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A NEW DENUDATINE TYPE C_{20}-DITERPENOID ALKALOID FROM *ACONITUM FISCHERI* VAR. *ARCUATUM*

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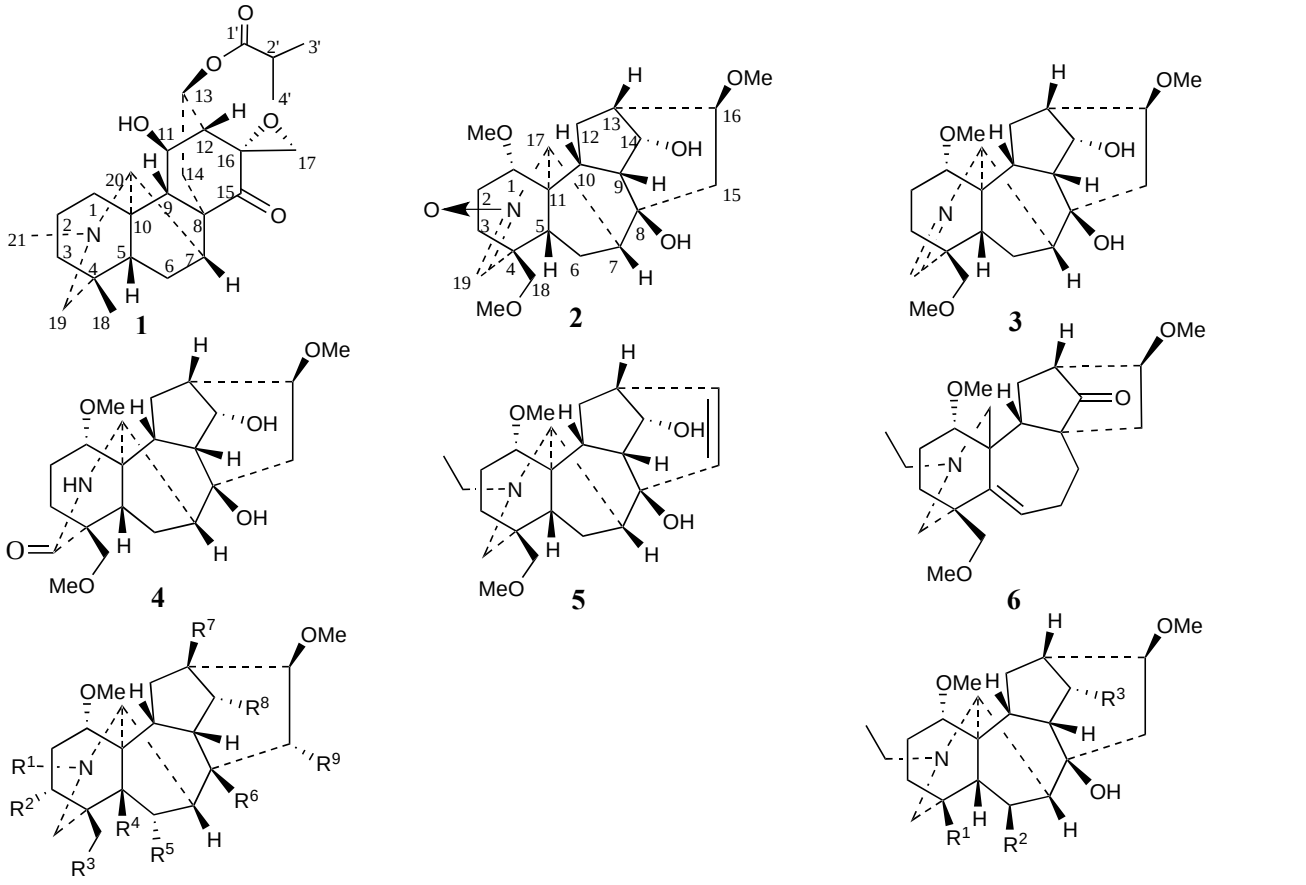
Abstract – A new denudatine-type C_{20}-diterpenoid alkaloid bearing a rare C-15 ketone carbonyl and 16,17-epoxy group, named arcutisine (**1**), together with 24 known compounds (**2-25**), were obtained from the roots of *Aconitum fischeri* var. *arcuatum*. Their structures were established by various spectroscopic analyses. Among them, compounds **1** and **2** were both isolated from a natural source for the first time. Besides, compounds **7** and **10** showed weak effect on anti-inflammatory activity with the inhibition rate of 33.5% and 33.7% at the concentration of 40 μM.

INTRODUCTION

Aconitum fischeri var. *arcuatum* (Ranunculaceae family) is widely distributed in Jilin Province, China. Diterpenoid alkaloids are typical ingredients displaying various bioactivities such as anticancer, analgesic activity, as well as vasodilator effects.[1] In previous studies, only two C_{20}-diterpenoid alkaloids and two C_{19}-diterpenoid alkaloids have been obtained from this plant.[2] Continuing investigations seeking new active compounds from *Aconitum fischeri* var. *arcuatum* led to the isolation of 25 alkaloids (Figure 1), including a new C_{20}-diterpenoid alkaloid, arcutisine (**1**). Arcutisine (**1**) is the first denudatine-type C_{20}-diterpenoid alkaloid with a rare C-15 ketone carbonyl and 16,17-epoxy group from a natural source. Twenty-four known compounds, nagadine nitrone (**2**),[3] nagadine (**3**),[4] piepunensine A (**4**),[5] liljestrandinine (**5**),[6] hemsleyaconitine G (**6**),[7] talatisamine (**7**),[5] 14-acetyltalatisamine (**8**),[8] 14-acetyl-8-methyltalatisamine (**9**),[9] acoforine (**10**),[9] 14-benzoylaconine (**11**),[10] indaconitine (**12**),[8] 3-deoxyaconitine (**13**),[10] columbidine (**14**),[9] hemsleyanine C (**15**),[11] cammaconine (**16**),[12] mesaconitine (**17**),[10] 14-benozylmesaconine (**18**),[10] hypaconitine (**19**),[10] *N*-deethyltalatisamine (**20**),[6] sachaconitine (**21**),[13] aconosine (**22**),[5] akiran (**23**),[14] dolaconine (**24**)[15] and vilmorine D (**25**)[16] were identified by various spectroscopic methods (HR-ESI-MS, IR, NMR) and comparison with literature. Compound **2**, a C_{19}-diterpenoid alkaloid with a nitrone functionality, was isolated from natural for the first time.

Meanwhile, compounds (**1-25**) were evaluated for their anti-inflammatory effects against LPS-induced nitric oxide (NO) production in RAW264.7 cells.



7 R^1=Et, R^2=H, R^3=OMe, R^4=H, R^5=H, R^6=OH, R^7=H, R^8=OH, R^9=H
8 R^1=Et, R^2=H, R^3=OMe, R^4=H, R^5=H, R^6=OH, R^7=H, R^8=OAc, R^9=H
9 R^1=Et, R^2=H, R^3=OMe, R^4=H, R^5=H, R^6=OMe, R^7=H, R^8=OAc, R^9=H
10 R^1=Et, R^2=H, R^3=OMe, R^4=H, R^5=H, R^6=OEt, R^7=H, R^8=OAc, R^9=H
11 R^1=Et, R^2=OH, R^3=OMe, R^4=H, R^5=OMe, R^6=OH, R^7=OH, R^8=OBz, R^9=OH
12 R^1=Et, R^2=OH, R^3=OMe, R^4=H, R^5=OMe, R^6=OAc, R^7=OH, R^8=OBz, R^9=H
13 R^1=Et, R^2=H, R^3=OMe, R^4=H, R^5=OMe, R^6=OAc, R^7=OH, R^8=OBz, R^9=OH
14 R^1=Et, R^2=H, R^3=OMe, R^4=H, R^5=H, R^6=OEt, R^7=H, R^8=OH, R^9=H
15 R^1=Et, R^2=H, R^3=OMe, R^4=OH, R^5=H, R^6=OH, R^7=H, R^8=OH, R^9=H
16 R^1=Et, R^2=H, R^3=OH, R^4=H, R^5=H, R^6=OH, R^7=H, R^8=OH, R^9=H
17 R^1=Me, R^2=OH, R^3=OMe, R^4=H, R^5=OMe, R^6=OAc, R^7=OH, R^8=OBz, R^9=OH
18 R^1=Me, R^2=OH, R^3=OMe, R^4=H, R^5=OMe, R^6=OH, R^7=OH, R^8=OBz, R^9=OH
19 R^1=Me, R^2=H, R^3=OMe, R^4=H, R^5=OMe, R^6=OAc, R^7=OH, R^8=OBz, R^9=OH
20 R^1=H, R^2=H, R^3=OMe, R^4=H, R^5=H, R^6=OH, R^7=H, R^8=OH, R^9=H
21 R^1=Et, R^2=H, R^3=H, R^4=H, R^5=H, R^6=OH, R^7=H, R^8=OH, R^9=H

22 R^1=H, R^2=H, R^3=H
23 R^1=OAc, R^2=OMe, R^3=OMe
24 R^1=H, R^2=H, R^3=OAc
25 R^1=H, R^2=H, R^3=OVr

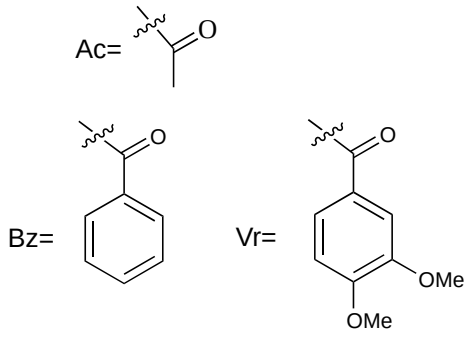


Figure 1. Structures of compounds **1-25**

RESULTS AND DISCUSSION

Compound **1** (white amorphous powder) showed a positive reaction with Dragendorff's reagent. Its molecular formula was deduced to be $C_{25}H_{35}NO_5$ by HR-ESI-MS. (*m/z* 430.2577 $[M + H]^+$, calcd. 430.2593). The IR (KBr) spectrum exibited absorption bands for hydroxyl group (3423 cm^{-1}) and

carbonyl group (1732 cm^{-1}). The NMR data revealed a typical *N*-methyl group [δ_H 2.24 (3H, s); δ_C 44.0, q], an isobutyryloxy group [δ_H 1.13, 1.15 (each 3H, d, J = 6.4 Hz); δ_C 18.8 q, 19.0 q] and a tertiary methyl group [δ_H 0.71 (3H, s); δ_C 26.3 q]. Its ^{13}C NMR data and DEPT spectra showed four methyls (δ_C 18.8, 19.0, 26.3 and 44.0), seven methylenes (δ_C 20.5, 24.6, 26.3, 34.2, 39.9, 49.8 and 59.1), eight methines (δ_C 34.1, 36.7, 50.2, 52.4, 54.9, 67.8, 69.0 and 71.7) and four quaternary carbons (δ_C 34.0, 45.8, 54.9, 58.2). The characteristic NMR data of **1** suggested it to be a C_{20}-diterpenoid alkaloid bearing a ketone carbonyl group (δ_C 207.3 s).

^1H and ^{13}C NMR resonances (δ_H 2.90, 2.93, ABq, J = 6.4 Hz; δ_C 49.8 t, 58.2 s) clearly indicated the presence of an epoxy moiety instead of a typical exocyclic double bond at the C_{16}-C_{17} position in C_{20}-diterpenoid alkaloids.[17] Moreover, correlations observed in the HMBCs between the signal at δ_H 2.90 (1H, d, J = 6.4 Hz, H-17α) with δ_C 50.2 (d, C-12) and 58.2 (s, C-16) confirmed the 16,17-epoxide group (Figure 2). The isobutyryloxy group could be positioned at C-13 according to the HMBC correlations from H-13 [δ_H 5.06 (1H, dd, J_1 = 8.4 Hz, J_2 = 4.4 Hz)] to Me_2CHCOO (δ_C 177.0 s). The existence of a remaining oxygenated carbon (δ_C 67.8 d) suggested that compound **1** possessed a hydroxyl group at C-11 in addition to the above-mentioned groups. This was also supported by the correlations between C-11 (δ_C 67.8 d)/H-13 (δ_H 5.06 dd, J_1 = 8.4 Hz, J_2 = 4.4 Hz) and C-11 (δ_C 67.8 d)/H-9 (δ_H 1.62 m) (Figure 2). The ketone carbonyl was located at C-15 based on the HMBC correlations from H-9 (δ_H 1.62 m), H-12 (δ_H 2.15 d, J = 3.0 Hz), H-14α (δ_H 2.51 m) and H-17β (δ_H 2.93 d, J = 6.4 Hz) to C-15 (δ_C 207.3 s). Comparison of the NMR data of **1** with those of sinchianine[18] revealed that they were both C_{20}-diterpenoids with a denudatine skeleton, except for the presence of a carbonyl group at C-15 and the ester side chain at C-13 in compound **1**. The planar structure of **1** was further verified by analysis of the HMBC and ^1H-^1H COSY spectra (Figure 2).

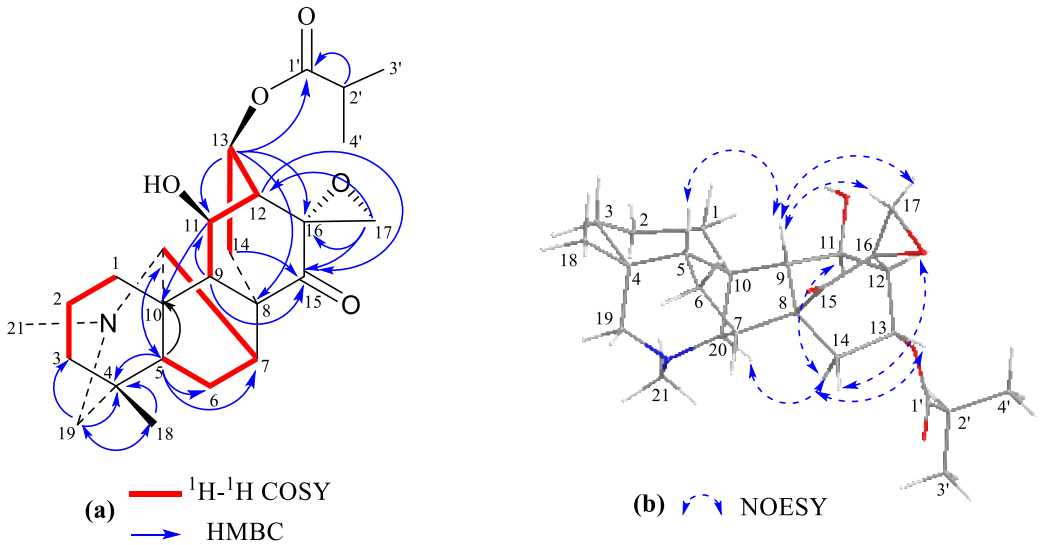


Figure 2. Key ^1H-^1H COSY (——), HMBC (⌒) and NOESY (⌒) correlations of compound **1**

The relative configuration of compound **1** was inferred from the key NOESY experiment (Figure 2). The NOESY correlations of H-5*β*/H-9 confirmed the *β*-position of H-9. Correlations from H-14 (δ_H 2.51 m) to H-20*α* revealed the *α*-orientation of H-14 (δ_H 2.51 m). Meanwhile, H-11 and H-13 were both installed at *α*-orientation based on the cross-peaks between H-11/H-14 (δ_H 2.51 m) and H-13/H-14 (δ_H 2.51 m) in the NOESY spectrum. H-9*β* showed a correlation with H-17 (δ_H 2.90, 2.93, ABq, J = 6.4 Hz), which indicated that the H-17 (δ_H 2.90, 2.93, ABq, J = 6.4 Hz) were at *β*-position, so the 16,17-epoxy group was deduced to be *α*-orientation. Thus, the structure of **1** was determined (Figure 1). The NMR data were shown in Table 1.

Table 1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) Data of **1** ($CDCl_3$, δ in ppm, J in Hz)

No.	δ_C	δ_H
1*α*	24.6 t	1.56 m
1*β*		1.42 m
2*α*	20.5 t	2.40 *
2*β*		1.48 m
3*α*	39.9 t	1.64 m
3*β*		1.20 m
4	34.0 s	—
5	52.4 d	1.18 m
6*α*	26.3 t	1.99 m
6*β*		1.76 m
7	36.7 d	2.35 m
8	54.9 s	—
9	54.9 d	1.62 m
10	45.8 s	—
11	67.8 d	4.07 d (9.8)
12	50.2 d	2.15 d (3.0)
13	69.0 d	5.06 dd (8.4, 4.4)
14*α*	34.2 t	2.51 m
14*β*		1.59 m
15	207.3 s	—
16	58.2 s	—
17*α*	49.8 t	2.90 ABq (6.4)
17*β*		2.93 ABq (6.4)
18	26.3 q	0.71 s
19*α*	59.1 t	2.23 *
19*β*		2.41 d (11.2)
20	71.7 d	3.12 s
21	44.0 q	2.24 s
13-O*C*OCH(Me)$_2$	177.0 s	—
13-OCO*CH*(Me)$_2$	34.1 d	2.54 m
13-OCOCH(*Me*)$_2$	19.0 q	1.13 d (6.4)
13-OCOCH(*Me*)$_2$	18.8 q	1.15 d (6.4)

*Overlapped signals

Compounds **3**, **5**, **8**, **12**, **16** were toxic for RAW264.7 cells at the concentration of 40 μM (Figure S10 in Supporting Information), while the other 20 compounds showed no significant cytotoxicity in RAW264.7 cells. Thus, these compounds were further used to evaluate anti-inflammatory activity. Compared with positive control, compounds **7** and **10** exhibited weak inhibitory activity against NO production in LPS-activated RAW264.7 macrophages with the inhibition rate of 33.5% and 33.7%, respectively (Table 2).

Table 2. Inhibition of NO (%)

Compd.	Inhibition (%)	Compd.	Inhibition (%)
1	2.7	**17**	19.2
2	1.8	**18**	23.6
4	27.1	**19**	17.0
6	30.7	**20**	28.5
7	33.5	**21**	27.4
9	18.0	**22**	32.6
10	33.7	**23**	31.5
11	24.3	**24**	32.0
13	19.9	**25**	27.0
14	14.6		
15	29.4	celecoxib	86.3

EXPERIMENTAL

General experimental procedure. Optical rotations were measured using a Perkin-Elmer 341 polarimeter. A Thermo Fisher Nicolet 6700 spectrometer was used for scanning IR spectroscopy (KBr pellets). HR-ESI-MS data were obtained by a Xevo G2QTOF/UPLC mass spectrometer (Waters). The NMR spectra were acquired with a Bruker AV 600 spectrometer relative to TMS as internal standard. Silica gel (Chengdu Kelong Chemical Co., Ltd.) and RP-18 silica gel (Merck) were utilized for column chromatography (CC). Spots were visualized by spraying with modified Dragendorff's reagent.

Plant material. *Aconitum fischeri* var. *arcuatum* was gathered from Linjiang, Jilin Province, P. R. China, in August 2017. A voucher specimen (Swjtu-An-201706) was deposited at Southwest Jiaotong University.

Extraction and isolation. 3.5 kg air-dried, powdered roots of *Aconitum fischeri* var. *arcuatum* were soaked with 95% EtOH (4 × 25 L) at room temperature for 3 days. Removal of the solvent afforded crude alkaloids-contained extract. Then the residue was suspended in H_2O and adjusted the pH to 2-3 by HCl solution. The suspension was extracted with petroleum ether (4 × 2 L) afterwards and the acidic aqueous layer was then basified to pH 9-10 using aqueous ammonia solution. Finally, the H_2O phase was extracted with CH_2Cl_2 to obtain the crude alkaloids (60 g).

The crude alkaloids (60 g) were chromatographed over silica gel column eluting with CH_2Cl_2/MeOH gradient system (*v/v*, 200:1) to give fractions A–F. Fraction A was separated by silica gel CC with petroleum ether (PE)/acetone/diethylamine (*v/v/v*, 90:1:0.1) to yield compounds **6** (48 mg), **9** (15 mg), **10** (15 mg) and **15** (80 mg). Column chromatography of fraction B (9.6 g) with PE/ethyl acetate (EtOAc)/ diethylamine (*v/v/v*, 80:1:0.2) as eluent afforded 4 fractions (Fr. B_1–Fr. B_4). Further silica gel CC purification of Fr. B_2 (700 mg) was accomplished by elution with CH_2Cl_2/MeOH (*v/v*, 200:1) to afford compounds **7** (37 mg), **8** (25 mg) and **17** (4 mg). Fraction B_3 (150 mg) was purified over RP-18 silica gel with MeOH /water (*v/v*, from 10 to 90%) to obtain **2** (7 mg), **3** (9 mg) and **4** (5 mg). Fraction B_4 (60 mg) was submitted to RP-18 silica gel CC eluting with MeOH/water (*v/v*, from 10 to 100%) to give compounds **19** (10 mg), **20** (3 mg) and **23** (6 mg). Compounds **1** (6 mg) and **21** (4 mg) were acquired by purifying Fr. C (2 g) using a CH_2Cl_2/MeOH (*v/v*, 200:1) mixture. Fraction E (8.6 g) was subjected to silica gel CC with CH_2Cl_2/MeOH (*v/v*, 200:1) to yield compounds **5** (9 mg), **13** (10 mg), **14** (20 mg), **24** (21 mg) and **25** (20 mg). Fraction F (14.6 g) was loaded onto a silica gel column eluting with CH_2Cl_2/MeOH (*v/v*, 50:1) to yield **11** (17 mg), **12** (20 mg), **16** (46 mg), **18** (80 mg) and **22** (52 mg).

Arcutisine (1)

White amorphous powder; $[\alpha]_D^{20}$ -2.8 (*c* 0.06, $CHCl_3$); IR (KBr) ν_{max} 3423, 2965, 2925, 2815, 1732, 1494, 1453, 1396, 1366, 1252, 1190, 1094, 1028, 997, 955 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) data and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 1; HR-ESI-MS at *m/z* 430.2577 $[M + H]^+$ (calcd. for $C_{25}H_{36}NO_5$, 430.2593).

Anti-inflammatory activity assay

In this study, the procedures of anti-inflammatory activity assay were conducted according to previous literature.[19] RAW264.7 macrophages were seeded onto 96-well plates (5×10^3 cells/well) and incubated at 37 °C with 5% CO_2 for 24 h. Cell viability of compounds **1-25** was first determined by MTT assay. Then the non-toxic compounds (cell viabilities exceeded 85%) were further evaluated for inhibitory activities, by measuring the nitrite concentration in the supernatant with Griess reagent. The absorbance at 492 nm was read using a micro plate reader. Celecoxib was used as positive control.

ACKNOWLEDGEMENTS

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