, *J. Nat. Prod.*, 1984, **47**, 474.
48. Q. J. Guo, Z. L. Yang, L. Chen, S. Huang, C. S. Guo, and C. G. Zhang, *Nat. Prod. Res. Dev.*, 2022, **34**, 1148.
49. X. Y. Duan, D. K. Zhao, and Y. Shen, *J. Asian Nat. Prod. Res.*, 2019, **21**, 323.