A New Diterpenoid and a New Diterpenoid Alkaloid from *Aconitum coreanum*

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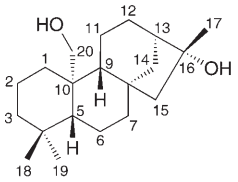
A new diterpenoid, guan fu diterpenoid A (**1**), and a new diterpenoid alkaloid, guan fu base S (**2**), were isolated from the Chinese medicinal herb *Aconitum coreanum* (LÈVL.) RAPAICS, together with five known diterpenoid alkaloids guan fu base P, guan fu base R, guan fu base G, guan fu base F, and guan fu base Z. The structures of the two new compounds were elucidated as *ent*-kaurane-16,20-diol (**1**) and (11β,13*S*)-2,3,15,16-tetradehydro-16,17-dihydrohetisan-11,13,14-triol 11,13-diacetate (**2**) on the basis of HR-MS and 2D-NMR analyses. This is the first report of an *ent*-kaurane diterpenoid in *Aconitum coreanum*.

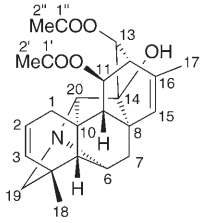
Introduction. – The roots of *Aconitum coreanum* (LÈVL.) RAPAICS, a well known traditional Chinese medicine called 'guanbaifu', has been widely used to treat various kinds of disorder over centuries [1]. Pharmacological studies and clinical practice demonstrated that its extract has anti-arrhythmic, analgesic, and anti-inflammatory effects [2]. Previous chemical studies of this herb have led to the isolation of more than 30 diterpenoid alkaloids, several sitosterols, and some organic acids [3]. Guan fu base A, one of the main diterpenoid alkaloids has been developed into a neo-type anti-arrhythmic drug [4]. This kind of C_{20}-diterpenoid alkaloids shows significant anti-arrhythmic activity, especially to treat ventricular premature beats and paroxysmal supraventricular tachycardia [5].

In searching for further bioactive constituents from the roots of *Aconitum coreanum*, we herein describe the isolation and identification of a new diterpenoid, *ent*-kaurane-16,20-diol[1]) (**1**), and a new diterpenoid alkaloid, (11β,13*S*)-2,3,15,16-tetradehydro-16,17-dihydrohetisan-11,13,14-triol 11,13-diacetate (**2**). Their structures were elucidated on the basis of spectroscopic methods, especially 2D-NMR techniques, including ^{1}H,^{13}C-HMQC, HMBC, and NOESY experiments. In addition, the five known diterpenoid alkaloids guan fu base P, guan fu base R, guan fu base G, guan fu base F, and guan fu base Z were also isolated from this plant and identified by comparing their physical and spectroscopic data with those reported in the literature.

[1]) The configuration of '*ent*-kaurane' is based on the IUPAC name; notice that the molecular skeleton of **1** with implied (5β,8α,9β,10α,13α,16β) configuration is named 'kaurane' by *Chem. Abstr.*



Guan fu diterpenoid A (**1**)



Guan fu base S (**2**)

Results and Discussion. – The pulverized, air-dried roots of *A. coreanum* were extracted with 95% EtOH. The residue of the extract was suspended in H_2O, and then successively extracted with AcOEt (pH > 10 adjusted by adding aqueous NH_3 solution) and BuOH. The AcOEt extract was concentrated to afford a crude residue containing alkaloids. Extensive purification of the residue by repeated column chromatography finally afforded compounds **1**, **2**, and five known alkaloids.

Compound **1** was obtained as colorless needles and had the molecular formula $C_{20}H_{34}O_2$, as deduced from its HR-ESI-MS (*m/z* 307.2651 ($[M+H]^+$)). From the ^{1}H- and ^{13}C-NMR (*Table 1*), DEPT, HMQC, HMBC (*Fig. 1* and *Table 1*), and NOESY (*Fig. 2* and *Table 1*) data, the structure of **1** was established as *ent*-kaurane-16,20-diol[1]).

The ^{13}C-NMR (DEPT) spectrum of **1** showed the presence of 20 C-atom signals at relatively high field: three Me (δ(C) 24.4, 34.2, and 22.7), ten CH_2 (δ(C) 35.1, 18.4, 42.2, 20.3, 38.5, 18.6, 25.9, 42.9, 58.6, and 61.5), three CH (δ(C) 56.9, 56.5, and 49.3), and four quaternary-C signals (δ(C) 33.0, 45.1, 42.9, and 79.4). In the ^{1}H-NMR spectrum, three angular Me signals at δ 1.35, 0.85, and 0.81 (each *s*) and one OCH_2 signal at δ 4.01–4.10 (*m*, 2 H) were observed. Since the unsaturation degree of **1** was four and there were no unsaturated C-atoms, the structure must have four rings. The ^{1}H,^{1}H-COSY and HMQC experiments indicated the presence of three fragments, *i.e.*, $CH_2CH_2CH_2$ (fragment *A*), $CHCH_2CH_2$ (fragment *B*), and $CHCH_2CH_2CHCH_2$ (fragment *C*). In the HMBC experiment (*Fig. 1* and *Table 1*), the long-range correlations CH_2(1)/C(10) and H–C(3)/C(4) identified fragment *A* as the C(1)–C(2)–C(3) part of ring A of the molecule. Similarly, the two-bond correlations H–C(5)/C(4) and C(10), and CH_2(7)/C(8) confirmed that fragment *B* consists of the C(5)–C(6)–C(7) part of the diterpenoid core. The HMBC cross-peaks H–C(9)/C(8), H–C(9)/C(10), and H–C(14)/C(8) verified that fragment *C* constitutes ring C of the diterpenoid skeleton. The HMBC cross-peaks H–C(15)/C(16), Me(17)/C(16) and the ^{13}C-NMR signal δ(C) 79.4 of C(16) showed the presence of a fragment *D*, *i.e.*, $CH_2C(OR)Me$ (R = H). The H–C(15)/C(8), H–C(14)/C(8), H–C(14)/C(13), and H–C(13)/C(16) correlations established that C(15), C(16), C(13), C(14), and C(8) constitute the five-membered ring D. Additionally, the two isolated Me groups were identified as C(18) and C(19) by the HMBC cross-peaks Me(18)/C(4) and Me(19)/C(4), and the isolated CH_2OH group was assigned to C(20) by the cross-peak CH_2(20)/C(10). The existence of an OH group was also confirmed by the IR absorptions at 3396 and 3348 cm^{-1}. The above data strongly suggested that **1** is a C_{20} *ent*-kaurane[1]) diterpenoid. Comparing its ^{13}C-NMR data with those of the know compound (16α)-*ent*-kaurane-16,17-diol [6] revealed that the signals were very similar, except for a downfield shift of the signal of C(20) and a highfield shift of the signal of C(17), thus testifying that **1** was an *ent*-kaurane-16,20-diol. The relative configuration of **1** was determined mainly by NOESY (*Fig. 2*) analyses, with the NOE cross-peaks Me(17)/H–C(13) and Me(17)/CH_2(15), so the Me group at C(16) was in the β-orientation, confirming the α-configuration of OH–C(16).

It is supposed that a diterpenoid functions as a precursor of a C_{20}-hetisine diterpenoid alkaloid [7]; however, diterpenoids were rarely isolated from *A. coreanum*.

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

	δ(C)	δ(H)	HMBC ($^1H \rightarrow {}^{13}C$)	NOESY
H_α–C(1)	35.1	2.16–2.19 (*m*)	C(2), C(10)	–
H_β–C(1)		0.57–0.63 (*m*)	C(2), C(3), C(9), C(10), C(20)	H–C(5), H–C(9)
H_α–C(2)	18.4	1.69–1.79 (*m*)	C(1)	–
H_β–C(2)		1.42–1.52 (*m*)	C(1), C(3), C(4)	–
H_α–C(3)	42.2	1.37–1.44 (*m*)	C(4), C(19)	–
H_β–C(3)		1.11–1.18 (*m*)	C(2), C(4), C(18), C(19)	H–C(18)
C(4)	33.0	–	–	–
H–C(5)	56.9	0.90 (*dd*, J = 12.5, 2.0)	C(4), C(6), C(10), C(19), C(20)	H_β–C(1), H–C(9)
H_α–C(6)	20.3	1.43–1.50 (*m*)	C(5), C(7)	H–C(20)
H_β–C(6)		1.30–1.32 (*m*)	C(5), C(7)	–
H_α–C(7)	38.5	1.95 (*d*, J = 11.0)	C(5), C(8), C(9), C(15)	–
H_β–C(7)		1.60–1.68 (*m*)	C(6), C(8), C(9)	–
C(8)	45.1	–	–	–
H–C(9)	56.5	1.05 (*d*, J = 8.5)	C(8), C(10), C(11), C(12)	H–C(1), H–C(5), H–C(15)
C(10)	42.9	–	–	–
H_α–C(11)	18.6	1.53–1.61 (*m*)	C(8), C(9), C(12)	–
H_β–C(11)		1.37–1.43 (*m*)	C(9), C(12)	–
H_α–C(12)	25.9	1.72–1.79 (*m*)	C(9), C(11), C(13)	H_α–C(14)
H_β–C(12)		1.43–1.50 (*m*)	C(9), C(11), C(14)	–
H–C(13)	49.3	1.80–1.84 (*m*)	C(12), C(14), C(15), C(16)	H–C(17)
H_α–C(14)	42.9	1.57–1.68 (*m*)	C(8), C(13), C(15)	H–C(12), H–C(20)
H_β–C(14)		1.47–1.54 (*m*)	C(7), C(8), C(13), C(15)	
H_α–C(15)	58.6	1.53–1.60 (*m*)	C(8), C(9), C(16)	H–C(17)
H_β–C(15)		1.53–1.60 (*m*)	C(8), C(9), C(16)	H–C(9), H–C(17)
C(16)	79.4	–	–	–
Me(17)	24.4	1.35 (*s*)	C(13), C(15), C(16)	H–C(13), H_α–C(15), H_β–C(15)
Me(18)	34.2	0.85 (*s*)	C(3), C(4), C(5), C(19)	H_β–C(3)
Me(19)	22.7	0.81 (*s*)	C(3), C(4), C(5), C(18)	H–C(20)
CH_2(20)	61.5	4.01–4.10 (*m*)	C(1), C(9), C(10)	H_α–C(6), H_α–C(14), H–C(19)

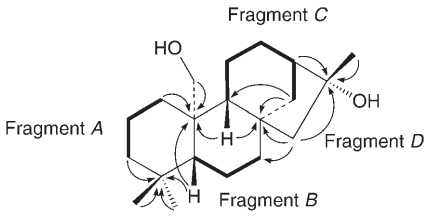


Fig. 1. *Key ^{1}H,^{1}H-COSY* (—) *and Key HMBC* (H → C) *data of* **1**

Thus, the now established presence of compound **1** could confirm the hypothesis of the biogenetic pathway of diterpenoid alkaloids involving a diterpenoid.

Compound **2** was isolated as white powder. The molecular formula of **2** was determined to be $C_{24}H_{29}NO_5$ by HR-ESI-MS (m/z 412.2108 ($[M+H]^+$)). The full

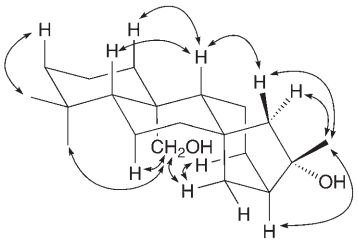


Fig. 2. *NOESY Data of* **1**

assignments of the ^{1}H- and ^{13}C-NMR signals (*Table 2*) of **2** were accomplished by a combination of HMQC, HMBC (*Fig. 3*), and NOESY (*Fig. 4*) data, which allowed to elucidate the structure of **2** as (11β,13*S*)-2,3,15,16-tetradehydro-16,17-dihydrohetisan-11,13,14-triol 11,13-diacetate.

Table 2. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$) *of* **2**. δ in ppm, J in Hz.

	δ(C)	δ(H)	HMBC ($^{1}H \rightarrow {}^{13}C$)	NOESY
H_α–C(1)	28.1	2.61–2.69 (*m*)	C(2), C(3), C(5), C(10)	H–C(11), H–C(13)
H_β–C(1)		2.02–2.10 (*m*)	–	–
H–C(2)	122.3	5.48–5.54 (*m*)	C(1), C(4)	–
H–C(3)	134.8	5.64 (*d*, J = 8.5)	C(1), C(4)	–
C(4)	39.2	–	–	–
H–C(5)	56.7	1.61 (br. *s*)	C(4), C(6), C(10), C(19), C(20)	–
H–C(6)	73.0	2.74 (br. *s*)	C(4), C(5), C(7), C(8), C(20)	–
H_α–C(7)	30.0	2.24 (*d*, J = 13.8)	C(6), C(8), C(14)	H–C(15)
H_β–C(7)		1.77 (*dd*, J = 13.8, 2.1)	C(8)	–
C(8)	48.5	–	–	–
H–C(9)	46.3	1.89 (*d*, J = 9.3)	C(7), C(8), C(10), C(12), C(14), C(20)	–
C(10)	46.6	–	–	–
H–C(11)	77.4	4.84 (*dd*, J = 9.3, 1.4)	C(9), C(10), C(12), C(13), C(16), C(1′)	H–C(1), H–C(13)
H–C(12)	45.7	2.68–2.70 (*m*)	C(9), C(11), C(13), C(14), C(16), C(17)	–
H–C(13)	79.6	4.88 (*t*, J = 2.0)	C(11), C(12), C(14), C(16), C(1″)	H–C(1), H–C(11)
C(14)	80.0	–	–	–
H–C(15)	126.0	5.27 (*t*, J = 1.4)	C(8), C(9), C(14), C(16), C(17)	H_α–C(7)
C(16)	139.7	–	–	–
Me(17)	19.4	1.86 (*s*)	C(12), C(15), C(16)	–
Me(18)	26.2	1.08 (*s*)	C(3), C(4), C(5), C(19)	–
H_α–C(19)	68.7	2.72 (*d*, J = 10.5)	C(3), C(4), C(5), C(18), C(20)	–
H_β–C(19)		2.23 (*d*, J = 10.5)	C(3), C(4), C(5), C(18), C(20)	H–C(20)
H–C(20)	64.5	3.10 (*s*)	C(6), C(8), C(10), C(14), C(19)	H_α–C(1), H_β–C(19)
*Me*C(1′)OO	21.3	2.04 (*s*)	C(1′)	–
Me*C*(1′)OO	170.5	–	–	–
*Me*C(1″)OO	21.2	2.08 (*s*)	C(1″)	–
Me*C*(1″)OO	169.7	–	–	–

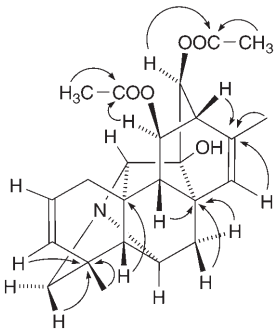


Fig. 3. *Key HMBC* (H → C) *data of* **2**

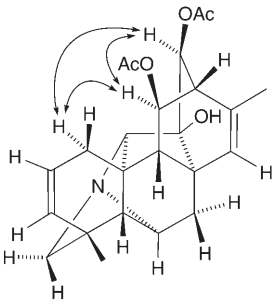


Fig. 4. *Key NOESY data of* **2**

The ^{13}C-NMR (DEPT) spectrum of **2** exhibited 20 signals attributed to the C_{20} diterpenoid core: two Me (δ(C) 19.4 and 26.2), three CH_2 (δ(C) 28.1, 30.0, and 68.7), and ten CH groups (δ(C) 122.3, 134.8, 56.7, 73.0, 46.3, 77.4, 45.7, 79.6, 126.0, and 64.5), and five quaternary C-atoms (δ(C) 39.2, 48.5, 46.6, 80.0, and 139.7). Apart from the above 20 C-atoms, two Me (δ(C) 21.2 and 21.3) and two C=O groups (δ(C) 169.7 and 170.5) were found. The ^{1}H-NMR spectrum indicated signals for two MeC=O groups (δ(H) 2.04 and 2.08, 2*s*), two Me groups (δ(H) 1.08 and 1.86, 2*s*), and three olefinic H-atoms (δ(H) 5.27, 5.48–5.54, and 5.64). The absence of ^{1}H-NMR signals for MeN, MeO, and aromatic-ring moieties suggested that there is no oxazolidine ring in the C_{20} skeleton and inferred that **2** is a C_{20} hetisine (=(2α,11α,13*R*)-hetisan-2,11,13-triol) [8] alkaloid. The ^{1}H,^{1}H-COSY and HMQC experiments indicated the presence of three fragments: CH_2CH=CH (fragment *A*), CHCHCH_2 (fragment *B*), and CHCHCHCH (fragment *C*). In the HMBC experiment (*Fig. 3* and *Table 2*), the long-range correlations H_α–C(1)/C(10) and H–C(3)/C(4) identified fragment *A* as the C(1)–C(2)–C(3) part of ring A of the molecule. Similarly, the two-bond correlations H–C(5)/C(10), and CH_2(7)/C(8) confirmed that fragment *B* consists of the C(5)–C(6)–C(7) part of ring B of the hetisine core. The HMBC cross-peaks H–C(9)/C(8), H–C(9)/C(10), and H–C(13)/C(14) verified that fragment *C* is part of the diterpenoid skeleton. The HMBC cross-peaks Me(17)/C(16), H–C(15)/C(16) and the ^{13}C-NMR signals at δ(C) 139.7 (C(16)) and 126.0 (C(15)) showed the presence of a fragment *D*, *i.e.*, MeC=CH. The H–C(15)/C(8), H–C(12)/C(16), and H–C(9)/C(8) correlations established that C(8), C(9), C(11), C(12), C(16), and C(15) constitute the six-membered ring C of the hetisine core. The two-bond correlations H–C(13)/C(14), H–C(12)/C(13), H–C(12)/C(11), and H–C(12)/C(16) and the three-bond correlations H–C(9)/C(14), H–C(15)/C(14), H–C(7)/C(14), H–C(11)/C(13), and H–C(13)/C(16) confirmed that C(8), C(14), C(13), and C(12) constitute a bridging ring across ring C connecting C(8) and C(12). Similarly, the five-membered rings C(8)–C(9)–C(10)–C(20)–C(14) and C(20)–N–C(6)–C(5)–C(10) were confirmed by the ^{1}H,^{13}C-HMBC cross-peaks H–C(9)/C(10), H–C(9)/C(8), H–C(9)/C(20), H–C(9)/C(14), H–C(20)/C(10), H–C(20)/C(14), and H–C(20)/C(8), and H–C(20)/C(6), H–C(20)/C(10), H–C(6)/C(5), H–C(5)/

C(6), and H–C(5)/C(10). The six-membered ring C(20)–N–C(19)–C(4)–C(5)–C(10) was confirmed by the ^{1}H,^{13}C-HMBC signals H–C(20)/C(19), H–C(20)/C(10), CH_2(19)/C(4), H–C(5)/C(4), and H–C(5)/C(10). These data strongly supported the structure of a C_{20} hetisine alkaloid for **2**. The two isolated MeCOO groups were positioned at C(11) and C(13) by means of the HMBC cross-peaks H–C(11)/C(1′) and H–C(13)/C(1″). The relative configuration of **2** was determined mainly by NOESY (*Fig. 4*) analyses, with the key NOEs H_α–C(1)/H–C(11), H_α–C(1)/H–C(13), and H–C(11)/H–C(13), confirmed that H–C(11) and H–C(13) were all on the α-side of the mean plane of the molecular skeleton.

The five known compounds guan fu base P [9], guan fu base R [10], guan fu base G [11], guan fu base F [12], and guan fu base Z [13] were also isolated and identified on the basis of their physical and spectroscopic data.

The authors are grateful to Prof. *Wei-Chun Wu* (Shenyang Pharmaceutical University) for collecting the plant material and for the identification of the plant.

Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh; *Qingdao Marine Chemical Plant*, Qingdao, P. R. China); *Sephadex LH-20* (*Pharmacia*). TLC: silica gel plates (*Qingdao Marine Chemical Plant*, Qingdao, P. R. China). M.p.: *XT-4* micro-melting-point apparatus; uncorrected. Optical rotations: *Perkin-Elmer-MC-241* polarimenter. IR Spectra: *Nicolet-Impact-410* FT-IR spectrometer; KBr pellets; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker-AV-500* spectrometer; δ in ppm, J in Hz. HR-ESI-MS: *Agilent-1100-LC/TOF MSD*; in *m/z*.

Plant Material. The roots of *Aconitum coreanum* were collected in Liaoning province, P. R. China, in October 2004, and identified by Prof. *Wei-Chun Wu* (Shenyang Pharmaceutical University). A voucher specimen (No. 20041013) was deposited in the center for instrumental analysis, China Pharmaceutical University, Nanjing, P. R. China.

Extraction and Isolation. The air-dried roots of *A. coreanum* (15 kg) were pulverized and extracted with 95% EtOH (3×). The extract was concentrated to a suitable volume, suspended in H_2O, and then successively extracted with alkalinized AcOEt (pH > 10, adjusted by aq. NH_3 soln.) for 3 cycles. The AcOEt extract was concentrated to afford a crude residue (130 g), which was further separated by CC (Al_2O_3, petroleum ether/AcOEt 10:1 → 1:2): *Fractions A–E*. *Fr. B* (27 g) was subjected to CC (SiO_2, petroleum ether/Me_2CO 10:1 → 2:1): *Fr. B1–B5*. *Fr. B1* (6.5 g) was purified by CC (SiO_2, petroleum ether/Me_2CO 100:1 → 5:1), and the resulting major components were purified by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1): **1** (12 mg), **2** (29 mg), guan fu base P (13 mg), guan fu base R (19 mg), guan fu base G (25 mg), guan fu base F (15 mg), and guan fu base Z (33 mg).

Guan Fu Diterpenoid A (= ent-*Kaurane-16,20-diol*; **1**): Colorless needles. M.p. 131–132°. $[\alpha]_D^{25} = -31.5$ ($c = 0.8$, $CHCl_3$). IR (KBr): 3396, 3348, 2918, 2849, 1470, 1057, 933. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 307.2651 ($[M+H]^+$; calc. 307.2637).

Guan Fu Base S (= *(11β,13*S*)-2,3,15,16-Tetradehydro-16,17-dihydrohetisan-11,13,14-triol 11,13-Diacetate*; **2**): White powder. M.p. 182–184°. $[\alpha]_D^{25} = +16.5$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3412, 1742, 1734, 1666, 1680, 1247, 1230. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 412.2108 ($[M+H]^+$; calc. 412.2124).

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