A Novel Asymmetric *ent*-Kaurane Dimer from *Isodon rubescens* var. *rubescens*

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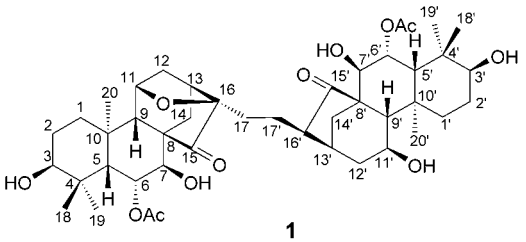
A novel asymmetric *ent*-kaurane dimer, xindongnin P (**1**), was isolated from *Isodon rubescens* var. *rubescens*. Its structure was elucidated by detailed spectroscopic analysis. Compound **1** contains a tetrahydrofuran moiety whose formation leads to inversion of configuration at C(16). This differentiates **1** from known related dimers, which were isolated before from the *Isodon* genus. A likely biogenetic pathway from the alleged monomer precursor **3** is proposed.

Introduction. – In our continuing phytochemical investigation of the *Isodon rubescens* complex, over 100 *ent*-kaurane diterpenoids and related compounds were reported [1–7]. Most of them were evaluated for their antitumor activities, and many showed significant cytotoxic effects. In addition, some new compounds were elucidated to contain novel chemical moieties by detailed spectroscopic analyses including 2D NMR and single-crystal X-ray diffraction. For example, an unusual C–C linkage between two subunits was found in three *ent*-kaurane dimers from *I. rubescens* var. *rubescens*. Their biotransformation from the normal *ent*-kauranoids isolated from the genus *Isodon* was also proposed [6]. They were believed to be interesting and mentioned in 'Hot off the press' in [8]. Our continued search for further dimers led to the isolation of a new xindongnin P (**1**) from this plant grown in the southeastern Dabie Mountains, along with a reported dimer, xindongnin M (**2**) [6]. Their structures were elucidated by analyses and comparison of their spectral data including HR-MS and 2D NMR spectra. Unlike the known dimers, xindongnin P (**1**) has an additional oxy- bridge whose formation involves inversion of the configuration at C(16). In this paper, we wish to report the isolation, structural elucidation, and a proposal for the biosynthesis of this new dimer.

Results and Discussion. – Compound **1**, obtained as an amorphous powder, gave a molecular-ion peak at *m/z* 807.4305 ($C_{44}H_{64}Na^+O_{12}$) in the HR-ESI-MS spectrum, consistent with the molecular formula $C_{44}H_{64}O_{12}$. Its ^{13}C-NMR spectum (*Table*) supported this formula, and exhibited C-signals of two different diterpene units bearing two AcO groups in total. On the basis of careful spectroscopic analyses and detailed comparison with the NMR data of the previously reported xindongnin M (**2**), compound **1** was elucidated to be a new asymmetric *ent*-kaurane dimer.



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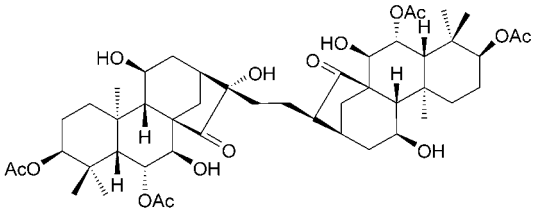

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Table. *^1H- (400 MHz) and ^{13}C-NMR (100 MHz) Data of* **1**. In C_5D_5N, δ in ppm, J in Hz.

	^{1}H	^{13}C		^{1}H	^{13}C
CH_2(1)	1.90–2.10[a])	37.4 (*t*)	CH_2(1′)	2.05[a]) and 1.64[b])	35.1 (*t*)
H_α–C(2)	1.90–2.10[a])	26.1 (*t*)	H_α–C(2′)	1.90–2.10[a])	26.2 (*t*)
H_β–C(2)	1.68–1.70[b])		H_β–C(2′)	1.68–1.70[b])	
H_α–C(3)	3.58 (*s*)	75.6 (*d*)	H_α–C(3′)	3.56 (*s*)	75.8 (*d*)
C(4)		38.1 (*s*)	C(4′)		38.1 (*s*)
H_β–C(5)	2.78 (br. *s*)	41.4 (*d*)	H_β–C(5′)	2.99 (br. *s*)	41.1 (*d*)
H_β–C(6)	5.70 (br. *s*)	72.6 (*d*)	H_β–C(6′)	5.70 (br. *s*)	72.4 (*d*)
H_α–C(7)	4.03 (*d*, *J* = 3.3)	71.9 (*d*)	H_α–C(7′)	4.06 (*d*, *J* = 3.3)	74.0 (*d*)
C(8)		53.2 (*s*)	C(8′)		50.7 (*s*)
H_β–C(9)	2.72 (*s*)	59.5 (*d*)	H_β–C(9′)	2.54 (*s*)	58.5 (*d*)
C(10)		38.4 (*s*)	C(10′)		38.4 (*s*)
H_α–C(11)	4.53 (br. *s*)	77.0 (*d*)	H_α–C(11′)	4.32 (*d*, *J* = 5.0)	64.0 (*d*)
H_α–C(12)	2.32[c])	38.2 (*t*)	H_α–C(12′)	2.30[c])	34.1 (*t*)
H_β–C(12)	1.82 (*m*)		H_β–C(12′)	1.90–2.10[a])	
H_α–C(13)	2.59 (*m*)	38.8 (*d*)	H_α–C(13′)	2.54 (*m*)	33.8 (*d*)
H_α–C(14)	2.28 (*d*, *J* = 12.0)	34.1 (*t*)	H_α–C(14′)	2.74 (*d*, *J* = 12.0)	36.1 (*t*)
H_β–C(14)	1.13 (*dd*, *J* = 4.0, 12.0)		H_β–C(14′)	1.42 (*dd*, *J* = 4.0, 12.0)	
C(15)		213.1 (*s*)	C(15′)		226.6 (*s*)
C(16)		85.7 (*s*)	C(16′)	2.40[d])	56.5 (*d*)
CH_2(17)	1.90–2.10[a])	27.3 (*t*)	CH_2(17′)	2.36–2.44[d])	21.2 (*t*)
Me–C(18)	1.30 (*s*)	30.0 (*q*)	Me–C(18′)	1.30 (*s*)	29.4 (*q*)
Me–C(19)	1.08 (*s*)	26.2 (*q*)	Me–C(19′)	1.10 (*s*)	26.1 (*q*)
Me–C(20)	1.44 (*s*)	21.8 (*q*)	Me–C(20′)	1.44 (*s*)	19.8 (*q*)
AcO–C(6)	2.16 (*s*)	170.0 (*s*)	AcO–C(6′)	2.11 (*s*)	170.0 (*s*)
		21.5 (*q*)			21.4 (*q*)
OH–C(3)	6.04 (*s*)		OH–C(3′)	5.93 (*s*)	
OH–C(7)	5.82 (*s*)		OH–C(7′)	5.70 (*s*)	
			OH–C(11′)	6.27 (*s*)	

[a])–[d]) Overlapping signals.

In the ^{13}C-NMR spectrum of **1** (see the *Table*), the characteristic signals of the unusual C–C linkage between two subunits were present: two significant upfield C=O signals (δ(C) 213.1 and 226.6), two non-oxy CH_2 (δ(C) 27.3 and 21.2), one oxygenated quaternary C-atom (δ(C) 85.7), and one non-oxy CH (δ(C) 56.5). Comparison of the ^{1}H- and ^{13}C-NMR data of **1** with those of the known dimer xindongnin M (**2**) confirmed this deduction, and further indicated that compound **1** was very similar to **2**. The AcO groups at C(3) and C(3′) of **2** were replaced by two OH groups in **1**. This was supported by the HMBC correlations of H–C(6) and H–C(6′) with two AcO C=O atoms, which allowed us to assign the two AcO groups at C(6) and C(6′) of **1**. The 1D-NMR and HMBC data for the remaining portion of the structure were almost identical with those of **2**, suggesting that compounds **1** and **2** should have the same oxygenation patterns.

However, according to the molecular formula, compound **1** had thirteen degrees of unsaturation, one more than the total of two subunits deduced above. And the chemical shifts of C(11) and C(16) were shifted upfield compared to corresponding signals of **2**, while that of C(15) was significantly shifted downfield. An oxy bridge between C(11) and C(16) could be established by the key HMBC correlation of H–C(11) with C(16) (see *Fig. 1*), which was compatible with the above observations. This oxy bridge differentiated **1** from the first three examples of this kind of dimer, namely xindongnins M–O. The configuration of C(16) was inverted.

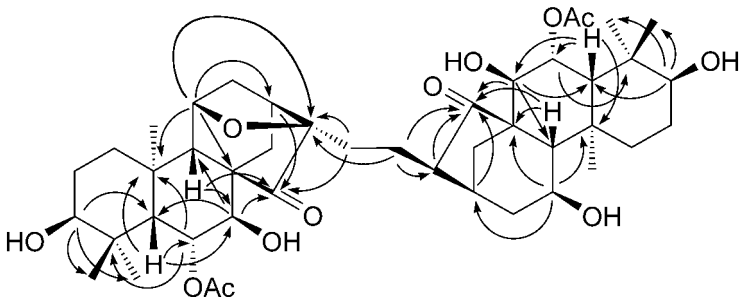


Fig. 1. *Key HMBC* (from H to C) *correlations of* **1**

The relative configuration of **1** was elucidated by ROESY experiments (*Fig. 2.*). The key NOEs of H–C(3) with Me(19), H–C(3′) with Me(19′), H–C(6) with H_β–C(5), H–C(6′) with H_β–C(5′), H–C(7) with H_β–C(14), H–C(7′) with H_β–C(14′), H–C(11) with H_α–C(1), H–C(11′) with H_α–C(1′) were observed, and suggested the presence of H_α–C(3), H_α–C(3′), H_β–C(6), H_β–C(6′), H_α–C(7), H_α–C(7′), H_α–C(11), and H_α–C(11′). The H_α–C(16′) was also assigned by the NOE of H_2–C(17′) with H_β–C(12′).

A possible route for the biogenesis of **1** from 'nomal' *ent*-kauranoids isolated from this plant is shown in the *Scheme*. A sequence involving a [4+2] cycloaddition of monomer **3** (namely xindongnin B, one of the major diterpenes of this plant) [5], hydrolysis of the formed enol ether **4,** and S_N2 substitution lead to dimer **1**. The possibility that **1** is an artifact produced during extraction and purification could be excluded because the isolation conditions were very mild and did not involve temperatures above 60° or any use of acids or bases.

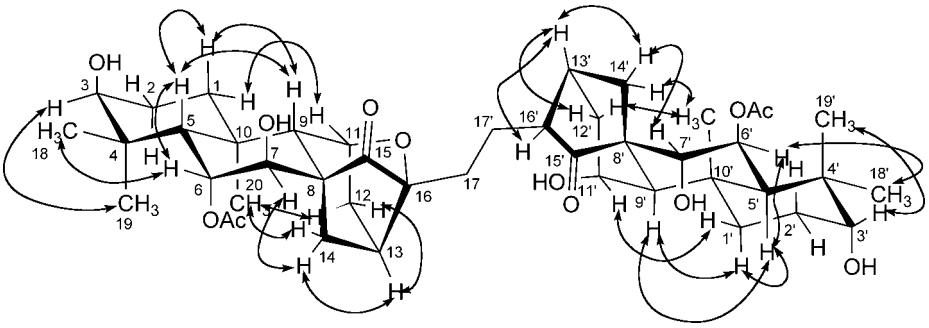
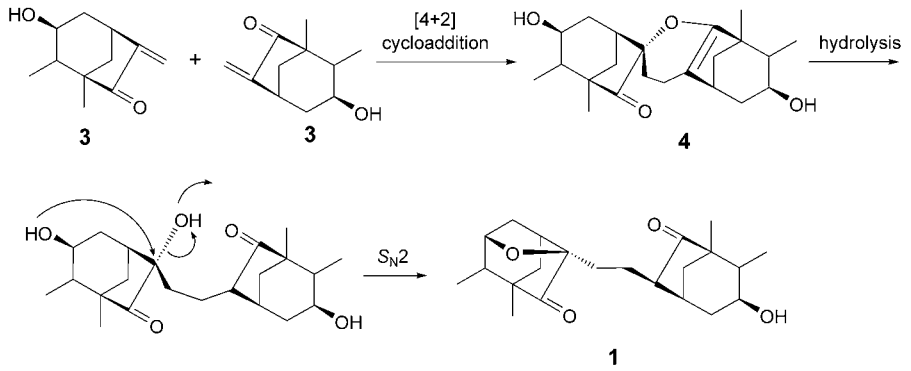


Fig. 2. *Key ROESY correlations of* **1**

Scheme. *Proposed Biogenesis of* **1**



Experimental Part

General. Optical rotations: *Jasco DIP-370* digital polarimeter. IR Spectra: *Bio-Rad FtS-135* spectrometer; KBr pellets; in cm^{-1}. 1D and 2D-NMR Spectra: *Bruker AM-400* and *DRX-500* spectrometers; δ in ppm, J in Hz; Me_4Si as internal standard, measured in C_5D_5N. MS: *VG Autospec-3000* spectrometer; in *m/z* (rel. %).

Plant Material. The plant material was collected in Shangcheng Prefecture (Aug. 2001), Henan Province of P. R. China and identified by Prof. *Zhong-Wen Lin*; the voucher specimen (KIB-09-01 Lin) is deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Science.

Extraction and Isolation. The dried and powdered leaves (1.0 kg) were extracted with 70% Me_2CO and filtered. The filtrate was concentrated and partitioned successively between petroleum ether and H_2O, then AcOEt and H_2O. The AcOEt extract (41 g) was submitted to column chromatography (silica gel (100–200 mesh, 500 g), $CHCl_3/Me_2CO$ 10:0, 9:1, 8:2, 7:3, 6:4, and 5:5). The $CHCl_3/Me_2CO$ 7:3 fraction was repeatedly subjected to further column chromatographed (silica gel) to afford a mixture of two compounds which was further separated by prep. HPLC (*HP1100*, *Zorbax SB-C18*, (9.4 mm × 250 mm), H_2O/MeOH 45:55 flow 3 ml/min, detection at 204 nm): **1** (8 mg) and **2** (20 mg).

*Xindongnin P (=(3β,6α,7β,11β,16*R*)-6-(Acetyloxy)-17-[(3β,6α,7β,11β)-6-(acetyloxy)-3,7,11-trihydroxy-15-oxo*-ent-*kauran-17-yl]-11,16-epoxy-3,7-dihydroxy*-ent-*kauran-15-one;* **1**): White amorphous powder. $[\alpha]_D^{22.0} = +136.36$ ($c = 0.022$, MeOH). IR (KBr): 3401, 2935, 2873, 1734, 1240, 1042. ^{1}H-NMR (C_5D_5N, 400 MHz) and ^{13}C-NMR (C_5D_5N, 100 MHz): *Table*. ESI-MS (pos.): 807 ($[M + Na]^+$). HR-ESI-MS (pos.): 807.4305 ($C_{44}H_{64}O_{12}Na^+$; calc. 807.4295).

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Received November 15, 2004