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Diterpenoid Alkaloids from the Lateral Root of *Aconitum carmichaelii*

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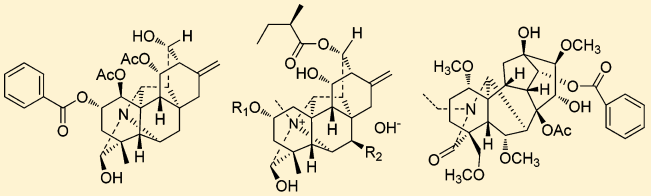
Supporting Information

ABSTRACT: Twenty-six new diterpenoid alkaloids, **1**–**26** (**1**–**4**: hetisan-type C_{20}-diterpenoid alkaloids; **5**–**26**: aconitane C_{19}-diterpenoid alkaloids), and two known analogues, hypaconitine **27** and benzoylmesaconine **28**, have been isolated from a water extract of the lateral root of *Aconitum carmichaelii*. Compounds **7** and **8** are rare examples of conformational isomers obtained from the same material. The conformation and conformational transformation of ring A in the C_{19}-diterpenoid alkaloids are discussed on the basis of NMR data analysis in combination with single-crystal X-ray crystallography of **6** and **27** by anomalous scattering of Cu Kα radiation. In preliminary analgesic and toxicity assays, the isomer with ring A in the chair conformation (**8** or **27**) was found to be more active than that with ring A in the boat conformation (**7** or **27a**). In addition, **15**, **16**, and **19** showed neuroprotective activity.



The parent and lateral roots of *Aconitum carmichaelii* Debx. (Ranunculaceae), which is widely distributed and cultivated in southwestern China, are indispensable drugs in traditional Chinese medicine.[1] The lateral roots, named "fu zi" in Chinese, are used in raw or prepared forms to treat diseases such as cadianeuria, neuralgia, and rheumatalgia in China, Japan, and Korea.[2,3] Previous chemical and pharmacological studies have shown that toxic aconitine C_{19}-diterpenoid alkaloids are the main active constituents of these drugs. Up to now, more than 35 aconitine alkaloids have been isolated from different parts of this plant, including aconitine, hypaconitine, mesaconitine, neoline, talatizamine (talatisamine),[4] isotalatizidine, karakoline (karacoline), monoacetyltalatizamine, senbusines A–C,[2] fuziline,[5] 14-acetyltaltizamine, lipoaconitine, lipohypaconitine, lipomesaconitine, lipodeoxyaconitine, benzoylaconitine, benzoylhypaconitine, benzoylmesaconitine,[6] hokbusines A and B,[7] beiwutine,[8] neojiangyouaconitine,[9] aldohypaconitine,[10] 14-*O*-acetylneoline, foresaconitine, crassicauline, 14-*O*-cinnamoylneoline, 14-*O*-anisoylneoline, 14-*O*-veratroylneoline, lipoforesaconitine, lipoyunanaconitine,[3] 8-*O*-cinnamoylneoline,[11] and karakanine.[12] In addition, two napelline C_{20}-diterpenoid alkaloids (songorine[5] and songoramine[12]), a hetisan C_{20}-diterpenoid alkaloid (ignavine[7]), and several other constituents[3b] have been reported. The complex structures, interesting chemistry, and noteworthy pharmacological effects of these alkaloids have been investigated in considerable detail.[13] A water extract of the lateral roots of *A. carmichaelii* has been investigated as part of a program to study the chemical diversity of traditional Chinese medicines and their biological effects.[14] We describe herein the isolation, structure elucidation, and biological assays of four new C_{20} (**1**–**4**) and 22 new C_{19} (**5**–**26**) diterpenoid alkaloids, together with two known analogues, hypaconitine **27** and benzoylmesaconine **28**, from the extract. Among the C_{19}-diterpenoid alkaloids, **5** and **8** have ring A (C-1, C-2, C-3, C-4, C-5, and C-11) in the chair conformation, while **6**, **7**, and **9**–**26** have ring A in the boat conformation. Compounds **7** and **8** are conformational isomers. The boat and chair conformations of ring A in C_{19}-diterpenoid alkaloids were demonstrated by detailed NMR data and single-crystal X-ray crystallographic analysis of **6** and **27** by anomalous scattering of Cu Kα radiation. The boat conformation of ring A in the C_{19}-diterpenoid alkaloids was speculated to be stabilized by an intramolecular hydrogen bond between the electron pair on the N atom and the C-1 or C-3 OH group in ring A.[15–17] However, acetylation of **7** and **8** afforded the same 3-monoacetate **7a** having ring A in the boat conformation. Since there was no OH group that would form intramolecular hydrogen bonds in **7a** and since ring A in **8** was transformed from the more stable chair to the less stable boat conformation during acetylation, we concluded that intramolecular hydrogen bonding is not the key factor in stabilizing the boat conformation of ring A in aconitine alkaloids. As conformational isomers have different chemical and physical properties,[5a,15,17a] the boat and chair conformation of ring A can be denoted by "A-*b*" and "A-*c*", respectively, in the nomenclature of C_{19}-diterpenoid alkaloids. In addition, because of the lack of clarity regarding the C-13 and C-19 configurations in hetisan C_{20}-diterpenoid alkaloids,[18] confusing trivial names are used for aconitine C_{20}-diterpenoid alkaloids in the literature;[2,4,6,7,15–17] hence, *R* and *S* representations are used for indicating the configurations at C-13 and C-19 in **1**–**4**, respectively, and systematic IUPAC-recommended nomenclature based on the

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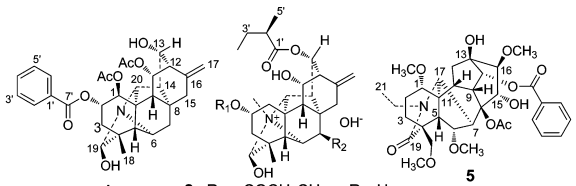
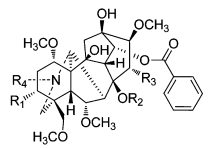
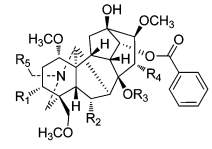
names of the parent alkaloids "hetisan" and "aconitane"[19] is used in this paper.



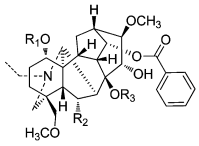
1 2 R_1 = $COCH_2CH_3$, R_2=H 3 R_1 = $COCH_2CH_3$, R_2=OH 4 R_1 = $COCH(CH_3)_2$, R_2=OH 5



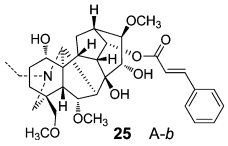
6 R_1 = R_3 = OH, R_2 = Ac, R_4 = CH_2CH_3, A-*b*
7 R_1 = R_3 = OH, R_2 = R_4 = CH_3, A-*b*
7a R_1 = OAc, R_3 = OH, R_2 = R_4 = CH_3, A-*b*
8 R_1 = R_3 = OH, R_2 = R_4 = CH_3, A-*c*
9 R_1 = R_3 = OH, R_2 = CH_3, R_4 = CH_2CH_3, A-*b*
10 R_1 = R_3 = OH, R_2 = H, R_4 = CH_3, A-*b*
11 R_1 = OH, R_2 = Ac, R_3 = H, R_4 = CH_2CH_3, A-*b*
12 R_1 = H, R_2 = Ac, R_3 = OH, R_4 = CH_3, A-*b*



13 R_1 = H, R_2 = OCH_3, R_3 = Ac, R_4 = OH, R_5 = CH_3, A-*b*
14 R_1 = R_3 = R_4 = H, R_2 = OCH_3, R_5 = CH_3, A-*b*
15 R_1 = R_4 = OH, R_2 = OCH_3, R_3 = H, R_5 = CH_3, A-*b*
16 R_1 = R_4 = OH, R_2 = OCH_3, R_3 = Bz, R_5 = H, A-*b*
17 R_1 = H, R_2 = OCH_3, R_4 = OH, R_3 = R_5 = CH_3, A-*b*
18 R_1 = R_2 = R_3 = H, R_4 = OH, R_5 = CH_3, A-*b*
27 R_1 = R_5 = H, R_2 = OCH_3, R_3 = Ac, R_4 = OH, A-*c*
27a R_1 = R_5 = H, R_2 = OCH_3, R_3 = Ac, R_4 = OH, A-*b*
28 R_1 = R_4 = OH, R_3 = R_5 = H, R_2 = OCH_3, A-*c*
28a R_1 = R_4 = OH, R_2 = OCH_3, R_3 = R_5 = H, A-*b*
28b R_1 = OH, R_2 = OCH_3, R_3 = R_5 = H, R_4 = OAc, A-*b*
28c R_1 = R_4 = OAc, R_2 = OCH_3, R_3 = R_5 = H, A-*b*



19 R_1 = R_3 = CH_3, R_2 = OH, A-*b*
20 R_1 = CH_3, R_2 = OH, R_3 = H, A-*b*
21 R_1 = CH_3, R_2 = OH, R_3 = CH_2CH_3, A-*b*
22 R_1 = CH_3, R_2 = R_3 = H, A-*b*
23 R_1 = R_2 = R_3 = H, A-*b*
24 R_1 = R_3 = H, R_2 = OCH_3, A-*b*
26 R_1 = CH_3, R_2 = OCH_3, R_3 = Ac, A-*b*



25 A-*b*

RESULTS AND DISCUSSION

The molecular formula of compound **1** was established as $C_{31}H_{35}NO_8$ on the basis of high-resolution electrospray ionization mass spectroscopy (HRESIMS) and NMR data (Experimental Section and Table 1). The IR spectrum of **1** showed absorptions due to the OH (3394 cm^{-1}) and carbonyl (1719 cm^{-1}) functionalities. The NMR data of **1** showed the presence of a tertiary methyl group, three methylenes, 11 methines (four of them oxymethines), three quaternary carbons, and an exocyclic terminal double bond, in addition to a benzoyl and two acetyl groups. These spectroscopic data suggested that **1** was a C_{20}-diterpenoid alkaloid triester.[7] The structure of **1** was confirmed by 2D NMR data analysis. The proton and corresponding carbon resonances in the 2D NMR spectra of **1** were assigned by the gradient heteronuclear single quantum coherence (gHSQC) experiment. The H-1/H-2/H_2-3, H-6/H_2-7, H-9/H-11, and H-14/H-13/H-12/H_2-17/H_2-15 coupling correlations in the $^1H-^1H$ correlation spectroscopy (COSY) spectrum of **1** revealed three vicinal and one allylic spin systems. The HMBC spectrum of **1** showed two- and three-bond correlations: H-1/C-2, C-3, C-5, C-10; H-5/C-9, C-10, C-20; H-9/C-5, C-10, C-20; and H-20/C-1, C-5, C-9. This demonstrated that the quaternary C-10 was linked to C-1, C-5, C-9, and C-20. HMBC correlations of H-5/C-18, C-19; H_3-18/C-3, C-4, C-5, C-19; and H-19/C-3, C-5, C-18 indicated that the quaternary C-4 was attached to C-3, C-5, C-18, and C-19, while H_2-7/C-8, C-9, C-14, C-15; H-9/C-7, C-8, C-14, C-15; H-14/C-7, C-8, C-9, C-15; and H_2-15/C-7, C-8, C-9, C-14 correlations indicated linkage of the quaternary C-8 with C-7, C-9, C-14, and C-15. HMBC correlations of H-20/C-6, C-8, C-13, C-14, C-19, together with their shifts, showed that C-20 was linked to C-14 and via the N atom to C-6 and C-19. H-5/H-6 and H-11/H-12 coupling correlations were not observed in the $^1H-^1H$ COSY spectrum of **1** because the dihedral angles for the two pairs of vicinal protons were nearly 90°. However; the connection between C-5 and C-6 was indicated by HMBC correlations of H-6/C-10 and H_2-7/C-5, while the connection between C-11 and C-12 was indicated by HMBC correlations of H-9/C-11, C-12; H-11/C-13, C-16; H-12/C-9, C-11, C-13, C-14; and H-13/C-11. The above information revealed the hetisan skeleton in **1**.[20] Furthermore, the HMBC spectrum showed correlations of H-1 and H-11 with the carbonyl carbons of the two acetoxy groups and a correlation of H-2 with the carbonyl carbon of the benzoyl moiety, indicating that the acetoxy groups were present at C-1 and C-11 and the benzoyloxy group at C-2 in **1**. The chemical shifts of H-13 and H-19 (Table 1) suggested OH groups at C-13 and C-19. Therefore, **1** had a planar structure, 1,11-diacetoxy-2-benzoyloxy-13,19-dihydroxyhetisan. In the nuclear Overhauser effect (NOE) difference spectrum of **1**, irradiation of H-20 enhanced the H-1, H-14, H-19, and H-2′/H-6′ resonances, while irradiation of H-13 enhanced the H-12 and H-14 resonances, indicating that these protons were oriented on the same side of the ring system. In addition, irradiation of H-6 enhanced the H-5 and H_3-18 resonances, while irradiation of H-11 enhanced the H-9, H-12, and H-15a resonances, indicating these protons were oriented on the other side of the ring system. The coupling constants $J_{1,2}$ (2.5 Hz), $J_{9,11}$ (9.0 Hz), and $J_{13,14}$ (9.0 Hz) were consistent with those of tadzhacotine,[20] the 19-dehydroxy analogue of **1** isolated from *Aconitum zwravschanicun*, and its structure was determined by X-ray crystallographic analysis. These data suggested that **1** had the same configuration as tadzhacotine, with a 19*β*-OH group. Since the absolute configuration of the hetisan nucleus was repeatedly confirmed by the X-ray crystallographic analysis of analogues isolated from species of the same genus,[21,22] it was proposed that the absolute configuration of the hetisan nucleus was retained in **1**. Thus, compound **1** was (+)-(13*R*,19*S*)-1*β*,11*α*-diacetoxy-2*α*-benzoyloxy-13,19-dihydroxyhetisan.

The spectroscopic data of compound **2** indicated that it was an analogue of **1**. Comparison of the NMR data of **2** with those of **1** (Table 1) indicated that the benzoyloxy and acetoxy groups in **1** were substituted by propionyloxy and 2-methylbutyryloxy moieties in **2**, respectively, and that the oxymethine (CH-1) group of the nucleus in **1** was replaced by a methylene unit (CH_2-1) in **2**. This was confirmed by the 2D NMR data analysis of **2**. In particular, COSY correlations of H_3-5′/H-2′/H_2-3′/H_3-4′ and HMBC correlations from H-2′, H_2-3′, H_3-5′, and H-13 to C-1′ confirmed the presence of the 2-methylbutyryloxy moiety at C-13 in **2**. HMBC correlations from H_2-2″ and H_3-3″ to C-1″, in combination with the H-2 shift, proved that the propionyloxy moiety was located at C-2. In addition, the NMR data of **2** indicated the presence of an *N*-CH_3 group [δ_H 3.03 (s) and δ_C 34.6],[18] suggesting that **2** was a quaternary-*N* base. This was confirmed by HMBC correlations of *N*-CH_3/C-6, C-19, C-20 and by (+)-HRESIMS, which showed a pseudomolecular ion peak at *m*/*z* 500.3020 (500.3007 calcd for $C_{29}H_{42}NO_6$). Since trifluoroacetic acid (TFA) was used in the

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Table 1. NMR Spectroscopic Data for Compounds 1−4.[a]

	1[b]		2[c]		3[d]		4[e]	
no.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1a	5.98 d (2.5)	70.3	3.35 brd (16.2)	31.8	3.36 d (16.8)	31.7	3.34 d (16.5)	31.8
1b			2.15 dd (16.2, 4.8)		2.15 dd (16.8, 5.4)		2.18 dd (16.5, 5.0)	
2	5.67 m	68.5	5.21 m	68.7	5.23 m	68.6	5.26 m	68.6
3a	2.17 brd (16.0)	33.2	2.02 brd (15.6)	36.5	2.03 brd (15.6)	36.4	1.97 brd (15.5)	36.5
3b	2.07 m		1.75 dd (15.6, 4.2)		1.77 dd (15.6, 4.2)		1.80 dd (15.5,4.5)	
4		41.7		40.8		40.6		40.6
5	2.52 brs	55.6	2.28 brs	58.7	2.41 brs	56.5	2.42 brs	56.4
6	4.44 brs	61.6	3.88 brs	66.8	3.77 brs	69.3	3.79 brs	69.4
7a	2.17 dd (11.0, 5.0)	34.3	2.20 dd (15.0, 3.0)	32.0	4.19 d (3.0)	67.4	4.19 brs	67.3
7b	2.09 m		2.05 dd (15.0, 3.0)					
8		44.9		44.7		52.0		51.9
9	2.66 d (9.0)	51.9	2.33 d (9.0)	55.7	2.68 d (10.2)	50.0	2.68 d (9.5)	50.1
10		54.2		52.2		50.1		50.2
11	5.32 d (9.0)	75.5	4.35 d (9.0)	74.0	4.29 brd (10.2)	73.8	4.29 d (9.5)	73.8
12	2.49 d (2.5)	49.5	2.60 brs	48.5	2.61 brs	48.7	2.61 d (2.5)	48.7
13	4.21 brd (9.0)	70.3	5.13 d (9.6)	73.2	5.18 brd (10.2)	73.1	5.19 brd (10.0)	73.2
14	2.64 d (9.0)	50.3	3.19 d (9.6)	45.7	3.21 brd (10.2)	44.3	3.21 d (10.0)	44.3
15a	2.57 brd (17.5)	33.4	2.40 d (17.4)	33.2	2.69 brd (18.0)	30.3	2.71 d (19.0)	30.2
15b	2.26 brd (17.5)		2.26 d (17.4)		2.20 d (18.0)		2.18 d (19.0)	
16		144.9		145.6		145.6		145.6
17a	4.87 brs	109.4	4.89 brs	109.3	4.92 brs	109.4	4.92 brs	109.3
17b	4.79 brs		4.74 brs		4.78 brs		4.78 brs	
18	1.17 s	22.0	1.09 s	22.3	1.07 s	22.1	1.08 s	22.1
19	5.38 brs	92.5	5.89 brs	101.0	5.99 brs	102.4	5.97 brs	102.6
20	4.99 s	62.5	4.30 s	73.6	4.31 s	72.9	4.27 s	73.0
N-CH_3			3.03 s	34.6	3.06 s	35.5	3.08 s	35.5
1′		130.8		176.0		176.0		176.0
2′	8.05 d (7.5)	130.4	2.33 heptet (7.2)	41.6	2.33 heptet (7.2)	41.5	2.31 heptet (7.5)	41.5
3′a	7.49 t (7.5)	129.6	1.66 d pentet (13.8,7.2)	26.8	1.64 d pentet (13.8,7.2)	26.9	1.64 d pentet (14.0,7.5)	26.9
3′b			1.44 d pentet (13.8,7.2)		1.44 d pentet (13.8,7.2)		1.42 d pentet (14.0,7.5)	
4′	7.65 t (7.5)	134.2	0.89 t (7.2)	11.7	0.88 t (7.2)	11.7	0.88 t (7.5)	11.7
5′	7.49 t (7.5)	129.6	1.15 d (7.2)	17.2	1.15 d (7.2)	17.4	1.17d (7.5)	17.4
6′	8.05 d (7.5)	130.4						
7′		165.5						

[a] ^{1}H NMR data (δ_H) were measured in Me_2CO-d_6 at 500 MHz for **1** and **4** and at 600 MHz for **2** and **3**, respectively. Proton coupling constants (J) in Hz are given in parentheses. ^{13}C NMR data (δ_C) were measured in Me_2CO-d_6 at 125 MHz for **1** and **4** and at 150 MHz for **2** and **3**, respectively. The assignments were based on ^{1}H−^{1}H COSY, HSQC, and HMBC experiments. [b] Data for OAc units in **1**: δ_H 1.90 (3H, s, OAc-1), 2.08 (3H, s, OAc-11); δ_C 171.0, 21.4 (OAc-1); 170.5, 21.2 (OAc-11). [c] Data for propionyl unit in **2**: δ_H 2.58 (1H, dq, J = 16.2, 7.2 Hz, H-2″a), 2.33 (1H, dq, J = 16.2, 7.2 Hz, H-2″b), 1.04 (3H, t, J = 7.2 Hz, H-3″); δ_C 173.9 (C-1″), 28.1 (C-2″), 9.1 (C-3″). [d] Data for propionyl unit in **3**: δ_H 2.59 (1H, dq, J = 16.2, 7.2 Hz, H-2″a), 2.32 (1H, dq, J = 16.2, 7.2 Hz, H-2″b), 1.05 (3H, t, J = 7.2 Hz, H-3″); δ_C 173.9 (C-1″), 28.1 (C-2″), 9.1 (C-3″). [e] Data for isobutyryl unit in **4**: δ_H 2.72 (1H, dq, J = 7.0 Hz, H-2″), 1.10 (3H, d, J = 7.0 Hz, H-3″), 1.11 (3H, d, J = 7.0 Hz, H-4″); δ_C 176.7 (C-1″), 34.5 (C-2″), 19.1 (C-3″), 20.0 (C-4″).

HPLC isolation procedure, **2** was expected to be obtained as a trifluoroacetate, but the absence of trifluoroacetic ion resonances in the ^{13}C NMR spectrum suggested that **2** was obtained as a quaternary amine hydroxide. This was supported by (−)-ESIMS, which exhibited a quasi-molecular ion at m/z 515 $[M - H]^-$. In the NOE difference spectrum of **2**, irradiation of H-2 enhanced the H-3b and H-1b resonances, and irradiation of H-3b enhanced the H_3-18 resonances. H-20 and H-3a resonances were enhanced upon irradiation of H-19. These enhancements revealed that the 2-propionyloxy and 19-OH groups in **2** were α- and β-oriented, respectively. Comparison of the coupling constants $J_{9,11}$ and $J_{13,14}$ in **2** with those for **1** (Table 1) suggested that **2** had the same 11α,13R configuration as **1**. Hydrolysis of **2** with 5% NaOH yielded a mixture of 2-methylbutyric acid and propionic acid with $[\alpha]^{20}_D$ +11.2 (c 0.06, MeOH), indicating a 2′S configuration. Therefore, compound **2** was defined as (−)-(13R,19S)-11α,19-dihydroxy-N-methyl-13-(S-2-methylbutyryloxy)-2α-propionyloxyhetisanium hydroxide.

The NMR and IR data of compound **3** were similar to those of **2** (Table 1 and Experimental Section). Comparison of the NMR data of **3** with those of **2** indicated that one methylene unit of the nucleus in **2** was replaced by an oxymethine [δ_H 4.19 (d, J = 3.0 Hz) and δ_C 67.4] in **3**. In addition, H-5, H-9, H-15a, C-6, and C-8 in **3** were deshielded by $\Delta\delta_H$ +0.13, $\Delta\delta_H$ +0.35, $\Delta\delta_H$ +0.29, $\Delta\delta_C$ +2.5, and $\Delta\delta_C$ +7.3 ppm, respectively. On the other hand, H-6, C-5, C-9, and C-15 were shielded by $\Delta\delta_H$ −0.11, $\Delta\delta_C$ −2.2, $\Delta\delta_C$ −5.7, and $\Delta\delta_C$ −2.9 ppm, respectively. This suggested that **3** was a 7-hydroxy derivative of **2**, which was confirmed by HRESIMS and 2D NMR data. In particular, the COSY correlation of H-6/H-7 and HMBC correlations of H-7/C-5, C-9, in combination with the shifts of these proton

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and carbon resonances, confirmed the OH-7 group in **3**. NOE enhancement of the *N*-CH_3 resonance upon irradiation of H-7 indicated that the OH-7 group was β-oriented. The configuration of the 2-methylbutyryloxy moiety in **3** was proved as described above. Therefore, compound **3** was determined to be (−)-(13*R*,19*S*)-7β,11α,19-trihydroxy-*N*-methyl-13-(*S*-2-methylbutyryloxy)-2α-propionyloxyhetisanium hydroxide.

Spectroscopic data showed that compound **4** was a higher homologue of **3** with an additional CH_2 unit (Table 1 and Experimental Section). Comparison of the NMR data of **3** and **4** demonstrated that the only difference between these compounds was replacement of the 2-propionyloxy moiety in **3** by a 2-isobutyryloxy moiety in **4**. Thus, **4** was assigned as (+)-(13*R*,19*S*)-2α-isobutyryloxy-7β,11α,19-trihydroxy-*N*-methyl-13-(*S*-2-methylbutyryloxy)hetisanium hydroxide, which was confirmed by 2D NMR and NOE difference experiments.

The molecular formula of compound **5** was $C_{34}H_{45}NO_{11}$ (by HRESIMS and NMR data). The NMR data indicated four methylene (one oxymethylene), nine methine (four oxymethines), and six quaternary carbons (a carbonyl and two oxygen-bearing), in addition to a benzoyl, an acetoxy, an *N*-ethyl, and four methoxy substituents, suggesting that **5** was a typical aconitane C_{19}-diterpenoid alkaloid.[7,16] Comparison of the NMR data of **5** with those of 3-deoxyaconitine[16] indicated that the CH_2-19 unit in deoxyaconitine was replaced by a carbonyl group (δ_C 173.0) in **5**, suggesting that **5** was 19-oxo-3-deoxyaconitine. The 2D NMR and NOE difference experiments confirmed the NMR data and configuration assignments of **5**. In particular, HMBC correlations of C-19 with H_2-3, H-5, H-17, and H_2-20 confirmed the 19-oxo group in **5**, while HMBC correlations of H-14/C-1′, OCH_3-1/C-1, OCH_3-6/C-6, OCH_3-16/C-16, and OCH_3-18/C-18 confirmed the locations of the benzoyloxy and four methoxy groups. The chair conformation of ring A in **5** was revealed by coupling constants $J_{1,2a} \approx J_{1,2b} \approx 7.5$ Hz.[2,10a,c,12b,15−17] Thus, compound **5** was assigned as (−)-(A-*c*)-8β-acetoxy-14α-benzoyloxy-*N*-ethyl-13β,15α-dihydroxy-1α,6α,16β,18-tetramethoxy-19-oxo-aconitane; the configuration of the aconitane nucleus was determined by X-ray crystallographic analysis of several analogues from *Aconitum* plants.[17]

Compound **6** had a planar structure and configuration consistent with those of aconifine,[23] as demonstrated by spectroscopic data analysis, including 2D NMR experiments. However, as compared with the reported data of aconifine, C-1, C-2, C3, and C-12 in **6** were shielded by $\Delta\delta_C$ −1.9, −1.8, −1.3, and −1.9 ppm, respectively, whereas C-17, C-18, and C-19 were deshielded by $\Delta\delta_C$ +3.8, +2.3, and +3.3 ppm. In particular, the ^{1}H NMR spectrum of **6** showed broad singlets ($W_{1/2} \approx 6$ Hz) due to H-1 and H-3, while H-3 in aconifine monoacetate was reported to be a quartet with coupling constants of 10 and 7 Hz (the corresponding data for aconifine were absent in the literature). This, combined with the chair conformation of ring A in aconifine, as determined by X-ray crystallographic analysis,[24] suggested that ring A in **6** was in the boat conformation. The boat conformation was confirmed by single-crystal X-ray crystallographic analysis of **6** (Figure 1). Thus, compound **6** was confirmed to be (−)-(A-*b*)-8β-acetoxy-14α-benzoyloxy-*N*-ethyl-3α,10β,13β,15α-tetrahydroxy-1α,6α,16β,18-tetramethoxyaconitane.

Although ring A in aconifine[23] and aconine[25] was reported to undergo chair (in $CDCl_3$)-to-boat (in pyridine-d_6) transformation, causing changes in the C-2, C-3, and C-4 shifts in these two solvents, the shift changes should be due to solvent

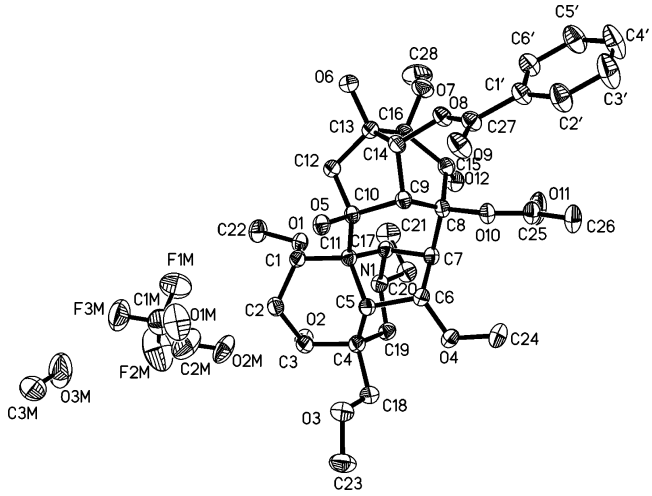

Figure 1. ORTEP diagram of compound **6**.

effects rather than the conformational transformation. This assumption was supported by the similarity between the splitting patterns and coupling constants for H-1 (dd, J = 7 and 10 Hz) in the triacetates of senbusines A and C in $CDCl_3$ and pyridine-d_6.[2]

While the spectroscopic data of compound **7** were similar to those of beiwutine,[26] NMR and HRESIMS data indicated replacement of the OAc group in beiwutine by an OCH_3 group in **7**. Thus, **7** was thought to be 8-deacetoxy-8-methoxybeiwutine, which was confirmed by 2D NMR data analysis. HMBC correlations from OCH_3, H-6, H-7, H-9, H-14, and H-15 to C-8 proved the presence of the OCH_3-8 group in **7**, while NOESY correlations of H-6/H_2-18/H-3/H-5/H-2b/H-1/H-12b/H-14/H-9/H-6/CH_3O-8/H-15/CH_3O-16 confirmed that the configuration of **7** was identical to that of beiwutine. Similarity of the splitting patterns and coupling constants of H-1 and H-3 in **6**, **7**, and beiwutine[26] indicated that ring A was in the boat conformation in these three compounds. Thus, compound **7** was determined to be (−)-(A-*b*)-14α-benzoyloxy-3α,10β,13β,15α-tetrahydroxy-1α,6α,8β,16β,18-pentamethoxy-*N*-methylaconitane.

Spectroscopic data, including 2D NMR data in $CDCl_3$ and acetone-d_6, indicated that compound **8** had the same planar structure and configuration as **7**. However, comparison of the NMR data of **8** and **7** in the same solvent, acetone-d_6, demonstrated that in **8** H-1, H-3, H-5, H-7, H-12b, H-17, and H_2-19 were shielded by more than $\Delta\delta_H$ −0.2 and that *N*-CH_3, C-17, C-18, and C-19 were shielded by more than $\Delta\delta_C$ −1.7 ppm. On the other hand, H-2b was deshielded by more than $\Delta\delta_H$ +0.5, and C-2, C-5, C-6, C-9, and C-12 were deshielded by more than $\Delta\delta_C$ +1.0 ppm. Particularly, the H-1 and H-3 resonances in **8** were split into doublets of doublets with coupling constants of about 6 and 8 Hz, respectively. Since the chemical shifts and coupling constants change because of the conformational change in ring A of aconitine derivatives,[16,17,27] the difference in the NMR data of **8** and **7** suggested that **8** was the conformational isomer of **7**, with ring A in the chair conformation. This was confirmed by the fact that acetylation of both **7** and **8** generated the same product, **7a**. However, the splitting patterns and coupling constants for H-1 and H-3 in the ^{1}H NMR spectrum of **7a** were consistent with those of **7**. This revealed that ring A in **7a** had the boat conformation. However, the assumption that the boat conformation of this ring in

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Table 2. ^{1}H NMR Spectroscopic Data for Compounds 5−12[a]

no.	5[b]	6[b]	7	7a[c]	8	9	10	11[b]	12[b]
1	3.34 t (7.5)	3.94 brs	4.01 brs	4.06 brs	3.80 dd (8.0, 6.0)	4.03 brs	4.01 brs	4.05 s	4.01 brs
2a	2.04 dd (12.5, 7.5)	2,34 brd (15.5)	2.42 brd (16.0)	2.43 brd (16.0)	2.37 m	2.43 brd (13.0)	2.39 brd (15.5)	2.44 brd (16.0)	2.04 brd (15.0)
2b	1.47 ddd (13.5, 12.5, 7.5)	1.39 ddd (15.5, 4.0, 4.0)	1.45 ddd (16.0, 4.0, 4.0)	1.60 ddd (16.0, 4.0, 4.0)	2.02 m	1.43 brd (13.0)	1.40 dt (15.5, 4.0)	1.45 brd (16.0)	1.39 dt (15.0, 4.5)
3a	1.75 m	4.20 brs	4.27 brd (4.0)	5.44 brd (4.0)	3.73 dd (8.0, 6.0)	4.31 brs	4.29 brd (4.0)	4.30 brs	2.07 dd (15.0, 4.5)
3b	1.75 m								1.86 dt (15.0, 4.5)
5	2.56 brd (7.5)	2.85 d (6.5)	2.85 brd (6.0)	2.91 brd (6.5)	2.52 d (6.0)	2.86 brd (6.0)	2.74 brd (7.0)	2.94 brd (5.0)	2.93 d (6.0)
6	4.05 d (7.5)	4.23 d (6.5)	4.23 brd (6.0)	4.26 brd (6.5)	4.07 d (6.0)	4.25 d (6.0)	4.28 d (7.0)	4.31 d (5.0)	4.31 d (6.0)
7	2.86 brs	3.07 brs	3.15 brs	3.20 brs	2.88 brs	3.10 brs	2.83 brs	3.36 brs	3.21 brs
9	2.71 dd (6.5, 5.0)	2.79 d (5.0)	2.57 d (5.0)	2.57 d (5.5)	2.49 t (5.5)	2.58 d (5.5)	2.51d (5.5)	2.88 d (5.0)	2.88 d (5.0)
10	2.45 ddd (13.0, 6.5, 6.0)								
12a	2.61 dd (15.0, 6.0)	2.39 d (15.5)	2.46 d (15.0)	2.39 d (15.0)	2.37 d (16.0)	2.46 d (15.0)	2.39 d (15.0)	2.42 d (15.0)	2.45 d (15.0)
12b	2.18 dd (15.0, 13.0)	2.15 d (15.5)	2.20 d (15.0)	2.23 d (15.0)	1.98 d (16.0)	2.20 d (15.0)	2.16 d (15.0)	2.23 d (15.0)	2.30 d (15.0)
14	4.90 d (5.0)	5.29 d (5.0)	5.34 d (5.5)	5.33 d (5.5)	5.32 d (5.5)	5.34 d (5.5)	5.36 d (5.5)	5.43 d (5.0)	5.42 d (5.0)
15a	4.52 d (5.0)	4.50 d (5.5)	4.73 d (5.5)	4.73 d (5.5)	4.65 t (5.5)	4.75 d (5.5)	4.77 d (5.5)	3.20 dd (16.0, 9.0)	4.60 d (5.5)
15b								2.52 dd (16.0, 6.5)	
16	3.27 d (5.0)	3.27 d (5.5)	3.39 d (5.5)	3.40 d (5.5)	3.20 d (5.5)	3.32 d (5.5)	3.23 d (5.5)	3.63 dd (9.0, 6.5)	3.52 d (5.5)
17	3.67 brs	3.62 brs	3.40 brs	3.46 brs	2.84 brs	3.40 brs	3.40 brs	3.36 brs	3.47 brs
18a	3.42 brd (9.0)	3.48 d (8.0)	3.63 d (8.0)	3.64 d (8.0)	3.70 d (8.5)	3.62 d (7.0)	3.61 d (8.5)	3.59 d (8.5)	3.55 d (8.0)
18b	4.02 brd (9.0)	3.30 d (8.0)	3.40 d (8.0)	3.35 d (8.0)	3.44 d (8.5)	3.40 d (7.0)	3.56 d (8.5)	3.45 d (8.5)	3.43 d (8.0)
19a		3.65 d (12.0)	3.70 d (10.0)	3.68 d (10.0)	2.80 d (10.0)	3.76 brd (10.0)	3.62 d (10.0)	3.84 brd (12.0)	3.51 brd (12.0)
19b		3.55 d (12.0)	3.41 d (10.0)	3.51 d (10.0)	2.78 d (10.0)	3.43 brd (10.0)	3.42 d (10.0)	3.30 brd (12.0)	3.32 brd (12.0)
20a	2.98 dq (13.0, 7.5)	3.30 m	3.15 brs	3.23 brs	2.37 brs	3.64 m	3.07 brs	3.57 m	3.19 s
20b	3.75 dq (13.0, 7.5)	3.03 m				3.40 m		3.39 m	
21	1.10 t (7.5)	1.36 t (7.5)				1.46 t (7.0)		1.46 t (7.0)	
OCH_3-1	3.27 s	3.36 s	3.42 s	3.42 s	3.25 s	3.44 s	3.42 s	3.44 s	3.35 s
OCH_3-6	3.11 s	3.20 s	3.35 s	3.36 s	3.26 s	3.34 s	3.31 s	3.31 s	3.32 s
OCH_3-8			3.17 s	3.16 s	3.12 s	3.17 s			
OCH_3-16	3.69 s	3.58 s	3.69 s	3.68 s	3.67 s	3.70 s	3.65 s	3.54 s	3.69 s
OCH_3-18	3.27 s	3.19 s	3.32 s	3.33 s	3.27 s	3.32 s	3.29 s	3.30 s	3.30 s
2′	8.05 d (7.5)	7.94 d (7.5)	8.07 d (7.5)	8.07 d (7.5)	8.06 d (7.5)	8.07 d (7.5)	8.07 d (7.5)	8.09 d (7.5)	8.06 d (7.5)
3′	7.55 t (7.5)	7.44 t (7.5)	7.52 t (7.5)	7.52 t (7.5)	7.50 t (7.5)	7.52 t (7.5)	7.47 t (7.5)	7.55 t (7.5)	7.56 t (7.5)
4′	7.67 t (7.5)	7.57 t (7.5)	7.64 t (7.5)	7.64 t (7.5)	7.62 t (7.5)	7.64 t (7.5)	7.60 t (7.5)	7.67 t (7.5)	7.69 t (7.5)
5′	7.55 t (7.5)	7.44 t (7.5)	7.52 t (7.5)	7.52 t (7.5)	7.50 t (7.5)	7.52 t (7.5)	7.47 t (7.5)	7.55 t (7.5)	7.56 t (7.5)
6′	8.05 d (7.5)	7.57 t (7.5)	8.07 d (7.5)	8.07 d (7.5)	8.06 d (7.5)	8.07 d (7.5)	8.07 d (7.5)	8.09 d (7.5)	8.06 d (7.5)

[a] ^{1}H NMR data (δ) were measured in Me_2CO-d_6 at 500 MHz for **5**−**12**. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on ^{1}H−^{1}H COSY, gHSQC, HMBC, and NOE experiments. [b] Data of OAc-8: δ_H 1.39 (3H, s) for **5**; 1.39 (3H, s) for **6**; 1.39 (3H, s) for **11**; 1.50 for **12**. [c] Data of 3-OAc in **7a**: δ_H 2.12 (3H, s).

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aconitine alkaloids is stabilized by intramolecular hydrogen bonding between the electron pair on the N atom and the OH-1 or OH-3 group[16,17] may not be valid because **7a** does not have any OH group that would be involved in intramolecular hydrogen bonding. Ring A in **8** underwent chair-to-boat conformational transformation upon acetylation. Thus, compound **8** was assigned the name (−)-(A-*c*)-14*α*-benzoyloxy-3*α*,10*β*,13*β*,15*α*-tetrahydroxy-1*α*,6*α*,8*β*,16*β*,18-pentamethoxy-*N*-methylaconitane.

The spectroscopic data of compound **9** demonstrated that it was a homologue of **7** with an *N*-CH_2CH_3 group replacing the *N*-CH_3 group. This was confirmed by the H-17/C-5, C-6, C-8, C-10, C-11, C-20 correlations in the HMBC spectrum of **9**. Hence, compound **9** was determined to be (−)-(A-*b*)-14*α*-benzoyloxy-*N*-ethyl-3*α*,10*β*,13*β*,15*α*-tetrahydroxy-1*α*,6*α*,8*β*,16*β*,18-pentamethoxyaconitane.

The HRESIMS data of compound **10** indicated that it was another homologue of **7** having the molecular formula $C_{31}H_{43}NO_{11}$. Comparison of the NMR data of **10** and **7** (Tables 2 and 5) revealed that the OCH_3 group of **7** was replaced by an OH group in **10**. In addition, in the case of **10**, the H-5, H-7, and H-16 resonances were shielded by $\Delta\delta_H$ −0.11, −0.32, and −0.16, respectively. The C-8 and C-16 resonances were shielded by $\Delta\delta_C$ −5.1 and −2.0 ppm, respectively. On the other hand, H-6, H-18b, C-7, and C-15 were deshielded by $\Delta\delta_H$ +0.05, $\Delta\delta_H$ +0.16, $\Delta\delta_C$ +5.8, and $\Delta\delta_C$ +4.8 ppm, respectively. This suggested that **10** was the 8-hydroxy derivative of **7**, which was confirmed by 2D NMR analysis. Therefore, compound **10** was identified as (−)-(A-*b*)-14*α*-benzoyloxy-3*α*,8*β*,10*β*,13*β*,15*α*-pentahydroxy-1*α*,6*α*,16*β*,18-tetramethoxy-*N*-methylaconitane.

HRESIMS indicated that the molecular formula of compound **11** was $C_{34}H_{47}NO_{11}$. Comparison of the NMR data of **11** and **7** demonstrated that the OCH_3-8, *N*-CH_3, and hydroxymethine (CHOH-15) units in **7** were replaced by OAc, *N*-CH_2CH_3, and methylene units in **11**, respectively. This suggested that **11** was (−)-(A-*b*)-8*β*-acetoxy-14*α*-benzoyloxy-*N*-ethyl-3*α*,10*β*,13*β*-trihydroxy-1*α*,6*α*,16*β*,18-tetramethoxyaconitane, which was supported by 2D NMR and NOE data.

Compound **12** was another derivative of **7**, as indicated by spectroscopic data. The NMR data revealed replacement of the OCH_3-8 group in **7** by an OAc-8 unit in **12**, while the OH-3 group in **7** was absent in **12**. This indicated that **12** was the 3-dehydroxy-8-acetoxy derivative of **7**, which was confirmed by 2D NMR and NOE analyses. In the NOE difference spectrum of **12**, enhancement of the OAc resonance upon irradiation of H-15 confirmed the 8*β*-OAc group. Thus, compound **12** was determined to be (−)-(A-*b*)-8*β*-acetoxy-14*α*-benzoyloxy-10*β*,13*β*,15*α*-trihydroxy-1*α*,6*α*,16*β*,18-tetramethoxy-*N*-methylaconitane.

The spectroscopic data of compound **13** demonstrated that it had a planar structure and configuration identical to that of 3-deoxyaconitine.[16,26,28] However, C-1, C-2, C-3, C-5, and C-18 in **13** were shielded by about $\Delta\delta_C$ −4.7, −4.3, −8.6, −7.7, and −2.7 ppm, respectively, as compared with the reported data for 3-deoxyaconitine in the same solvent, $CDCl_3$, whereas C-19 was deshielded by about $\Delta\delta_C$ +3.5 ppm. The H-1 resonance appeared as a broad singlet ($W_{1/2} \approx 6$ Hz) in the ^{1}H NMR spectrum of **13** (acetone-d_6 or $CDCl_3$), while the ^{1}H NMR data of 3-deoxyaconitine did not include the H-1 resonance.[16,26,28] This indicated that **13** was the conformational isomer of 3-deoxyaconitine with ring A in the boat conformation, since ring A in the latter was confirmed to be in the chair conformation by X-ray crystallographic analysis.[29] Thus, compound **13** was confirmed to be (−)-(A-*b*)-8*β*-acetoxy-14*α*-benzoyloxy-*N*-ethyl-13*β*,15*α*-dihydroxy-1*α*,6*α*,16*β*,18-tetramethoxyaconitane.

Comparison of the NMR data of **14** with those of **13** (Tables 3 and 5) indicated the absence of acetyl and 15-OH groups in **14**. The four methoxy groups were proved to be at C-1, C-6, C-16, and C-18 by 2D NMR data analysis. COSY correlations of H-10/H-9/H-14 and H_2-15/H-16 and HMBC correlations of H-9/C-7, C-8, C-12, C-13, C-14 and H-14/C-8, C-9, C-13, C-16, together with their shifts, confirmed the presence of two hydroxyl groups at C-8 and C-13. Therefore, compound **14** was (−)-(A-*b*)-14*α*-benzoyloxy-*N*-ethyl-8*β*,13*β*-dihydroxy-1*α*,6*α*,16*β*,18-tetramethoxyaconitane.

The spectroscopic data of compound **15** revealed that it had a planar structure and configuration identical to that of benzoylaconitine.[30] However, the difference between the NMR data of **15** and benzoylaconitine was identical to that between the NMR data of **6** and aconifine. This demonstrated that **15** was the conformational isomer of benzoylaconitine with ring A in the boat conformation. Therefore, compound **15** was determined as (−)-(A-*b*)-14*α*-benzoyloxy-*N*-ethyl-3*α*,8*β*,13*β*,15*α*-tetrahydroxy-1*α*,6*α*,16*β*,18-tetramethoxyaconitane.

Compound **16** was the conformational isomer of manshuritine,[31] with ring A in the boat conformation, as indicated by spectroscopic data and confirmed by 2D NMR experiments. The difference in the NMR data of **16** and manshuritine was similar to that in the NMR data of **15** and benzoylaconitine. Thus, compound **16** was assigned the name (−)-(A-*b*)-8*β*,14*α*-dibenzoyloxy-*N*-ethyl-3*α*,13*β*,15*α*-trihydroxy-1*α*,6*α*,16*β*,18-tetramethoxyaconitane.

The spectroscopic data of compound **17** indicated that it was the conformational isomer of neojiangyouaconitine, with ring A in the boat conformation. This was confirmed by 2D NMR and NOE data analysis, which amended the NMR assignment of this compound. Therefore, compound **17** was determined as (−)-(A-*b*)-14*α*-benzoyloxy-*N*-ethyl-13*β*,15*α*-dihydroxy-1*α*,6*α*,8*β*,16*β*,18-pentamethoxyaconitane.

Compound **18** was a homologue of **14**. ^{1}H−^{1}H COSY correlations of H-6a/H-6b/H-7 and HMBC correlations from H-7 to C-5, C-8, C-9, C-11, and C-17, together with the shifts of these proton and carbon resonances, indicated the absence of any substituent at C-6 in **18**. The COSY cross-peak of H-15/H-16 and HMBC correlations of H-14/C-8, C-9, C-13, C-16 and H-15/C-7, C-8, C-16, in combination with their shifts, demonstrated the presence of OH groups at C-8, C-13, and C-15. In addition, the HMBC correlations of H-14/C-7′, OCH_3-1/C-1, OCH_3-16/C-16, and OCH_3-18/C-18 confirmed the locations of the OCH_3 and benzoyloxy substituents in **18**. Therefore, compound **18** was determined to be (−)-(A-*b*)-14*α*-benzoyloxy-*N*-ethyl-8*β*,13*β*,15*α*-trihydroxy-1*α*,16*β*,18-trimethoxyaconitane, whose configuration was supported by the NOE difference data.

The spectroscopic data of **19** indicated that it was an isomer of **14**. The vicinal coupling correlations of H-10/H-9/H-14/H-13 and H-15/H-16, together with their shifts, in the ^{1}H−^{1}H COSY spectrum of **19** indicated that OH-13 in **14** was replaced by OH-15 in **19**. This was confirmed by correlations from H-13 and H-9 to C-10 and C-14 in the HMBC spectrum of **19**. HMBC correlations of OCH_3-1/C-1, OCH_3-8/C-8, OCH_3-16/C-16, and OCH_3-18/C-18 confirmed the locations of the OCH_3 groups in **19**. HMBC correlations of H-6/C-4, C-5, C-7, C-8, C-17, along with their shifts, revealed the presence of an

Table 3. ^{1}H NMR Spectroscopic Data of Compounds 13−18[a]

no.	**13** ($CDCl_3$)[b]	**13** (Me_2CO-d_6)[b]	**14** (Me_2CO-d_6)	**15** (Me_2CO-d_6)	**16** (Me_2CO-d_6)[c]	**17** (Me_2CO-d_6)	**18** (Me_2CO-d_6)
1	3.72 brs	3.61 brs	3.70 brs	3.67 brs	3.76 brs	3.68 brs	3.70 brs
2a	2.03 dd (15.0, 4.5.0)	2.02 brd (14.0)	2.02 dd (15.0, 4.5)	2.36 brd (16.0)	2.40 brd (14.5)	2.01 ddd (14.5, 5.0)	2.00 dd (15.0, 4.5)
2b	1.48 dt (15.0, 4.5)	1.34 m	1.49 dt (15.0, 4.5)	1.63 dt (10.5, 4.0)	1.67 brd (14.5)	1.45 ddd (14.5, 14.5, 5.0)	1.58 ddd (15.0, 15.0, 4.5)
3a	2.06 dd (15.0, 4.5)	2.04 m	2.05 dd (15.0, 4.5)	4.30 brs	4.35 brs	1.98 dd (14.5, 5.0)	1.92 dd (15.0, 4.5)
3b	1.91 ddd (15.0, 4.5)	1.34 m	1.83 dt (4.5, 15.0)			1.91 ddd (14.5, 14.5, 5.0)	1.78 ddd (15.0, 15.0, 4.5)
5	2.71 d (6.5)	2.57 d (6.0)	2.56 d (6.0)	2.50 d (6.5)	3.34 d (5.5)	2.56 d (5.5)	2.13 d (7.5)
6	4.35 d (6.5)	4.11 d (6.0)	4.31 d (6.0)	4.29 d (6.5)	4.56 d (5.5)	4.21 d (5.5)	1.98 dd (15.0, 7.5)
7	3.15 brs	2.95 brs	2.59 brs	2.74 brs	3.30 brs	3.07 brs	1.79 dd (15.0, 7.5)
9	2.96 dd (7.0, 4.5)	2.90 dd (5.0, 4.0)	2.65 dd (7.0, 5.0)	2.54 dd (7.0, 5.0)	2.58 dd (5.5, 5.0)	2.57 dd (6.0, 5.0)	2.83 d (7.5)
10	2.68 ddd (13.0, 7.0, 5.0)	2.35 m	2.54 ddd (13.0, 7.0, 5.0)	2.48 ddd (12.5, 7.0, 4.5)	2.59 m	2.53 ddd (12.5, 6.0, 4.5)	2.50 dd (7.5, 5.0)
12a	2.46 dd (14.0, 13.0)	2.39 dd (12.0, 13.0)	2.36 dd (14.0, 13.0)	2.30 dd (14.0, 12.5)	2.45 dd (14.0, 12.5)	2.38 dd (14.0, 12.5)	2.43 ddd (13.0, 5.0, 5.0)
12b	1.88 dd (14.0, 5.0)	1.82 brd (12.0)	1.78 dd (14.0, 5.0)	1.88 dd (14.0, 4.5)	2.04 dd (14.0, 5.0)	1.82 dd (14.0, 4.5)	2.30 dd (14.0, 13.0)
14	4.97 d (4.5)	4.92 d (4.0)	4.98 d (5.0)	4.91 d (5.0)	4.98 d (5.0)	4.87 d (5.0)	4.88 d (5.0)
15a	4.60 d (5.5)	4.54 d (4.0)	2.77 dd (16.0, 8.5)	4.75 d (6.0)	4.71 d (5.5)	4.73 d (5.5)	4.68 d (6.0)
15b			2.58 dd (16.0, 6.0)				
16	3.41 d (5.5)	3.28 d (4.0)	3.56 dd (8.5, 6.0)	3.24 d (6.0)	3.57 d (5.5)	3.32 d (5.5)	3.22 d (6.0)
17	3.63 brs	3.79 brs	3.51 brs	3.57 brs	3.24 s	3.54 brs	3.60 brs
18a	3.54 d (8.0)	3.50 d (8.0)	3.60 d (8.0)	3.61 brd (8.5)	3.55 d (8.0)	3.42 d (8.0)	3.22 d (9.0)
18b	3.38 d (8.0)	3.01 d (8.0)	3.50 d (8.0)	3.55 brd (8.5)	3.39 d (8.0)	3.42 d (8.0)	3.19 d (9.0)
19a	3.48 brd (12.0)	3.45 brs	3.53 brd (12.0)	2.63 d (12.5)	3.73 d (12.0)	3.51 d (10.0)	3.13 brd (10.5)
19b	3.29 brd (12.0)	3.45 brs	3.26 brd (12.0)	2.42 d (12.5)	3.36 d (12.0)	3.25 d (10.0)	3.04 brd (10.5)
20a	3.60 m	3.18 m	3.60 m	3.54 dq (13.0, 7.5)	3.24 s	3.61 dq (13.0, 7.5)	3.42 dq (10.5, 7.0)
20b	3.34 m	3.11 m	3.38 m	3.26 dq (13.0, 7.5)		3.51 dq (13.0, 7.5)	3.14 dq (10.5, 7.0)
21	1.58 t (7.0)	1.51 brt (7.0)	1.51 t (7.0)	1.47 t (7.5)		1.55 t (7.0)	1.50 t (7.0)
OCH_3-1	3.39 s	3.45 s	3.37 s	3.43 s	3.41 s	3.38 s	3.36 s
OCH_3-6	3.31 s	3.23 s	3.34 s	3.31 s	3.26 s	3.34 s	
OCH_3-8						3.18 s	
OCH_3-16	3.73 s	3.79 s	3.53 s	3.68 s	3.73 s	3.73 s	3.64 s
OCH_3-18	3.31 s	3.40 s	3.29 s	3.28 s	3.05 s	3.32 s	3.27 s
2′	8.05 d (7.5)	8.01 d (7.5)	8.07 d (7.5)	8.05 d (7.5)	7.78 d (7.5)	8.07 d (7.5)	8.00 d (7.5)
3′	7.56 t (7.5)	7.49 t (7.5)	7.50 t (7.5)	7.47 t (7.5)	7.20 t (7.5)	7.53 t (7.5)	7.43 t (7.5)
4′	7.69 t (7.5)	7.62 t (7.5)	7.62 t (7.5)	7.60 t (7.5)	7.36 t (7.5)	7.64 t (7.5)	7.55 t (7.5)
5′	7.56 t (7.5)	7.49 t (7.5)	7.50 t (7.5)	7.47 t (7.5)	7.20 t (7.5)	7.53 t (7.5)	7.43 t (7.5)
6′	8.05 d (7.5)	8.01 d (7.5)	8.07 d (7.5)	8.05 d (7.5)	7.78 d (7.5)	8.07 d (7.5)	8.00 d (7.5)

[a] ^{1}H NMR data (δ) were measured at 500 MHz for **13**−**18**. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on ^{1}H−^{1}H COSY, gHSQC, HMBC, and NOE experiments. [b] Data of OAc-8: δ_H 1.51 (3H, s) in $CDCl_3$ and 1.49 (3H, s) in (Me_2CO-d_6) for **13**. [c] Data of 8-OBz in **16**: δ_H 7.86 (2H, d, 7.5 Hz, H-2″ and H-6″), 7.47 (2H, t, 7.5 Hz, H-3″ and H-5″), 7.60 (1H, t, 7.5 Hz, H-4″).

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Table 4. ^{1}H NMR Spectroscopic Data of Compounds 19–26[a]

no.	19	20	21[b]	22	23	24	25	26[c]
1	3.70 brs	3.68 brs	3.72 brs	3.76 brs	4.19 brs	4.19 brs	4.16 brs	3.71 brs
2a	2.00 dd (14.0, 4.5)	2.02 dd (15.0, 4.2)	2.02 dd (14.0, 4.5)	2.04 dd (15.0, 4.5)	1.74 m	1.69 m	1.68 m	2.03 dd (15.0, 4.5)
2b	1.49 ddd (14.0, 14.0, 4.5)	1.50 ddd (15.0, 15.0, 4.2)	1.50 ddd (14.0, 7.5, 4.5)	1.58 ddd (15.0, 15.0, 5.5)	1.72 m	1.68 m	1.68 m	1.49 ddd (15.0, 15.0, 4.5)
3a	2.05 dd (13.5, 4.5)	2.07 (12.0, 4.2)	2.04 (15.0, 4.5)	1.95 dd (15.0, 5.5)	1.96 m	2.01 m	2.02 dd (14.4, 4.2)	2.05 dd (15.0, 4.5)
3b	1.86 ddd (13.5, 13.5, 4.5)	1.77 ddd (12.0, 12.0, 4.2)	1.84 ddd (15.0, 15.0, 4.5)	1.81 ddd (15.0, 15.0, 4.5)	1.94 m	1.95 m	1.93 ddd (14.4, 14.4, 5.4)	1.96 ddd (15.0, 15.0, 4.5)
5	2.33 d (6.0)	2.29 d (6.6)	2.34 d (6.5)	2.16 d (7.5)	2.07 d (7.2)	2.42 d (6.5)	2.41 d (7.2)	2.69 d (6.0)
6a	4.83 d (6.0)	4.88 d (6.6)	4.87 d (6.5)	2.10 dd (15.0, 7.5)	2.10 dd (15.0, 7.2)	4.34 brd (6.5)	4.34 d (7.2)	4.39 d (6.0)
6b				1.82 dd (15.0, 7.5)	1.87 dd (15.0, 7.2)			
7	3.03 brs	2.70 brs	3.02 brs	2.89 d (7.5)	2.89 d (7.2)	2.73 brs	2.69 brs	3.15 s
9	2.50 dd (5.5, 4.0)	2.49 dd (5.4, 4.8)	2.54 dd (5.0, 4.5)	2.49 m	2.48 dd (5.4, 4.8)	2.48 dd (6.0, 4.5)	2.42 dd (6.0, 4.8)	2.90 dd (6.0, 5.4)
10	2.40 m	2.37 ddd (12.6, 5.4, 3.0)	2.41ddd (12.5, 5.0, 2.5)	2.35 m	2.29 ddd (9.0, 5.4, 3.0)	2.33 m	2.29 m	2.56 m
12a	2.39 dd (12.5, 12.5)	2.36 dd (12.6, 12.6)	2.39 dd (12.5, 12.5)	2.33 dd (14.0, 13.0)	2.28 dd (9.0, 3.0)	2.31 m	2.27 m	2.45 m
12b	1.63 brd (12.5)	1.61 dd (12.6, 3.0)	1.63 dd (12.5, 2.5)	1.55 dd (14.0, 5.0)	1.78 brd (9.0)	1.81 brd (10.0)	1.78 brd (9.6)	1.69 m
13	2.64 dd (5.5, 4.5)	2.57 dd (6.0, 4.2)	2.65 dd (6.0, 4.5)	2.59 dd (5.0, 4.0)	2.57 dd (5.4, 4.8)	2.58 dd (6.0, 4.5)	2.54 dd (6.0, 4.8)	2.68 dd (6.6, 4.8)
14	5.00 dd (4.5, 4.0)	5.00 dd (4.8, 4.2)	4.99 dd (4.5, 4.5)	5.04 dd (4.5, 4.0)	5.02 dd (4.8, 4.8)	5.05 dd (4.5, 4.5)	4.93 t (4.8)	5.11 dd (4.8, 4.8)
15	4.47 d (6.0)	4.52 d (5.4)	4.48 d (6.0)	4.54 d (6.5)	4.50 d (6.6)	4.57 d (6.5)	4.51 d (6.6)	4.41 d (6.0)
16	3.16 d (6.0)	3.07 d (5.4)	3.17d (6.0)	3.08 d (6.5)	3.12 d (6.6)	3.12 d (6.5)	3.10 d (6.6)	3.25 d (6.0)
17	3.46 brs	3.47 brs	3.47 brs	3.53 brs	3.54 brs	3.53 brs	3.50 brs	3.50 s
18a	3.80 d (8.0)	3.86 d (8.4)	3.79 d (8.0)	3.27 d (8.5)	3.26 d (9.0)	3.62 d (8.0)	3.61d (8.4)	3.55 d (7.8)
18b	3.60 d (8.0)	3.51 d (8.4)	3.61 d (8.0)	3.22 d (8.5)	3.23 d (9.0)	3.51 d (8.0)	3.52 d (8.4)	3.34 d (7.8)
19a	3.72 brd (12.0)	3.72 m	3.72 brd (12.0)	3.15 d (12.0)	3.17 d (12.6)	3.54 d (12.0)	3.52 brd (12.6)	3.55 d (12.0)
19b	3.29 brd (12.0)	3.17 m	3.28 brd (12.0)	3.05 d (12.0)	3.01 d (12.6)	3.19 d (12.0)	3.18 brd (12.6)	3.29 d (12.0)
20a	3.54 m	3.45 m	3.53 dq (12.5, 7.0)	3.43 m	3.42 m	3.47 m	3.45 m	3.49 m
20b	3.28 m	3.18 m	3.27 dq (12.5, 7.0)	3.17 m	3.20 m	3.20 m	3.22 m	3.42 m
21	1.51 t (7.0)	1.51 t (7.2)	1.51 t (7.0)	1.49 t (7.0)	1.46 t (7.2)	1.50 t (7.0)	1.48 t (7.2)	1.54 t (6.6)
OCH_3-1	3.37 s	3.36 s	3.38 s	3.38 s				3.36 s
OCH_3-6						3.37 s	3.38 s	3.33 s
OCH_3-8	3.27 s							
OCH_3-16	3.38 s	3.34 s	3.40 s	3.34 s	3.34 s	3.36 s	3.37 s	3.41 s
OCH_3-18	3.27 s	3.27 s	3.28 s	3.31 s	3.30 s	3.30 s	3.29 s	3.30 s
2′	8.03 d (7.5)	8.03 d (7.2)	8.05 d (7.5)	8.07 d (7.5)	8.01 d (7.2)	8.08 d (7.5)	7.87 m	7.97 d (7.8)
3′	7.51 t (7.5)	7.42 t (7.2)	7.50 t (7.5)	7.46 t (7.5)	7.41 t (7.2)	7.47 t (7.5)	7.42 m	7.51 t (7.8)
4′	7.63 t (7.5)	7.55 t (7.2)	7.63 t (7.5)	7.59 t (7.5)	7.54 t (7.2)	7.60 t (7.5)	7.43 m	7.62 t (7.8)
5′	7.51 t (7.5)	7.42 t (7.2)	7.50 t (7.5)	7.46 t (7.5)	7.41 t (7.2)	7.47 t (7.5)	7.42 m	7.51 t (7.8)
6′	8.03 d (7.5)	8.03 d (7.2)	8.05 d (7.5)	8.07 d (7.5)	8.01 d (7.2)	8.08 d (7.5)	7.87 m	7.97 d (7.8)
7′							6.53 d (16.2)	
8′							7.72 d (16.2)	

[a] ^{1}H NMR data (δ) were measured in Me_2CO-d_6 at 500 MHz for **19**, **21**, **22**, and **24** and at 600 MHz for **20** and **23**, respectively. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on ^{1}H–^{1}H COSY, gHSQC, HMBC, and NOE experiments. [b] Data of OEt-8 in **21**: δ_H 3.62 (2H, m), 0.72 (3H, t, J = 7.0 Hz). [c] Data of the OAc-8 in **26**: δ_H 1.71 (3H, s).

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OH-6 group. In the NOE difference spectrum of **19**, irradiation of H-1 enhanced the H-10 resonance, and irradiation of H-6 enhanced the H-5, H-7, OCH_3-8, and H-9 resonances. Irradiation of H-14 enhanced the H-9, H-10, and H-13 resonances, while irradiation of H-15 caused enhancement of the OCH_3-16 and H-7 resonances. These enhancements revealed that these protons were cofacial. Therefore, compound **19** was assigned the name (−)-(A-*b*)-14*α*-benzoyloxy-*N*-ethyl-6*α*,15*α*-dihydroxy-1*α*,8*β*,16*β*,18-tetramethoxyaconitane.

Compound **20** was indicated to be a demethyl analogue of **19** by spectroscopic data. Comparison of the NMR data of **20** and **19** demonstrated that H-7 and C-8 in **20** were shielded by $\Delta\delta_H$ −0.31 and $\Delta\delta_C$ −4.4 ppm, respectively. On the other hand, H-6, H-15, C-7, and C-15 were deshielded by $\Delta\delta_H$ +0.08, $\Delta\delta_H$ +0.07, $\Delta\delta_C$ +6.8, and $\Delta\delta_C$ +4.1 ppm, respectively. This suggested that the OCH_3-8 moiety in **19** was replaced by an OH-8 group in **20** and was confirmed by the 2D NMR and NOE difference data for **20**. Thus, compound **20** was defined as (−)-(A-*b*)-14*α*-benzoyloxy-*N*-ethyl-6*α*,8*β*,15*α*-trihydroxy-1*α*,16*β*,18- trimethoxyaconitane.

The spectroscopic data of compound **21** indicated that it differed from **19** only in that an OCH_3 group was substituted by an OCH_2CH_3 unit. 2D NMR analysis, especially the HMBC correlation of OCH_2CH_3/C-8, confirmed that the OCH_2CH_3 group was at C-8 in **21**. Thus, compound **21** was determined to be (−)-(A-*b*)-14*α*-benzoyloxy-8*β*-ethoxy-*N*-ethyl-6*α*,15*α*-dihydroxy-1*α*,16*β*,18-trimethoxyaconitane.

Comparison of the NMR data of compound **22** with those of **20** indicated that the OH-6 group in **22** was absent and that C-5 and C-7 were shielded by $\Delta\delta_C$ −3.8 and −10.3 ppm, respectively. $^1H-^1H$ COSY correlations of H-5/H_2-6/H-7 and HMBC correlations of H_2-6/C-4, C-7, C-5, C-8, C-11, C-17 confirmed that **22** was the 6-dehydroxy derivative of **20**. Therefore, compound **22** was assigned as (−)-(A-*b*)-14*α*-benzoyloxy-*N*-ethyl-8*β*,15*α*-dihydroxy-1*α*,16*β*,18-trimethoxyaconitane.

The NMR data of compound **23** showed that there were only two OCH_3 groups and that C-1 was shielded by $\Delta\delta_C$ −10.2 ppm, as compared to those of **22**, whereas H-1 and C-2 were deshielded by $\Delta\delta_H$ +0.43 and $\Delta\delta_C$ +6.6 ppm, respectively. This revealed that **23** was an analogue of **22**, with the OCH_3-1 group substituted by an OH-1 group. This was supported by 2D NMR data that amended the NMR assignment of **23**. Therefore, compound **23** was confirmed to be (−)-(A-*b*)-14*α*-benzoyloxy-*N*-ethyl-1*α*,8*β*,15*α*-trihydroxy-16*β*,18-dimethoxyaconitane.

Comparison of the NMR data of compound **24** with those of **23** indicated that the methylene unit (CH_2-6) in **23** was replaced by an OCH_3-substituted methine moiety (CH_3OCH-6) in **24**. Meanwhile, the H-5, C-5, and C-7 resonances in **24** were deshielded by $\Delta\delta_H$ +0.35, $\Delta\delta_C$ +3.2, and $\Delta\delta_C$ +8.1 ppm, respectively, and H-7 was shielded by $\Delta\delta_H$ −0.16 ppm. This suggested that **24** was the 6*α*-methoxy derivative of **23**, which was proved by HMBC correlations of H-6/C-4, C-5, C-7, C-8, C-17, OCH_3 and NOESY correlations of H-6 with H-5, H-7, and H-9. Thus, **24** was assigned the name (−)-(A-*b*)-14*α*-benzoyloxy-*N*-ethyl-1*α*,8*β*,15*α*-trihydroxy-6*α*,16*β*,18-trimethoxyaconitane.

Compound **25** differed from **24** only in the replacement of the benzoyl group by a cinnamoyl moiety, as indicated by HRESIMS and NMR data and confirmed by 2D NMR and NOE difference experiments. Therefore, compound **25** was confirmed to be (−)-(A-*b*)-14*α*-cinnamoyloxy-*N*-ethyl-1*α*,8*β*,15*α*-trihydroxy-6*α*,16*β*,18-trimethoxyaconitane.

Compound **26** was the conformational isomer of penduline (3,13-dideoxyaconitine),[32] with ring A in the boat conformation, as indicated by spectroscopic data and confirmed by 2D and NOE experiments. Thus, **26** was determined as (−)-(A-*b*)-8*β*-acetoxy-14*α*-benzoyloxy-*N*-ethyl-15*α*-hydroxy-1*α*,6*α*,16*β*,18-tetramethoxyaconitane.

The known compounds were identified as hypaconitine **27** and benzoylmesaconine **28** by comparison of their spectroscopic data with those reported in the literature.[4,6] Although aconitane alkaloids with ring A in the chair[2,10a,c,12b,15−17,23,24,26,33] and boat[3a,5a,11,12b,15,16,34−36] conformations have been reported, the conformation of ring A has not been clarified. Compounds **7** and **8** are the first examples of conformational isomers of aconitane alkaloids simultaneously isolated from the extract of the same plant. Because of the different physical and chemical properties of the conformational isomers, the conformation of ring A in aconitane alkaloids should be defined by structure elucidation and nomenclature. ^{13}C NMR analysis of aconitane alkaloids[16] showed that the ^{13}C chemical shift changes for C-1, C-2, C-3, and C-12 upon protonation of the nitrogen in pyrodelphine with HCl and AcOH were due to the conformational change in ring A. This result prompted us to examine this conformational change, which possibly occurred during HPLC isolation using a mobile phase containing TFA, in all the compounds considered. Hypaconitine **27** and benzoylmesaconine **28** were obtained without the use of TFA. The chair conformation of ring A in **27** and **28** was indicated by NMR spectra recorded in acetone-d_6 (Supporting Information, Table S7), especially by the H-1 resonance at δ 3.10 (dd, J = 10.5 and 6.5 Hz) for **27** and the H-3 resonance at δ 3.83 (dd, J = 12.5 and 5.0 Hz) for **28**. This was confirmed by X-ray crystallographic analysis of **27** (Supporting Information, Figure S2).[33] Two samples (**27a** and **28a**) were prepared by passing **27** and **28** through an HPLC column using 30% MeCN in H_2O (containing 0.1% TFA) as the mobile phase. ^1H NMR spectra showed that H-1 in **27a** and H-3 in **28a** gave broad singlet peaks and resonated at δ 3.70 and 4.30, respectively. In addition, as compared with those of **27**, the C-1, C-2, C-3, and C- 5 resonances in the case of **27a** were shielded by $\Delta\delta_C$ −4.3, −4.7, −7.9, and −5.1 ppm, respectively. This change was consistent with that observed for pyrodelphine after protonation of the nitrogen atom[16] and with the difference in the NMR data between **7** and **8**. This indicated that ring A in **6**, **7**, and **9**−**26** would change to the boat conformation during HPLC isolation using a mobile phase containing TFA. As discussed in the structure determination of **8**, intramolecular hydrogen bonding is not the key to the stabilization of the boat conformation of ring A in aconitane alkaloids. This was further supported by acetylation of **28** to yield two products, 15-monoacetate **28b** and 3,15-diacetate **28c**, in which ring A was in the boat conformation, as revealed by NMR data. We speculate that the boat conformation of ring A is stabilized by protonation of the nitrogen atom[16] in the solution state. This speculation was supported by the X-ray crystallographic analysis of **6**: the molecular ratio was 1:0.5:0.5 for **6**/TFA/CH_3OH in the crystal state, and no intramolecular hydrogen bonds were formed between OH-3 and the nitrogen atom. ^{13}C resonances of TFA were not observed in the ^{13}C NMR spectra of **6**, **7**, and **9**−**26**, but the appearance of an exchangeable broad resonance (around δ 7.5−9.5) with integration less of than one proton in the ^1H NMR spectra of **6**, **7**, **9**−**22**, and **24**−**26** confirmed the

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Table 5. ^{13}C NMR Spectroscopic Data of Compounds 5−8, 7a, and 9−14[a]

no.	5[b]	6[b]	7	7a[c]	8	9	10	11[b]	12[b]	13[b]	13[d]	14
1	82.5	77.9	78.2	78.0	78.1	78.1	78.4	78.0	79.0	81.4	80.3	82.1
2	26.3	31.0	31.1	28.9	35.0	30.9	31.0	30.9	23.2	22.2	22.0	22.0
3	33.8	70.3	70.1	72.4	69.8	70.3	70.3	70.2	27.6	27.4	26.6	27.6
4	47.7	43.7	44.0	43.3	44.1	43.7	44.0	43.7	38.6	39.0	38.1	38.9
5	49.0	40.2	39.0	39.1	41.5	39.6	40.2	40.3	39.3	43.0	41.4	40.8
6	84.5	82.6	82.6	82.3	84.0	82.7	82.2	82.8	82.1	82.4	81.9	81.7
7	51.7	45.4	43.1	43.2	42.4	43.4	48.9	49.8	45.1	45.5	44.7	43.5
8	90.1	88.6	81.7	81.6	81.8	81.7	76.6	82.7	88.6	90.9	90.0	74.2
9	43.4	53.1	54.3	54.1	56.0	54.4	54.1	53.8	53.0	44.0	42.9	45.4
10	41.2	78.6	79.3	79.3	79.4	79.3	79.4	78.5	78.6	39.8	39.5	54.8
11	49.8	56.4	56.8	56.6	56.9	56.7	56.3	57.1	56.4	51.0	50.1	50.2
12	36.3	47.0	47.4	47.6	48.7	47.5	47.4	46.6	47.4	36.8	37.7	36.9
13	75.1	75.4	76.0	76.0	76.2	76.0	75.8	76.1	75.4	74.9	73.8	75.6
14	79.7	78.7	79.3	79.2	80.0	79.3	79.5	78.7	78.8	79.4	78.2	80.1
15	79.6	80.1	77.7	77.5	78.7	77.7	82.5	42.2	80.2	79.3	78.4	43.3
16	91.6	90.3	93.7	93.6	94.9	94.2	91.7	83.0	90.0	91.1	89.6	83.7
17	60.2	65.0	67.7	68.1	64.1	65.3	68.1	66.8	67.2	62.8	60.7	63.9
18	79.2	77.2	77.3	76.9	75.1	77.3	78.1	77.2	78.9	78.8	77.5	79.3
19	173.0	50.4	52.2	52.2	50.5	50.6	52.6	50.2	59.2	58.2	56.8	58.9
20	41.4	51.0	41.9	42.6	42.9	50.5	42.2	50.9	42.7	50.7	50.1	51.5
21	13.2	11.1				11.2		11.0		10.8	10.0	10.9
OCH_3-1	55.6	55.3	55.4	55.7	55.7	55.1	55.4	55.3	56.0	55.8	55.6	55.9
OCH_3-6	57.8	59.1	59.1	59.2	58.8	59.1	58.3	59.2	59.3	59.3	58.6	58.2
OCH_3-8			50.4	50.4	49.9	50.4						
OCH_3-16	61.3	61.7	62.3	62.2	62.2	62.5	61.1	59.6	61.6	61.8	61.6	59.3
OCH_3-18	59.2	59.3	59.2	59.3	58.9	59.2	59.2	59.0	59.1	58.9	59.1	59.1
1′	130.9	130.6	131.2	131.1	131.7	131.2	131.4	130.9	130.6	130.6	129.1	131.5
2′	130.3	130.3	130.4	130.4	130.4	130.4	130.6	130.4	130.4	130.3	129.6	130.6
3′	129.6	129.7	129.3	129.3	129.2	129.3	129.2	129.6	129.7	129.7	129.9	129.2
4′	134.2	134.5	133.9	133.9	133.6	133.9	133.7	134.4	134.4	134.4	133.7	133.8
5′	129.6	129.7	129.3	129.3	129.2	129.3	129.2	129.6	129.7	129.7	129.9	129.2
6′	130.3	130.3	130.4	130.4	130.4	130.4	130.6	130.4	130.4	130.3	129.6	130.6
7′	166.2	166.1	166.3	166.3	166.5	166.3	166.5	166.3	166.1	166.1	165.7	166.5

[a]Data (δ) were measured in Me_2CO-d_6 at 125 MHz for **5**−**8**, **7a**, and **9**−**14**. The assignments were based on ^{1}H−^{1}H COSY, gHSQC, HMBC, and NOE experiments. [b]Data of OAc-8: δ_C 173.0 and 21.5 for **5**; 172.9 and 21.3 for **6**; 170.0 and 21.3 for **11**; 172.9 and 21.4 for **12**; and 173.0 and 21.4 for **13**. [c]Data of OAc-3 in **7a**: δ_C 170.3 and 21.0. [d]Data (δ) were measured in $CDCl_3$ for **13** at 125 MHz, data of OAc-8: δ_C 170.4 and 21.2.

protonation of the nitrogen atom by TFA contamination. This result was further supported by the ^{19}F NMR spectra of these compounds, which showed ^{19}F resonances at around δ −76 ppm, indicating the presence of TFA. Since acidification and saponification are commonly employed in the extraction and isolation of the aconitane alkaloids,[7b,8,12b,15,18,26,30a,32b] conformational transformation should occur. This, as well as the effects of the conformational change on biological activity, must be investigated in detail.

On the basis of the common pharmacological effects of aconitane alkaloids,[13a] the analgesic effects of two pairs of conformational isomers **7**/**8** and **27**/**27a** were tested. In the acetic acid-induced writhing test,[37] **7** and **8** exhibited analgesic effects with 35 ± 8% and 56 ± 12% reduction of writhes, respectively, as compared to the blank control, at a dose of 5 mg/kg (ip), while both **27** and **27a** caused death of mice. Subsequent acute toxicity evaluation showed that **27** and **27a** had LD_{50} values of 2.65 and 4.37 mg/kg (ip), respectively. At a nontoxic dose of 0.5 mg/kg (ip), **27** and **27a** exhibited analgesic effects with 36 ± 9% and 30 ± 11% reduction of writhes, respectively. This indicated that the isomer with ring A in the chair conformation (**8** or **27**) was more active than that with ring A in the boat conformation (**7** or **27a**). In addition, the effects of all the isolates against neurotoxicity induced by serum deprivation in PC12 cells were investigated by the methyl thiazolyl tetrazolium (MTT) method.[38] The results showed significant inhibition of MTT reduction by serum deprivation; at a concentration of 10 μM, **15**, **16**, and **19** increased cell viability from 55.1 ± 4.2% to 70.8 ± 5.7%, 74.7 ± 4.6%, and 75.8 ± 7.6%, respectively, indicating that they may be effective in treating neurodegenerative disorders. The isolates were also assessed for their activities against Fe^{2+}-cystine-induced rat liver microsomal lipid peroxidation,[39] DL-galactosamine-induced WB-F344 cell damage,[40] and nitric oxide production in mouse peritoneal macrophages,[41] as well as for their cytotoxicity against several human cancer cell lines,[42] but were found to be inactive at a concentration of 10 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a Rudolph Research Autopol III automatic polarimeter, and UV spectra were obtained using a Cary 300 spectrometer. Circular dichroism (CD) spectra were recorded using a JASCO J-815 CD spectrometer. IR spectra were recorded using a Nicolet 5700 FT-IR microscope (transmission). 1D and 2D NMR spectra were acquired at 500 or 600 MHz for ^{1}H and at 125 or 150 MHz for ^{13}C using INOVA 500 MHz or SYS 600 MHz spectrometers; Me_2CO-d_6 and

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Table 6. ^{13}C NMR Spectroscopic Data of Compounds 15−26 and 27a[a]

no.	15	16[b]	17	18	19	20	21[c]	22	23	24	25	26[d]
1	77.9	80.6	81.6	82.0	81.7	82.0	81.8	82.0	71.8	71.8	70.9	81.3
2	31.7	30.1	22.2	22.0	22.0	22.0	22.0	22.0	28.4	28.5	27.6	22.2
3	70.3	70.1	27.3	25.2	27.2	27.2	27.2	25.2	25.8	27.9	27.1	27.4
4	43.8	44.2	38.9	38.5	38.8	38.7	38.8	38.5	38.5	39.0	38.1	38.9
5	42.0	45.5	42.6	39.9	44.2	43.9	44.3	40.0	40.2	43.4	42.5	42.6
6	82.6	82.8	82.6	24.6	71.3	71.9	71.3	24.9	25.0	82.9	82.0	82.9
7	45.4	44.3	43.6	41.3	44.7	51.5	45.2	41.2	41.3	49.4	48.4	45.5
8	90.3	91.5	83.5	78.3	83.4	79.0	83.1	78.4	78.3	78.4	77.5	91.2
9	53.1	42.8	44.9	43.7	45.2	45.6	45.2	44.1	44.2	45.1	44.7	44.1
10	78.7	40.3	40.6	40.6	43.8	44.2	43.8	43.9	43.9	44.0	43.1	43.1
11	55.3	52.2	51.4	50.3	51.6	51.4	51.6	50.5	49.8	50.4	49.6	51.0
12	47.0	36.0	37.4	37.2	29.5	29.8	29.9	29.4	30.1	30.3	29.6	30.6
13	75.4	75.1	75.3	75.4	38.2	39.7	38.3	39.5	39.3	39.4	38.3	39.5
14	80.1	79.4	79.9	80.2	76.5	76.7	76.5	76.6	76.8	76.8	75.6	75.8
15	78.6	79.7	76.5	81.6	75.1	79.2	75.7	79.2	79.2	79.0	78.0	76.8
16	88.6	90.8	94.7	92.6	93.5	91.1	93.6	91.7	91.6	91.4	90.5	89.8
17	65.0	67.3	63.3	63.7	63.6	63.4	63.6	63.6	64.4	64.0	63.1	62.4
18	77.2	77.1	78.9	78.4	79.3	79.2	79.2	78.5	78.8	79.5	78.7	78.8
19	50.4	51.4	58.1	57.7	58.6	59.0	58.2	57.7	57.3	58.5	57.7	58.1
20	51.0	42.8	50.4	50.2	50.2	50.2	50.2	50.1	50.1	50.2	49.3	50.6
21	11.4		10.9	10.8	10.9	10.9	10.9	10.7	10.7	10.9	10.0	10.7
OCH_3-1	56.3	55.5	55.8	56.0	55.7	55.7	55.7	56.0				55.7
OCH_3-6	59.1	58.9	59.3							58.1	57.3	59.0
OCH_3-8			50.4		50.6							
OCH_3-16	61.7	61.7	62.4	61.3	57.4	57.7	57.5	57.6	57.5	57.6	56.7	58.1
OCH_3-18	59.3	59.2	59.0	59.4	59.2	59.2	59.2	59.4	59.4	59.2	58.3	59.2
1′	130.6	129.8	131.1	131.5	131.5	131.6	131.5	131.6	131.6	131.5	134.7	130.9
2′	130.3	129.3	130.4	130.6	130.3	130.5	130.3	130.6	130.6	130.6	128.2	130.2
3′	129.7	129.1	129.3	129.2	129.3	129.2	129.2	129.2	129.2	129.2	128.9	129.7
4′	134.5	133.9	133.9	133.6	133.8	133.6	133.8	133.6	133.6	133.6	130.2	134.2
5′	129.7	129.1	129.3	129.2	129.3	129.2	129.2	129.2	129.2	129.2	128.9	129.7
6′	130.3	129.3	130.4	130.6	130.3	130.5	130.3	130.6	130.6	130.5	128.2	130.2
7′	161.9	166.5	166.3	166.6	166.1	166.3	166.0	166.3	166.3	166.2	144.6	165.9
8′											118.5	
9′											165.6	

[a]Data (δ) were measured in Me_2CO-d_6 at 125 MHz for **15**−**19**, **21**, **22**, and **24** and at 150 MHz for **20**, **23**, **25**, and **26**, respectively. [b]Data of OBz-8 in **16**: δ_C 130.2 (C-1″), 130.3 (C-2″), 130.2 (C-3″), 134.6 (C-4″), 130.2 (C-5″),130.3 (C-6″), and 167.7 (C-7″). [c]Data of OEt-8: δ_C 58.2 and 15.7 for **21**. [d]Data of OAc-8 in **26**: δ_C 173.1 and 21.7.

$CDCl_3$ solvent peaks were used as references. ESIMS data were measured using a Q-Trap LC/MS/MS (Turbo Ionspray Source) spectrometer. HRESIMS data were measured using an Agilent Technologies 6520 Accurate Mass Q-TOF LC/MS spectrometer. Column chromatography (CC) was performed using basified silica gel (200−300 mesh, Qingdao Marine Chemical Inc. China) and a Sephadex LH-20 column (Amersham Biosciences, Inc., Shanghai, China). Preparative thin-layer chromatography (TLC) was performed on high-performance silica gel preparative TLC plates ($HSGF_{254}$, glass precoated, Yantai Jiangyou Silica Gel Development Co., Ltd., Yantai, China). HPLC separation was performed using an instrument consisting of a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual-wavelength absorbance detector, with a Prevail (250 × 10 mm i.d.) preparative column packed with C18 (10 μM). TLC was carried out on glass precoated silica gel GF_{254} plates. Spots were observed under UV light or by spraying the developed plates with Dragendorff's reagent or a mixture of 5% H_2SO_4−ethanol, followed by heating. Unless otherwise noted, all the chemicals were obtained from commercially available sources and used without further purification.

Plant Material. The lateral root of *Aconitum carmichaelii* was collected in June 2009 from the culture field in Jiangyou, Sichuan Province, People's Republic of China. Plant identity was verified by Dr. Yan Ren (Chengdu University of TCM, Sichuan 610075, China). A voucher specimen (no. ID-S-2383) was deposited at the herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China.

Extraction and Isolation. The air-dried lateral roots of *A. carmichaelii* (50 kg) were powdered and extracted with H_2O (3 × 150 L × 6 h) at 40 °C. The H_2O extract was concentrated to 120 L under reduced pressure, subjected to chromatography over a macroporous adsorbent resin (HPD-110, 19 kg) column (20 × 200 cm), and eluted successively with H_2O (50 L), 30% EtOH (120 L), 50% EtOH (120 L), and 95% EtOH (100 L) to afford the corresponding fractions A−D. After removal of the solvent, C (3.5 kg) was chromatographed over MCI gel CHP 20P with successive elution using H_2O (10 L), 30% EtOH (30 L), 50% EtOH (20 L), and 95% EtOH (10 L) to give fractions C1−C4. Fraction C3 (200 g) was fractionated via silica gel CC, with gradient elution [$CHCl_3$/MeOH (100:1−1:4)] to yield C3-1−C3-7, based on TLC analysis. Fraction C3-2 (10.4 g) was separated using basified silica gel (pH 8−9) CC eluted with a petroleum ether/Me_2CO/diethylamine (25:1:1 to 4:1:1) mixture to afford C3-2-1−C3-2-7. Fraction C3-2-2 (3.0 g) was crystallized from MeOH to give **27** (1.2 g). Fraction C3-3 (1.1 g) was subjected to RP HPLC (30% MeCN in H_2O, containing 0.1% TFA) to give C3-3-1−C3-3-6. Fraction C3-3-2 (250 mg) was separated by PTLC (petroleum ether/Me_2CO/diethylamine, 3:1:1) to yield **22** (13 mg) and **23** (30 mg). Fraction C3-4 (3.4 g) was subjected to CC over Sephadex LH-20,

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eluted using a $CHCl_3$/MeOH (1:1) mixture, to afford C3-4-1−C3-4-3. Fraction C3-4-2 (298 mg) was purified by RP HPLC (50% MeCN in H_2O, containing 0.1% TFA) to obtain **1** (20 mg). Fraction C3-4-3 (1.1 g) was separated by basified silica gel CC (petroleum ether/Me_2CO/diethylamine, 20:1:1 to 5:1:1) to give C3-4-3-1−C3-4-3-6. Fraction C3-4-3-2 (236 mg) was purified by RP HPLC (30% MeCN in H_2O, containing 0.1% TFA) to yield **6** (10.3 mg) and **7** (50 mg). Fraction C3-5 (7.8 g) was subjected to RP flash chromatography (30−95% MeOH in H_2O, containing 0.1% TFA) to give C3-5-1−C3-5-5. Fraction C3-5-2 (3.6 g) was subjected to basified silica gel CC (petroleum ether/Me_2CO/diethylamine, 30:1:1 to 1:1:1) to give C3-5-2-1−C3-5-2-3. Fraction C3-5-2-2 (3.2 g), using RP HPLC (35% MeCN in H_2O, containing 0.1% TFA), afforded C3-5-2-2-1−C3-5-2-2-3. Fraction C3-5-2-2-3 was further separated by RP HPLC (55% MeOH in H_2O, containing 0.1% TFA) to yield **14** (15.2 mg), **15** (20.5 mg), **16** (6.7 mg), **17** (7.1 mg), **18** (23.4 mg), and **19** (23.3 mg). Fraction C3-5-3 (2.4 g) was chromatographed on basified silica gel (petroleum/Me_2CO/diethylamine, 50:1:1, 25:1:1, and 10:1:1 successively) to give C3-5-3-1−C3-5-3-3. Separation of C3-5-3-2 (716 mg) by CC over Sephadex LH-20 (petroleum/$CHCl_3$/MeOH, 5:5:1) gave C3-5-3-2-1 and C3-5-3-2-2, which were further separated by RP HPLC (45% MeOH in H_2O, containing 0.1% TFA) to yield **9** (20.5 mg), **10** (20.3 mg), **11** (6.6 mg), and **12** (4.3 mg). Fraction C3-5-4 (95 mg) was separated by PTLC (petroleum ether/Me_2CO/diethylamine, 3:1:1) and then RP HPLC (50% MeOH in H_2O; 0.1% TFA) to yield **5** (13.3 mg). Fraction C3-6 (6.2 g), using RP MPLC (35% MeCN in H_2O; 0.1% TFA), gave C3-6-1−C3-6-4. Fraction C3-6-2 was subjected to CC over Sephadex LH-20 eluted with petroleum ether/$CHCl_3$/MeOH (5:3:1) to give C3-6-2-1−C3-6-2-4. Fraction C3-6-2-1 (611 mg) was separated into C3-6-2-1-1−C3-6-2-1-3 by PTLC (petroleum ether/Me_2CO/diethylamine, 3:1:1) and then purified by RP HPLC (MeOH in H_2O; 0.1% TFA) to afford **13** (5.1 mg), **20** (20.2 mg), and **21** (0.5 mg) from C3-6-2-1-1 (58 mg) and **24** (89.0 mg), **25** (10.3 mg), and **26** (12.3 mg) from C3-6-2-1-3 (183 mg). Fraction C3-6-3 was separated by basified silica gel CC eluted with petroleum ether/Me_2CO/diethylamine (15:1:1 to 5:1:1) to give C3-6-3-2−C3-6-3-4. C3-6-3-3 (1.2 g) was subjected to Sephadex LH-20 CC (CH_3Cl−MeOH, 1:1) and then RP HPLC (30% MeCN in H_2O, containing 0.1% TFA) to afford **2** (13.1 mg), **3** (14.6 mg), and **4** (4.3 mg). Fraction C3-6-4 was fractionated by basified silica gel CC, eluted with petroleum ether/Me_2CO/diethylamine (10:1:1 to 2:1:1), to give C3-6-4-1 and C3-6-4-2. Further separation of C3-6-4-1 (43 mg) and C3-6-4-2 (386 mg) by PTLC (petroleum ether/Me_2CO/diethylamine, 3:1:1) afforded **8** (10 mg) and **28** (152 mg), respectively.

*(+)-(13R,19S)-1β,11α-Diacetoxy-2α-benzoyloxy-13,19-dihydroxyhetisan (**1**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ +18.1 (*c* 0.51, MeOH); UV (MeOH) λ_{max} (log ε) 229 (3.84), 274(2.94) nm; CD (MeOH) 226 ($\Delta\varepsilon$ +3.68), 253 ($\Delta\varepsilon$ −0.32); IR (KBr) ν_{max} 3394, 3187, 2921, 2850, 1719, 1692 (sh), 1647, 1469, 1420, 1318, 1205, 1188, 1138, 801, 720, 649 cm^{-1}; ^1H NMR (Me_2CO-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 1; (+)-ESIMS *m*/*z* 550 $[M + H]^+$; (+)-HRESIMS *m*/*z* 550.2445 $[M + H]^+$ (calcd for $C_{31}H_{36}NO_8$, 550.2435).

*(−)-(13R,19S)-11α,19-Dihydroxy-N-methyl-13-(S-2-methylbutyryloxy)-2α-propionyloxyhetisanium hydroxide (**2**):* white, amorphous powder (MeOH); $[\alpha]^{20}_D$ −24.8 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 201 (3.88); IR (KBr) ν_{max} 3413, 2976, 2939, 1738, 1684, 1464, 1421, 1355, 1306, 1275, 1201, 1178, 1142 1097, 1074, 1057, 1021,970, 930, 898, 881, 834, 801, 767, 721 cm^{-1}; ^1H NMR (Me_2CO-d_6, 600 MHz) data, see Table 1; ^{13}C NMR (Me_2CO-d_6, 150 MHz) data, see Table 1; (+)-ESIMS *m*/*z* 500 $[M - OH]^+$; (−)-ESIMS *m*/*z* 515 $[M - H]^-$; (+)-HRESIMS *m*/*z* 500.3020 $[M - OH]^+$ (calcd for $C_{29}H_{42}NO_6$, 500.3007).

*(−)-(13R,19S)-7β,11α,19-Trihydroxy-N-methyl-13-(S-2-methylbutyryloxy)-2α-propionyloxyhetisanium hydroxide (**3**):* white, amorphous powder (MeOH); $[\alpha]^{20}_D$ −26.1 (*c* 0.19, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.76) nm; IR (KBr) ν_{max} 3394, 3083, 2942, 2852, 1732 (sh), 1678, 1453, 1367, 1320, 1204, 1139, 1052, 956, 845, 802, 722 cm^{-1}; ^1H NMR (Me_2CO-d_6, 600 MHz) data, see Table 1; ^{13}C NMR (Me_2CO-d_6, 150 MHz), see Table 1; (+)-ESIMS *m*/*z* 516 $[M - OH]^+$; (+)-HRESIMS *m*/*z* 516.2968 $[M - OH]^+$ (calcd for $C_{29}H_{42}NO_7$, 516.2956).

*(+)-(13R,19S)-2α-Isobutyryloxy-7β,11α,19-trihydroxy-N-methyl-13-(S-2-methylbutyryloxy)hetisanium hydroxide (**4**):* white, amorphous powder (MeOH); $[\alpha]^{20}_D$ +10.2 (*c* 0.37, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.87) nm; IR (KBr) ν_{max} 3394, 2977, 2924, 2850, 1736 (sh), 1712 (sh),1683, 1464, 1421, 1355, 1307, 1274, 1200, 1141, 1076, 1057, 1021, 964, 930, 901, 881, 840, 801, 766, 723 cm^{-1}; ^1H NMR (Me_2CO-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 1; (+)-ESIMS *m*/*z* 530 $[M - OH]^+$; (+)-HRESIMS *m*/*z* 530.3117 $[M - OH]^+$ (calcd for $C_{30}H_{44}NO_7$, 530.3112).

*(−)-(A-c)-8β-Acetoxy-14α-benzoyloxy-N-ethyl-13β,15α-dihydroxy-1α,6α,16β,18-tetramethoxy-19-oxo-aconitane (**5**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −28.4 (*c* 0.46, MeOH); CD (MeOH) 220 ($\Delta\varepsilon$ +0.03), 237 ($\Delta\varepsilon$ +0.20); IR (KBr) ν_{max} 3524, 3410, 2934, 2879, 2850, 1720, 1680, 1643, 1454, 1379, 1306, 1278, 1208, 1133, 1085, 1055, 987, 961, 927, 900, 843, 803, 725 cm^{-1}; ^1H NMR (Me_2CO-d_6, 500 MHz) data, see Table 2; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 5; (+)-ESIMS *m*/*z* 644 $[M + H]^+$; (+)-HRESIMS *m*/*z* 644.3054 $[M + H]^+$ (calcd for $C_{34}H_{46}NO_{11}$, 644.3065), 666.2876 $[M + Na]^+$ (calcd for $C_{34}H_{45}NO_{11}Na$, 666.2885).

*(−)-(A-b)-8β-Acetoxy-14α-benzoyloxy-N-ethyl-3α,10β,13β,15α-tetrahydroxy-1α,6α,16β,18-tetramethoxyaconitane (**6**):* colorless needles (MeOH); $[\alpha]^{20}_D$ −7.9 (*c* 0.53, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.74), 273 (2.70) nm; CD (MeOH) 215 ($\Delta\varepsilon$ +1.43), 222 ($\Delta\varepsilon$ +0.96), 234 ($\Delta\varepsilon$ +1.58); IR (KBr) ν_{max} 3437, 3066, 2949, 1728, 1690, 1494, 1454, 1276, 1199, 1123, 1102, 934, 718 cm^{-1}; ^1H NMR (Me_2CO-d_6, 500 MHz) data, see Table 2; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 5; (+)-ESIMS *m*/*z* 662 $[M + H]^+$; (+)-HRESIMS *m*/*z* 662.3187 $[M + H]^+$(calcd for $C_{32}H_{46}NO_{11}$, 662.3171).

*X-ray Crystallography of Compound **6**.* $C_{34}H_{47}NO_{12}$, M = 661.75, orthorhombic, $P2_12_12_1$, a = 10.296(3) Å, b = 15.228(3) Å, c = 23.631(6) Å, $\alpha = \beta = \gamma = 90°$, V = 3705.0(16) $Å^3$, Z = 4, D_{calcd} = 1.448 g cm^{-3}, 6980 reflections independent, 6223 reflections observed ($|F|^2 \geq 2\sigma|F|^2$), R_1 = 0.0656, wR_2 = 0.1784, S = 1.045.

The data were collected on a Rigaku MicroMax 002+ diffractometer with Cu Kα radiation by using the ω-scan technique to a maximum 2θ value of 145.38°. The crystal structures were solved by direct methods by using SHELXS-97, and all non-hydrogen atoms were refined anisotropically using the least-squares method. All hydrogen atoms were positioned by geometrical calculations and difference Fourier overlapping calculation. The absolute configuration was determined on the basis of the Flack parameter −0.1(2). Crystallographic data for the structure of **6** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 809199. Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk).

*(−)-(A-b)-14α-Benzoyloxy-3α,10β,13β,15α-tetrahydroxy-1α,6α,8β,16β,18-pentamethoxy-N-methylaconitane (**7**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −5.32 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.03), 272 (2.98) nm; CD (MeOH) 217 ($\Delta\varepsilon$ +2.37), 231 ($\Delta\varepsilon$ +4.16), 255 ($\Delta\varepsilon$ +0.34); IR (KBr) ν_{max} 3361, 3086, 2940, 2832, 1719, 1685, 1603, 1453, 1413, 1317, 1278, 1201, 1108, 1071, 1008, 973, 935, 880, 832, 800, 764, 716, 681 cm^{-1}; ^1H NMR (Me_2CO-d_6, 500 MHz) data, see Table 2; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 5; (+)-ESIMS *m*/*z* 620 $[M + H]^+$; (+)-EIMS *m*/*z* 619 $[M]^{+\cdot}$, 604 $[M - CH_3]^{+\bullet}$, 589 $[M - 2CH_3]^{+\bullet}$, 588 $[M - CH_3O]^{+\bullet}$; (+)-HRESIMS *m*/*z* 620.3071 $[M + H]^+$ (calcd for $C_{32}H_{46}NO_{11}$, 620.3065).

*(−)-(A-c)-14α-Benzoyloxy-3α,10β,13β,15α-tetrahydroxy-1α,6α,8β,16β,18-pentamethoxy-N-methylaconitane (**8**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −2.07 (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.82), 272 (2.75) nm; CD (MeOH) 218 ($\Delta\varepsilon$ +0.11), 227 ($\Delta\varepsilon$ −0.02), 243 ($\Delta\varepsilon$ +0.24); IR (KBr) ν_{max} 3393, 3363, 3190, 2921, 2850, 1678, 1647, 1469, 1421, 1278, 1203, 1135, 1105, 1070, 1045, 973, 801, 720, 648 cm^{-1}; ^1H NMR (Me_2CO-d_6, 500 MHz) data, see Table 2;

^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 5; (+)-ESIMS m/z 620 $[M + H]^+$; (+)-EIMS m/z 619 $[M]^{+\bullet}$, 604 $[M - CH_3]^{+\bullet}$, 589 $[M - 2CH_3]^{+\bullet}$; (+)-HRESIMS m/z 620.3061 $[M + H]^+$ (calcd for $C_{32}H_{46}NO_{11}$, 620.3065).

*(−)-(A-b)-14α-Benzoyloxy-N-ethyl-3α,10β,13β,15α-tetrahydroxy-1α,6α,8β,16β,18-pentamethoxyaconitane (**9**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −13.4 (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.05), 272 (2.90) nm; CD (MeOH) 210 ($\Delta\varepsilon$ +0.07), 230 ($\Delta\varepsilon$ +0.08); IR (KBr) ν_{max} 3371, 3091, 2935, 2824, 1713, 1684, 1605, 1492, 1453, 1426, 1400, 1353, 1283, 1250, 1203, 1136, 1111, 1071, 976, 940, 930, 882, 841, 801, 721, 711 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 500 MHz) data, see Table 2; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 5; (+)-ESIMS m/z 634 $[M + H]^+$; (+)-HRESIMS m/z 634.3216 $[M + H]^+$ (calcd for $C_{34}H_{48}NO_{11}$, 634.3222).

*(−)-(A-b)-14α-Benzoyloxy-3α,10β,8β,13β,15α-pentahydroxy-1α,6α,16β,18-tetramethoxy-N-methylaconitane (**10**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −2.34 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε) 228 (3.99), 272 (3.24) nm; IR (KBr) ν_{max} 3370, 3093, 2922, 2849, 1711, 1679, 1492, 1425, 1352, 1282, 1250, 1202, 1136, 1111, 1092, 1071, 976, 940, 882, 841, 800, 721, 711, 682, 558 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 500 MHz) data, see Table 2; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 5; (+)-ESIMS m/z 606 $[M + H]^+$; (−)-ESIMS m/z 604 $[M - H]^-$; (+)-HRESIMS m/z 606.2909 $[M + H]^+$ (calcd for $C_{31}H_{44}NO_{11}$, 606.2909).

*(−)-(A-b)-8β-Acetoxy-14α-benzoyloxy-N-ethyl-3α,10β,13β-trihydroxy-1α,6α,16β,18-tetramethoxyaconitane (**11**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −3.78 (*c* 0.55, MeOH); UV (MeOH) λ_{max} (log ε) 228 (3.88), 273 (3.12) nm; CD (MeOH) 217 ($\Delta\varepsilon$ +0.34), 232 ($\Delta\varepsilon$ +0.73); IR (KBr) ν_{max} 3390, 2937, 2831, 1716, 1681, 1453, 1376, 1280, 1247, 1205, 1137, 1108, 1049, 978, 945, 840, 801, 720 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 500 MHz) data, see Table 2; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 5; (+)-ESIMS m/z 646 $[M + H]^+$; (+)-HRESIMS m/z 646.3221 $[M + H]^+$ (calcd for $C_{34}H_{48}NO_{11}$, 646.3222).

*(−)-(A-b)-8β-Acetoxy-14α-benzoyloxy-10β,13β,15α-trihydroxy-1α,6α,16β,18-tetramethoxy-N-methylaconitane (**12**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −24.3 (*c* 0.51, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.09), 272 (2.93) nm; CD (MeOH) 217 ($\Delta\varepsilon$ +0.24), 234 ($\Delta\varepsilon$ +0.89); IR (KBr) ν_{max} 3486, 3067, 2943, 2831, 1721, 1687, 1454, 1375, 1279, 1247, 1202, 1181, 1112, 1029, 977, 946, 835, 801, 741, 718 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 500 MHz) data, see Table 2; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 5; (+)-ESIMS m/z 632 $[M + H]^+$; (+)-HRESIMS m/z 632.3082 $[M + H]^+$ (calcd for $C_{33}H_{46}NO_{11}$, 632.3065).

*(−)-(A-b)-8β-Aacetoxy-14α-benzoyloxy-N-ethyl-13β,15α-dihydroxy-1α,6α,16β,18-tetramethoxyaconitane (**13**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −7.40 (*c* 0.31, MeOH); UV (MeOH) λ_{max} (log ε) 229 (3.71), 272 (2.55) nm; CD (MeOH) 228 ($\Delta\varepsilon$ +0.37), 245 ($\Delta\varepsilon$ +0.47), 257 ($\Delta\varepsilon$ +0.48); IR (KBr) ν_{max} 3487, 3067, 2941, 2831, 1778, 1721, 1692, 1453, 1371, 1280, 1201, 1137, 1113, 1055, 1027, 800, 714 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 500 MHz) data, see Table 3; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 5; (+)-ESIMS m/z 630 $[M + H]^+$; (+)-HRESIMS m/z 630.3279 $[M + H]^+$(calcd for $C_{34}H_{48}NO_{10}$, 630.3273).

*(−)-(A-b)-14α-Benzoyloxy-N-ethyl-8β,13β-dihydroxy-1α,6α,16β,18-tetramethoxyaconitane (**14**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −10.2 (*c* 0.79, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.01), 273 (3.23) nm; CD (MeOH) 217 ($\Delta\varepsilon$ −0.09), 234 ($\Delta\varepsilon$ +0.22), 255 ($\Delta\varepsilon$ −0.03); IR (KBr) ν_{max} 3407, 3076, 2943, 2832, 1693, 1453, 1415, 1364, 1323, 1281, 1202, 1118, 1057, 1026, 981, 958, 835, 802, 719 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 500 MHz) data, see Table 3; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 5; (+)-ESIMS m/z 572 $[M + H]^+$; (+)-HRESIMS m/z 572.3222 $[M + H]^+$(calcd for $C_{32}H_{46}NO_8$, 572.3218).

*(−)-(A-b)-14α-Benzoyloxy-N-ethyl-3α,8β,13β,15α-tetrahydroxy-1α,6α,16β,18-tetramethoxyaconitane (**15**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −16.3 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 228 (3.85), 273 (3.14) nm; CD (MeOH) 216 ($\Delta\varepsilon$ +0.80), 229 ($\Delta\varepsilon$ +1.61). ^{1}H NMR (Me_2CO-d_6, 600 MHz) data, see Table 3; ^{13}C NMR (CD_3COCD_3, 150 MHz) data, see Table 6; (+)-ESIMS m/z 604 $[M + H]^+$; (+)-HRESIMS m/z 604.3117 $[M + H]^+$ (calcd for $C_{32}H_{46}NO_{10}$, 604.3116).

*(−)-(A-b)-8β,14α-Dibenzoyloxy-N-ethyl-3α,13β,15α-trihydroxy-1α,6α,16β,18-tetramethoxyaconitane (**16**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −2.11 (*c* 0.33, MeOH); CD (MeOH) 219 ($\Delta\varepsilon$ −0.28), 243 ($\Delta\varepsilon$ +0.55); ^{1}H NMR (Me_2CO-d_6, 500 MHz) data, see Table 3; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 6; (+)-ESIMS m/z 694 $[M + H]^+$; (+)-HRESIMS m/z 694.3232 $[M + H]^+$ (calcd for $C_{38}H_{48}NO_{11}$, 694.3222).

*(−)-(A-b)-14α-Benzoyloxy-N-ethyl-13β,15α-dihydroxy-1α,6α,8β,16β,18-pentamethoxyaconitane (**17**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −13.9 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.91), 273 (2.76) nm; CD (MeOH) 218 ($\Delta\varepsilon$ +0.10), 228 ($\Delta\varepsilon$ +0.25), 251 ($\Delta\varepsilon$ −0.22); IR (KBr) ν_{max} 3353, 3071, 2940, 2832, 1717, 1691, 1453, 1278, 1200, 1175, 1172, 1076, 978, 800, 717 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 500 MHz) data, see Table 3; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 6; (+)-ESIMS m/z 602 $[M + H]^+$; (+)-HRESIMS m/z 602.3332 $[M + H]^+$ (calcd for $C_{33}H_{48}NO_9$, 602.3324).

*(−)-(A-b)-14α-Benzoyloxy-N-ethyl-8β,13β,15α-trihydroxy-1α,16β,18-trimethoxyaconitane (**18**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −10.1 (*c* 0.69, MeOH); UV (MeOH) λ_{max} (log ε) 229 (3.54), 272 (2.39) nm; CD (MeOH) 214 ($\Delta\varepsilon$ +1.68), 232 ($\Delta\varepsilon$ +1.18), 254 ($\Delta\varepsilon$ −0.10); IR (KBr) ν_{max} 3361, 3199, 3069, 2922, 2851, 1677, 1634, 1468, 1454, 1423, 1322, 1281, 1202, 1180, 1132, 1050, 1027, 835, 801, 718 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 500 MHz) data, see Table 3; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 6; (+)-ESIMS m/z 558 $[M + H]^+$; (+)-HRESIMS m/z 558.3062 $[M + H]^+$(calcd for $C_{31}H_{44}NO_8$, 558.3061).

*(−)-(A-b)-14α-Benzoyloxy-N-ethyl-6α,15α-dihydroxy-1α,8β,16β,18-tetramethoxyaconitane (**19**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −17.3 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.02), 272 (2.88) nm; CD (MeOH) 218 ($\Delta\varepsilon$ +0.19), 232 ($\Delta\varepsilon$ +0.40); IR (KBr) ν_{max} 3394, 3187, 3010, 2921, 2850, 1646, 1469, 1420, 1343, 1300, 1245, 1187, 1119, 721, 646 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 500 MHz) data, see Table 4; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 6; (+)-ESIMS m/z 572 $[M + H]^+$; (+)-HRESIMS m/z 572.3214 $[M + H]^+$ (calcd for $C_{32}H_{46}NO_8$ 572.3218).

*(−)-(A-b)-14α-Benzoyloxy-N-ethyl-6α,8β,15α-trihydroxy-1α,16β,18-trimethoxyaconitane (**20**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −6.40 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε) 228 (3.97), 273 (2.83) nm; CD (MeOH) 231 ($\Delta\varepsilon$ +0.08), 270 ($\Delta\varepsilon$ +0.01); IR (KBr) ν_{max} 3386, 3083, 2933, 2854, 1679, 1452, 1367, 1318, 1280, 1204, 1137, 1074, 1052, 842, 801, 720 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 600 MHz) data, see Table 4; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 6; (+)-ESIMS m/z 558 $[M + H]^+$; (+)-HRESIMS m/z 558.3063 $[M + H]^+$ (calcd for $C_{31}H_{44}NO_8$, 558.3061).

*(−)-(A-b)-14α-Benzoyloxy-8β-ethoxy-N-ethyl-6α,15α-dihydroxy-1α,16β,18-trimethoxyaconitane (**21**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −12.0 (*c* 0.42, MeOH); UV (MeOH) λ_{max} (log ε) 232 (4.02), 272 (2.91) nm; CD (MeOH) 217 ($\Delta\varepsilon$ +0.75), 231 ($\Delta\varepsilon$ +1.50), 256 ($\Delta\varepsilon$ −0.07); IR (KBr) ν_{max} 3370, 3075, 2936, 2893, 2831, 1692, 1453, 1392, 1280, 1201, 1176, 1126, 1074, 1045, 1008, 954, 830, 800, 718 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 500 MHz) data, see Table 4; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 6; (+)-ESIMS m/z 558 $[M + H]^+$; (+)-ESIMS m/z 586 $[M + H]^+$; (+)-HRESIMS m/z 586.3383 $[M + H]^+$ (calcd for $C_{33}H_{48}NO_8$, 586.3374).

*(−)-(A-b)-14α-Benzoyloxy-N-ethyl-8β,15α-dihydroxy-1α,16β,18-trimethoxyaconitane (**22**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −7.70 (*c* 0.52, MeOH); UV (MeOH) λ_{max} (log ε) 228 (3.97), 271 (2.91) nm; CD (MeOH) 227 ($\Delta\varepsilon$ +0.19), 249 ($\Delta\varepsilon$ −0.15); IR (KBr) ν_{max} 3409, 3071, 2936, 2897, 1679, 1452, 1319, 1279, 1204, 1131, 1095, 1048, 836, 801, 720 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 500 MHz) data, see Table 4; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 6; (+)-ESIMS m/z 542 $[M + H]^+$; (+)-HRESIMS m/z 542.3116 $[M + H]^+$ (calcd for $C_{31}H_{44}NO_7$, 542.3112).

*(−)-(A-b)-14α-Benzoyloxy-N-ethyl-1α,8β,15α-trihydroxy-16β,18-dimethoxyaconitane (**23**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −11.1 (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ε) 229 (3.98), 274 (3.19) nm; CD (MeOH) 226 ($\Delta\varepsilon$ +0.33), 239 ($\Delta\varepsilon$ +0.17), 255 ($\Delta\varepsilon$ +0.28); IR (KBr) ν_{max} 3314, 2955, 2918, 2849, 1732, 1670, 1539,

1464, 1397, 1260, 1198, 1100, 1027, 801, 722 cm^{-1}; ^1H NMR (Me_2CO-d_6, 600 MHz) data, see Table 4; ^{13}C NMR (Me_2CO-d_6, 150 MHz) data, see Table 6; (+)-ESIMS m/z 528 $[M + H]^+$; (+)-HRESIMS m/z 528.2958 $[M + H]^+$ (calcd for $C_{30}H_{42}NO_7$, 528.2956).

*(−)-(A-b)-14α-Benzoyloxy-N-ethyl-1α,8β,15α-trihydroxy-6α,16β,18-trimethoxyaconitane (**24**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −13.0 (*c* 0.58, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.83), 272 (2.66) nm; CD (MeOH) 219 ($\Delta\varepsilon$ −0.12), 229 ($\Delta\varepsilon$ −0.01), 250 ($\Delta\varepsilon$ −0.51); IR (KBr) ν_{max} 3359, 3077, 2936, 1692, 1602, 1453, 1279, 1200, 1176, 1113, 1076, 1043, 1002, 956, 891, 829, 800, 718 cm^{-1}; ^1H NMR (Me_2CO-d_6, 500 MHz) data, see Table 4; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 6; (+)-ESIMS m/z 558 $[M + H]^+$; (+)-HRESIMS m/z 558.3070 $[M + H]^+$ (calcd for $C_{31}H_{44}NO_8$, 558.3061).

*(−)-(A-b)-14α-Cinnamoyloxy-N-ethyl-1α,8β,15α-trihydroxy-6α,16β,18-trimethoxyaconitane (**25**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −4.63 (*c* 1.06, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.02), 277 (3.97) nm; CD (MeOH) 227 ($\Delta\varepsilon$ −0.49), 253 ($\Delta\varepsilon$ +0.33), 280 ($\Delta\varepsilon$ +0.56); IR (KBr) ν_{max} 3373, 3063, 2941, 2828, 1678, 1639, 1451, 1312, 1282, 1202, 1114, 982, 936, 863, 833, 802, 771, 720, 686 cm^{-1}; ^1H NMR (Me_2CO-d_6, 600 MHz) data, see Table 4; ^{13}C NMR (Me_2CO-d_6, 150 MHz) data, see Table 6; (+)-ESIMS m/z 584 $[M + H]^+$; (+)-HRESIMS m/z 584.3223 $[M + H]^+$ (calcd for $C_{33}H_{46}NO_8$, 584.3218).

*(−)-(A-b)-8β-acetoxy-14α-benzoyloxy-N-ethyl-15α-hydroxy-1α,6α,16β,18-tetramethoxyaconitane (**26**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −37.9 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ε) 228 (3.82), 274 (3.18) nm; ^1H NMR (Me_2CO-d_6, 500 MHz) data, see Table 4; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 6; (+)-ESIMS m/z 614 $[M + H]^+$; (+)-HRESIMS m/z 614.3328 $[M + H]^+$ (calcd for $C_{34}H_{48}NO_9$, 614.3324).

*(+)-(A-b)-8β-Acetoxy-14α-benzoyloxy-13β,15α-dihydroxy-1α,6α,16α,18-tereamethoxy-N-methylaconitane (**27a**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ +4.40 (*c* 0.49, MeOH); ^1H NMR (Me_2CO-d_6 500 MHz) data, see Supporting Information, Table S7; ^{13}C NMR (Me_2CO-d_6 125 MHz) data, see Supporting Information, Table S8.

*(−)-(A-b)-14α-Benzoyloxy-3α,8β,10β,13β,15α-pentahydroxy-1α,6α,16α,18-tereamethoxy-N-methylaconitane (**28a**):* amorphous powder (MeOH); $[\alpha]^{20}_D$ −7.5 (*c* 0.87, MeOH); ^1H NMR (Me_2CO-d_6, 500 MHz) data, see Supporting Information, Table S7; ^{13}C NMR (Me_2CO-d_6 125 MHz) data, see Supporting Information, Table S8.

*Hydrolysis of **2**−**4**.* Compound **2** (5 mg), **3** (5 mg), or **4** (2 mg) was dissolved in 95% EtOH (1.2 mL), and NaOH (10 mg) was added. The solution was stirred at 40 °C for 24 h, acidified with HCl (2 N), and then evaporated under reduced pressure. The residue was dissolved in $CHCl_3$/MeOH (1:l, 2 mL) and subjected to CC over Sephadex LH-20, eluting with $CHCl_3$/MeOH (1:l), to afford a mixture with $[\alpha]^{20}_D$ +11.2 (*c* 0.06, MeOH). The ^1H NMR spectrum of the mixture showed signals corresponding to 2-methylbutyric acid and propanoic acid.

*Acetylation of Compounds **7**, **8**, and **28**.* Compound **7** (9.0 mg), **8** (4.5 mg), or **28** (12.0 mg) was dissolved in pyridine (1.0 mL), and acetic anhydride (0.2 mL) was added. The solution was kept at room temperature overnight and evaporated to dryness under reduced pressure. The residue was separated by RP HPLC (40% MeCN/H_2O, containing 0.1% TFA) to give **7a** (8 and 4 mg) from **7** and **8** and **28b** (3 mg) and **28c** (10 mg) from **28**, respectively. **7a**: amorphous powder (MeOH); $[\alpha]^{20}_D$ −3.05 (*c* 0.42, MeOH); ^1H NMR (Me_2CO-d_6 500 MHz) data, see Table 2; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 5; (+)-ESIMS m/z 662 $[M + H]^+$. **28b**: amorphous powder (MeOH); $[\alpha]^{20}_D$ −5.4 (*c* 0.46, MeOH); ^1H NMR(Me_2CO-d_6, 500 MHz) data, see Supporting Information, Table S7; ^{13}C NMR (Me_2CO-d_6 125 MHz) data, see Supporting Information, Table S8; (+)-ESIMS m/z 632 $[M + H]^+$. **28c**: amorphous powder (MeOH); $[\alpha]^{20}_D$ −8.7 (*c* 0.57, MeOH); ^1H NMR (Me_2CO-d_6, 500 MHz) data, see Supporting Information, Table S7; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Supporting Information, Table S8; (+)-ESIMS m/z 674 $[M + H]^+$.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Copies of IR, MS, 1D and/or 2D NMR, and CD spectra for compounds **1**−**28**, **27a**, and **28a**−**c**. This can be accessed free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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