New Labdane-Type Diterpenoids from *Amentotaxus argotaenia*

by **Qin Yang**[a])[b]), **Guan Ye***[a]), **Jia-Quan Feng**[b]), and **Wei-Min Zhao***[b])

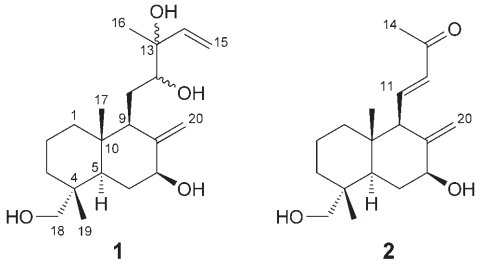
[a]) Central Research Institute of the Shanghai Pharmaceutical Group, Shanghai 201203, P. R. China (phone/fax: +86-21-50806623; e-mail: yg4847@yahoo.com.cn)

[b]) Department of Natural Products Chemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Rd., Zhangjiang Hi-tech Park, Shanghai 201203, P. R. China (phone/fax: +86-21-50806052; e-mail: wmzhao@mail.shcnc.ac.cn)

A new labdane diterpene, amentotaxin A (**1**), and an unprecedented dinorlabdane diterpene, amentotaxin B (**2**), were isolated from the stems of *Amentotaxus argotaenia* (Hance) PILGER, together with seven known compounds. Their structures were identified by in-depth spectroscopic and mass-spectrometric analyses.

Introduction. – *Amentotaxus* (Taxaceae), including approximately five species, is presently confined to eastern Asia, though its fossil remains of the Tertiary Age are found in North America and Europe [1]. Unlike the famous antitumor *Taxus* plants of the same family, which have been studied extensively, *Amentotaxus* plants have drawn little attention so far. Previous investigations on these plants mainly focused on morphology and taxonomy, and phytochemical studies have only been carried out in recent years, terpenoids and flavonoids being the major secondary metabolites of this genus [2–10]. Among the isolates from *Amentotaxus*, several cassane-type diterpenes with potential anti-inflammatory effects, as well as *ent*-kaurane-type diterpenes with anticancer effects were found [5][6].

Amentotaxus argotaenia (Hance) PILGER, an endangered tree endemic to China, has been used in Chinese folk medicine to treat poisonous-snake bites, eczema, abdominal pain, bone fracture, and trauma [11]. As a continuation of our efforts to find new bioactive natural products from herbal medicines, we investigated the chemical components of *A. argotaenia*. As a result, two new diterpenes were isolated, compounds **1** and **2**, together with seven known compounds. Herein, we report the isolation and structure elucidation of these compounds.



Results and Discussion. – Compound **1** was isolated as a colorless, amorphous powder. It was assigned the molecular formula $C_{20}H_{34}O_4$ by positive-ion HR-ESI-MS analysis (*m/z* 361.2361 ($[M+Na]^+$)). The IR spectrum of **1** showed the presence of OH (3384, 1153, 1045 cm^{-1}) and Me (1385 cm^{-1}) groups. A total of 20 resonances in the ^{13}C-NMR spectrum (*Table*) indicated a diterpene, and comparison of its NMR spectra with those of diterpenes isolated previously from this genus suggested that **1** was a labdane with an exocyclic CH_2 group [δ(C) 103.4 (C(20))], a Me(19) group (δ(C) 17.7), a HO–CH_2(18) moiety (δ(C) 69.9), and an angular Me(17) group (δ(C) 15.2). The ^{1}H-NMR spectrum (*Table*) of **1** supported the above analysis, showing two sets of Me signals at δ(H) 0.56 (*s*, Me(17)) and 0.59 (*s*, Me(19)), an exocyclic methylidene group at δ(H) 4.53 and 5.08 (2 br. *s*, CH_2(20)), and an oxygenated CH_2 at δ(H) 2.79 (*d*, J = 10.4 Hz, H_a–C(18)) and 3.17 (*d*, J = 10.8 Hz, H_b–C(18)).

Table. *^{13}C- and ^{1}H-NMR Data for* **1** *and* **2**. At 100 and 400 MHz, resp., in (D_6)DMSO (**1**) or CD_3OD (**2**); δ in ppm, J in Hz. Labdane atom numbering.

Atom	**1**		**2**	
	δ(C)	δ(H)	δ(C)	δ(H)
1	38.3 (*t*)	0.84–0.87 (*m*, H_α), 1.52–1.53 (*m*, H_β)	41.6 (*t*)	1.48–1.50 (*m*)
2	18.5 (*t*)	1.50–1.52 (*m*)	19.9 (*t*)	0.99–1.02 (*m*)
3	35.2 (*t*)	1.08–1.10 (*m*, H_α), 1.46–1.50 (*m*, H_β)	36.7 (*t*)	1.27–1.29 (*m*)
4	37.5 (*s*)		39.4 (*s*)	
5	45.2 (*d*)	1.51–1.52 (*m*)	46.6 (*d*)	1.58–1.59 (*m*)
6	33.4 (*t*)	1.78–1.84 (*m*, H_α), 1.01–1.05 (*m*, H_β)	33.9 (*t*)	1.93–2.00 (*m*, H_α), 1.32–1.37 (*m*, H_β)
7	72.1 (*d*)	3.67–3.73 (*m*)	73.8 (*d*)	4.02–4.05 (*m*)
8	151.7 (*s*)		152.7 (s)	
9	49.9 (*d*)	1.84–1.86 (*m*)	60.8 (*d*)	2.53 (*d*, J = 10.4)
10	38.4 (*s*)		40.7 (*s*)	
11	24.9 (*t*)	1.26–1.30 (*m*, H_α), 1.54–1.58 (*m*, H_β)	148.4 (*d*)	6.98 (*dd*, J = 15.9, 10.3)
12	73.9 (*d*)	3.08–3.11 (*m*)	135.0 (*d*)	6.10 (*d*, J = 15.7)
13	74.9 (*s*)		201.5 (*s*)	
14	144.7 (*d*)	5.91 (*dd*, J = 17.2, 10.7)	27.7 (*q*)	2.29 (*s*)
15	111.9 (*t*)	4.96 (*dd*, J = 10.9, 2.5, H_a), 5.14 (*dd*, J = 17.2, 2.5, H_b)		
16	23.6 (*q*)	1.08 (*s*)		
17	15.2 (*q*)	0.56 (*s*)	16.5 (*q*)	0.97 (*s*)
18	69.9 (*t*)	2.79 (*d*, J = 10.4, H_a), 3.17 (*d*, J = 10.8, H_b)	72.0 (*t*)	3.00 (*d*, J = 11.1, H_a), 3.39 (*d*, J = 11.1, H_b)
19	17.7 (*q*)	0.59 (*s*)	18.8 (*q*)	0.77 (*s*)
20	103.4 (*t*)	4.53 (br. *s*, H_a), 5.08 (br. *s*, H_b)	106.6 (*t*)	4.59 (br. *s*, H_a), 5.18 (br. *s*, H_b)

Inspection of the ^{1}H,^{1}H-COSY spectrum of **1** enabled us to identify two CH(OH)–CH_2–CH spin systems, corresponding to the fragments C(5)–C(6)–C(7) and C(9)–C(11)–C(12). Assignment of the former fragment was corroborated by

HMBC correlations between H–C(5) at δ(H) 1.51–1.52 (*m*) and both C(17) and C(4), between H–C(7) at δ(H) 3.67–3.73 (*m*) and C(8), and between CH_2(20) at δ(H) 4.53/5.08 and C(7) (*Fig. 1*). Examination of the downfield signals in the ^{1}H-NMR spectrum of **1** showed an *ABX* system at δ(H) 4.96 (*dd*, J = 10.9, 2.5 Hz, H_a–C(15)), 5.14 (*dd*, J = 17.2, 2.5 Hz, H_b–C(15)), and 5.91 (*dd*, J = 17.2, 10.7 Hz, H–C(14)), indicating a singly substituted C=C bond. Attachment of this C=C bond was pinned down by the HMBC correlation of the quaternary, oxygenated C(13) atom with Me(16), and by HMBC interactions of H–C(16) at δ(H) 1.08 with both C(12) and C(14), as well as of H–C(14) at δ(H) 5.91 with C(12) (*Fig. 1*). The second spin system, C(9)–C(11)–C(12), was located similarly, based on the HMBC correlations between H–C(9) at δ(H) 1.84–1.86 (*m*) and C(17), C(1), and C(7), and between H–C(20) at δ(H) 4.53/5.08 and C(9). Thus, the planar structure of **1** was established.

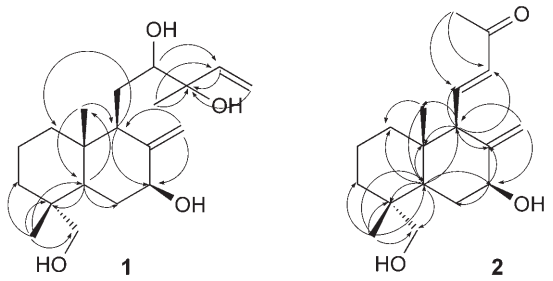


Fig. 1. *Key HMBC (H → C) correlations for* **1** *and* **2**

The relative configuration of **1** was derived by analysis of its ROESY spectrum, in which correlation signals were found between H–C(5) and H–C(7), between H–C(5) and Me(18)[1]), and between H–C(7) and H–C(9), which, thus, implied α-H atoms at positions 7 and 9 (*Fig. 2*). Therefore, compound **1** was finally identified as labda-8(20),14-diene-7,12,13,18-tetrol, and given the trivial name *amentotaxin A*.

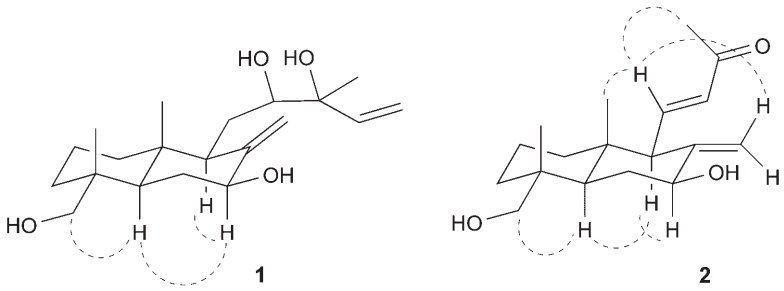


Fig. 2. *Key ROESY (H → H) correlations for* **1** *and* **2**

Compound **2** was obtained as a colorless crystal. The molecular formula of **2** was $C_{18}H_{28}O_3$, as indicated by HR-ESI-MS (m/z 315.1940 ($[M+Na]^+$)). Its ^{1}H- and ^{13}C-NMR data (*Table*) resemble those of **1**, except that there were several signals

[1]) Tentatively assigned to be α-orientated, based on biogenetic grounds.

absent. Strong absorption bands for OH groups (3377 cm^{-1}) were found in the IR spectrum, as well as a methylidene function (899 cm^{-1}). An α,β-unsaturated methyl ketone was indicated by NMR [δ(H) 6.10 (*d*, $J = 15.7$ Hz, H–C(12)); δ(C) 135.0 (C(12)); δ(H) 6.98 (*dd*, $J = 15.9$, 10.3 Hz, H–C(11)); δ(C) 148.4 (C(11)); δ(C) 201.5 (C(13); δ(H) 2.29 (*s*, Me(14)); δ(C) 27.7 (C(14))] and IR (1664 cm^{-1}). The α,β-unsaturated C=C bond was assigned (*E*)-configuration, as demonstrated by a large coupling constant (15.9 Hz) between H–C(11) and H–C(12), and by ROESY correlations between H–C(14) and H–C(11) (*Fig. 2*). The ^{1}H-NMR spectrum of **2** further showed two Me groups at δ(H) 0.77 (*s*, Me(19)) and 0.97 (*s*, Me(17)), an oxygenated CH_2 group at δ(H) 3.00 (*d*, $J = 11.1$ Hz, H_a–C(18)) and 3.39 (*d*, $J =$ 11.1 Hz, H_b–C(18)), as well as two olefinic, exocyclic H-atoms at δ(H) 4.59 (br. *s*, H_a–C(20)) and 5.18 (br. *s*, H_b–C(20)).

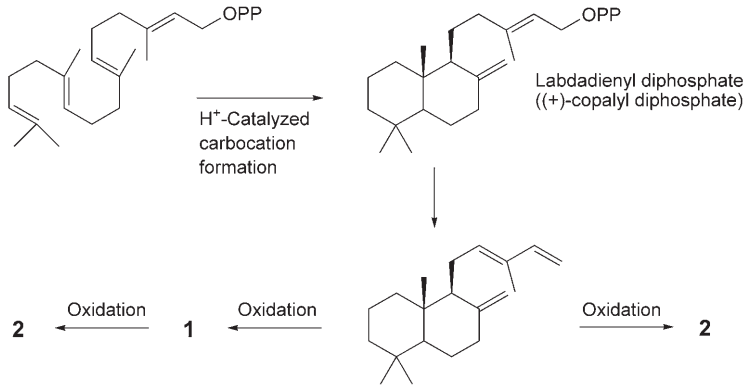
The ^{13}C-NMR spectrum of **2** (*Table*) showed the resonances of two regular Me groups, an additional Me group in α-position to an sp^2-hybridized C-atom, one oxygenated and four non-oxygenated CH_2 moieties, two olefinic C-atoms, one oxygenated CH, two tertiary sp^3 C-atoms, and two quaternary sp^3 C-atoms. From these data, a bicyclic structure identical to that of **1** was secured. The linkages of the peripheral residues were confirmed by analysis of the ^{1}H,^{1}H-NMR coupling constant between H–C(11) at δ(H) 6.98 (*dd*, $J = 15.9$, 10.3 Hz) and H–C(9) at δ(H) 2.53 (*d*, $J = 10.4$ Hz), in addition to HMBC interactions between H–C(9) and C(11) and C(12), and between H–C(12) and C(9), indicating that C(9) was connected to C(11). Other key HMBC correlations are shown in *Fig. 1*.

The α-orientation of H–C(7) and H–C(9) was indicated by ROESY interactions between H–C(5) and CH_2(18), between H–C(9) and both H–C(5) and H–C(7), and between H–C(11) and Me(14), CH_2(20), and Me(17) (*Fig. 2*). From these data, the structure of compound **2** was identified as 7,18-dihydroxy-15,16-dinorlabda-8(20),11-dien-13-one, and given the trivial name *amentotaxin B*.

Compound **2** is a rare dinorlabdane diterpene. To our best knowledge, only six structurally related compounds have been reported before [12–18], all of them being 15,16-dinor-diterpenoids with different oxygenation patterns, suggesting that they may be oxidized derivatives of a common C_{20} labdane precursor. The biosynthesis of these compounds has not been studied, except for the biomimetic synthesis of tobacco labdanoids [19]. A possible biogenetic pathway [19–22] leading to **1** and **2** is proposed in the *Scheme*.

The seven known compounds were identified as 1-(4-hydroxy-3-methoxyphenyl)-2-{2-methoxy-4-[(*E*)-3-hydroxyprop-1-enyl]phenoxy}propane-1,3-diol [23][24], '7,8-*erythro*-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan' [25–27], '7,8-*threo*-7,9,9′-trihydroxy-3,3′- dimethoxy-8-*O*-4′-neolignan' [25–27], vanillic acid 4-*O*-β-glucoside [28], '7,8-*erythro*-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan 4-*O*-β-D-glucopyranoside' [27], '7,8-*threo*-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan 4-*O*-β-D-glucopyranoside' [27], and '3,4-dimethoxyphenyl-1-*O*-β-apiofuranosyl-(1 → 2)-β-D-glucopyranoside' [29], by comparison of their NMR and MS data with those reported in the literature.

Scheme



Experimental Part

General. Column chromatography (CC): silica gel (200–300 or 100–200 mesh; *Qingdao Marine Chemical Factory*, P. R. China); C_{18} reverse-phase (RP) silica gel (20–45 μm; *Fuji Silica Chemical*, Japan) or *Sephadex LH-20* (*Pharmacia*). Gel filtration: macroporous resin (*Nankai Chemical*, P. R. China). Thin-layer chromatography (TLC): silica gel *GF-254* plates (*Yantai Marine Chemical Co., Ltd*, P. R. China). Prep. HPLC: *Agilent 1100* system with *Zorbax SB-C_{18}* column (9.4 mm × 25 cm). Optical rotations: *Perkin-Elmer 341* polarimeter. IR Spectra: *Perkin-Elmer 577* spectrophotometer; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker AM-400* spectrometer; δ in ppm, J in Hz. ESI-MS: *Finnigan LCQ-DECA* instrument. HR-ESI-MS: *Waters Q-TOF* mass spectrometer; in m/z.

Plant Material. The stems of *Amentotaxus argotaenia* (Hance) PILGER were collected in June 2006 in the suburb of Chongqing, P. R. China, and identified by Prof. *Si-Rong Yi* (Institute of Medicine Plantation, Chongqing). A voucher specimen (No. 2006610) was deposited at the herbarium of the Institute of Materia Medica, Shanghai, P. R. China.

Extraction and Isolation. The powdered, air-dried stems of *A. argotaenia* (2.4 kg) were extracted with refluxing 95% EtOH (3 × 90 min). After solvent removal, the residue was partitioned successively between H_2O and AcOEt and then BuOH to afford an AcOEt-soluble fraction (24.5 g) and a BuOH-soluble fraction (31.5 g). The AcOEt-soluble fraction was purified by CC (SiO_2; $CHCl_3$/MeOH 20:1 → 2:1) to afford six fractions (*Fr. 1–Fr. 6*). *Fr. 2* (1.20 g) was resubjected to CC (SiO_2; $CHCl_3$/MeOH 15:1 → 5:1), which gave four fractions, which were further separated by CC (*Sephadex LH-20*) and semi-prep. HPLC (*Zorbax SB-C_{18}*) to yield **2** (8 mg), '7,8-*erythro*-7,9,9′- trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan' (8 mg), '7,8-*threo*-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan' (7 mg), and vanillic acid 4-*O*-β-glucoside (7 mg), resp. *Fr. 3* (0.45 g) was purified by CC (*Sephadex LH-20*) to afford crystalline (4-hydroxy-3-methoxyphenyl)-2-{2-methoxy-4-[(*E*)-3-hydroxyprop-1-enyl]phenoxy}propane-1,3-diol (13 mg). *Fr. 4* (0.37 g) was first purified by CC (1. SiO_2; 2. *Sephadex LH-20*) and then by prep. TLC, which provided **1** (7 mg). Purification of *Fr. 1* was not pursued due to its low polarity, and *Fr. 5* and *Fr. 6* only contained trace amounts of material. The above BuOH-soluble fraction (31.5 g) was subjected to gel filtration (macroporous resin), eluting with H_2O (*Fr. A*) and 95% EtOH (*Fr. B*). A portion (1.20 g) of *Fr. B* (4.65 g) was subjected to CC (*RP-C_{18}*), which gave *Fr. B-1* (50 mg) and *Fr. B-2* (26 mg). Further purification by semi-prep. HPLC (*Zorbax SB-C_{18}*) gave '7,8-*erythro*-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan 4-*O*-β-D-glucopyranoside' (8 mg) and '7,8-*threo*-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan 4-*O*-β-D-glucopyranoside' (10 mg) from *Fr. B-1*, and '3,4-dimethoxyphenyl-1-*O*-β-apiofuranosyl-(1→2)-β-D-glucopyranoside' (7 mg) from *Fr. B-2*, resp.

Amentotaxin A (= *Labda-8(20),14-diene-7,12,13,18-tetrol*; **1**). Colorless, amorphous powder. $[\alpha]_D^{23} = +18$ ($c = 0.10$, MeOH). IR: 3384, 2928, 2870, 1647, 1385, 1292, 1153, 1045, 1024. ^{1}H- and ^{13}C-NMR: see *Table*. ESI-MS: 361 ($[M+Na]^+$). HR-ESI-MS: 361.2361 ($[M+Na]^+$, $C_{20}H_{34}NaO_4^+$; calc. 361.2355).

Amentotaxin B (*=7,18-Dihydroxy-15,16-dinorlabda-8(20),11-dien-13-one*; **2**). Colorless needles. $[\alpha]_D^{23} = +29$ ($c = 0.10$, MeOH). IR: 3377, 2928, 2870, 1664, 1622, 1387, 1290, 1151, 1043, 899, 756. ^{1}H- and ^{13}C-NMR: see *Table*. ESI-MS: 315 ($[M+Na]^+$). HR-ESI-MS: 315.1940 ($[M+Na]^+$, $C_{18}H_{28}NaO_3^+$; calc. 315.1936).

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