# A Novel Asymmetric *ent*-Kauranoid Dimer from *Isodon enanderianus*

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**Further investigation on the aerial parts of *Isodon enanderianus* afforded a novel asymmetric *ent*-kauranoid dimer, *enanderinanin J* (1). The structure of the dimer was elucidated by means of spectroscopic methods (including 2D NMR techniques). *Enanderinanin J* was a dimer of *xerophilusin A* and probably formed by [4+2] cycloaddition.**

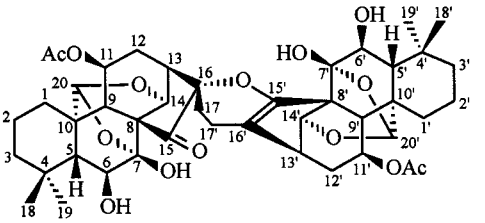
**Keywords** *Isodon enanderianus*, Labiatae, *ent*-kauranoid, asymmetric dimer, *enanderinanin J*

## Introduction

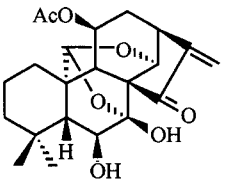
*Isodon enanderianus* (Hand.-Mazz.) H. W. Li (Labiatae), widely distributed in the southern parts of Yunnan Province, has been used in Chinese traditional folk medicine as anti-inflammatory and detoxified agent.[1] Previous phytochemical investigation has shown the presence of a series of *ent*-kaurenoids in this plant.[2,3] Further investigation of this plant resulting in the isolation of a novel asymmetric *ent*-kauranoid dimer, named *enanderinanin J* (**1**). The structure of **1** has been elucidated as an asymmetric dimer of xerophilusin A (**2**),[4] which possessed the basic skeleton of 7, 20∶14, 20-diepoxy *ent*-kauranoid (Fig. 1). This paper deals with the isolation and structural elucidation of **1**.

## Results and discussion

*Enanderianin J* (**1**) was obtained as amorphous powder, and the HREIMS (calcd for 808.3670, found 808.3694) indicated the molecular formula of $C_{44}H_{56}O_{14}$,




**1**




**2**

**Fig. 1** Structures of *enanderinanin J* (**1**) and *xerophilusin A* (**2**).

which suggested 17 degrees of unsaturation. It displayed a single spot on TLC (silica gel) developed in several solvent systems and its homogeneity was confirmed by its NMR spectral behavior. In $^{13}C$ NMR spectrum, the carbon signals were mostly occuring in pairs, which differed from maoecrystal M,[5] a symmetric dimer, only exhibiting half of signals. Therefore, it was assumed that **1** was an asymmetric dimer.

The hypothesis was rationalized through the analysis of NMR data. Comparison of the $^{13}C$ NMR spectral data of **1** (Table 1) with those of **2** showed that the two substructure encompassing rings A—C with their associated substituents in **1** were identical with those in **2**. The

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Received November 27, 2001; revised March 19, 2002; accepted April 29, 2002.

prominent features distinguishing two units in **1** from **2** were the replacement of the olefin signals seen in Table 1 [$^{13}$C NMR $\delta$: 150.0 (s, C-16) and 117.6 (t, C-17)] by methlyene signals [$^{13}$C NMR $\delta$: 18.2 (t, C-17′) and 21.7 (t, C-17)] and quaternary carbon signals [$^{13}$C NMR $\delta$: 86.6 (s, C-16) and 111.2 (s, C-16′)]. Furthermore, the methylene carbon resonance of C-12 ($\delta$ 32.7) in **2** was shifted to higher field [$\delta$ 25.8 (C-12′) and 26.7 (C-12)] and the carbonyl carbon of C-15 ($\delta$ 199.1) in **2** was shifted to lower field [$\delta$ 203.6 (C-15)] and transferred to an olefin group [$\delta$ 111.2 (s, C-16′) and 149.1 (s, C-15′)], respectively. The linkage of two units in **1** now became the pivotal step for determining the structure of **1**. The unsaturation degree of two units was 16. However, the extra degree of unsaturation required by the molecular formula indicated the presence of an additional ring. In HMBC spectrum (Fig. 2), the correlations were clearly observed among H-17 ($\delta$ 2.44, overlap) [with C-15 ($\delta$ 203.6, s), C-16 ($\delta$ 86.6, s), C-16′ ($\delta$ 111.2, s) and C-17′ ($\delta$ 18.2, t)], H-17′ ($\delta$ 2.23, overlap) [with C-16 ($\delta$ 86.6, s), C-17 ($\delta$ 21.7, t), C-15′ ($\delta$ 149.1, s) and C-16′ ($\delta$ 111.2, s)]. As a consequence, the two units were linked by a six-membered dihydropyran ring.

The remaining question was the determination of the configuration at C-16. The $\beta$-orientation of methylene at C-16 was deduced from the upfield shift of C-12 ($\Delta\delta$ −6.0) caused by a $\gamma$-steric compression effect between 16$\beta$-methylene and H-12$\beta$. The stereochemistry of C-16 was also verified by the ROESY correlation (Fig. 3). NOE cross peak of H-17 with H-12$\beta$ was observed, which indicated the $\beta$-orientation of methylene at C-16. Therefore, the configuration at C-16 in **1** was assigned as *S* from the evidence mentioned above and with consideration of the configuration at C-16 in an analogue, isodo-

**Table 1** $^{13}$C NMR data of compounds **1** and **2** (**1** in $C_5D_5N$; **2** in acetone-$d_6$)

| Carbon | 1 | 2 | Carbon | 1 |
|---|---|---|---|---|
| 1 | 27.4 (t) | 28.1 (t) | 1′ | 28.6 (t) |
| 2 | 18.7 (t) | 19.2 (t) | 2′ | 19.1 (t) |
| 3 | 40.9 (t) | 41.7 (t) | 3′ | 41.1 (t) |
| 4 | 33.5 (s) | 34.1 (s) | 4′ | 33.6 (s) |
| 5 | 63.1 (d) | 63.2 (d) | 5′ | 61.8 (d) |
| 6 | 72.3 (d) | 72.7 (d) | 6′ | 72.3 (d) |
| 7 | 101.5 (s) | 101.0 (s) | 7′ | 100.2 (s) |
| 8 | 55.7 (s) | 56.0 (s) | 8′ | 49.7 (s) |
| 9 | 53.8 (d) | 52.7 (d) | 9′ | 45.2 (d) |
| 10 | 43.5 (s) | 44.0 (s) | 10′ | 42.8 (s) |
| 11 | 67.8 (d) | 67.9 (d) | 11′ | 66.7 (d) |
| 12 | 26.7 (t) | 32.7 (t) | 12′ | 25.8 (t) |
| 13 | 38.9 (d) | 39.4 (d) | 13′ | 38.9 (d) |
| 14 | 69.7 (d) | 70.0 (d) | 14′ | 72.4 (d) |
| 15 | 203.6 (s) | 199.1 (s) | 15′ | 149.1 (s) |
| 16 | 86.6 (s) | 150.0 (s) | 16′ | 111.2 (s) |
| 17 | 21.7 (t) | 117.6 (t) | 17′ | 18.2 (t) |
| 18 | 31.0 (q) | 31.5 (q) | 18′ | 31.2 (q) |
| 19 | 23.0 (q) | 23.4 (q) | 19′ | 23.2 (q) |
| 20 | 98.3 | 98.7 (d) | 20′ | 99.1 (d) |
| 11-OAc | 169.5 (s) | 170.0 (s) | 11′-OAc | 170.0 (s) |
| | 21.0 (q) | 21.3 (q) | | 21.7 (q) |

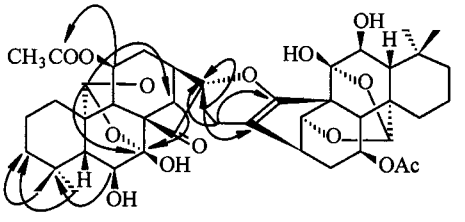



**Fig. 2** Selected HMBC correlations of **1**.

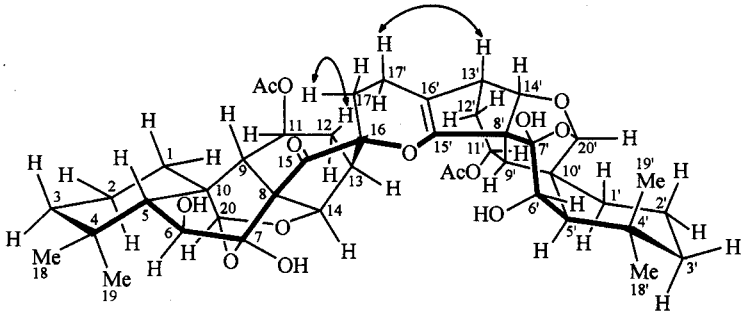
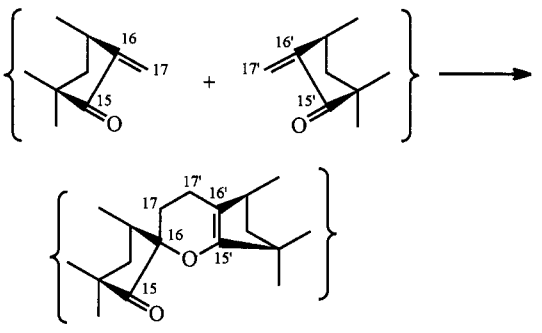



**Fig. 3** Key ROESY correlations of **1**.

pharicin E.[6] Thus, the structure of **1** was determined to be an asymmetric dimer of **2** linked by a six-membred dihydropyran ring.

The six-membered ring of **1** should be formed by condensation between the olefin group and the $\alpha,\beta$-unsaturated ketone group in the ring D of the monomer **2**, the mechanism being probably through a [4+2] cycloaddition (Scheme 1).

**Scheme 1**



## Experimental

*General procedures*

Optical rotation was taken on a SEPA-300 polarimeter. IR spectral data were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets. UV spectrum was obtained on a UV 210A spectrometer. MS spectra were recorded on a VG Auto Spec-3000 spectrometer. 1D- and 2D-NMR spectra were run on a Bruker AM-400 and a DRX-500 instrument with TMS as internal standard, respectively.

*Plant material*

The aerial parts of *Isodon enanderianus* were collected from Shiping county, Yunnan Province, in 1997 and identified by Prof. Zhong-Wen Lin. A voucher specimen (KIB 99-10-15) is deposited in the Laboratory of Phytochemistry, Kunming Institute of Botany, Chinese Academy of Science.

*Extraction and isolation*

The air-dried and powdered plants (7.8 kg) were extracted with 70% acetone (3 × 20 L) at room temperature for 3 days each time. The extract was concentrated and filtered and the filtrate was partitioned with EtOAc. The EtOAc extract (391 g) was subjected to column chromatography on a Si gel column and eluted with $CHCl_3$ containing increasing amounts of $Me_2CO$ system to give seven fractions (I—VII). Fraction IV (45 g) was further subjected to CC over Si gel [petroleum ether-EtOAc ($V$: $V$=4:1)], followed by RP-18 column [MeOH-$H_2O$ ($V$: $V$=7:3)] to yield **1** (41 mg).

*Enanderinanin J* (**1**) Amorphous powder, $[\alpha]_D^{16.5}$ −123.3 (*c* 0.440, MeOH); UV no absorption; $^1$H NMR ($C_5D_5N$, 400 MHz) $\delta$: 5.74 (d, $J$=5.4 Hz, 1H, H-14$\beta$), 5.38 (d, $J$=6.9 Hz, 1H, H-11$\alpha$), 5.42 (s, 1H, H-20), 4.19 (brs, 1H, H-6$\alpha$), 3.48—3.51 (m, 1H, H-13$\alpha$), 2.90 (s, 1H, H-9$\beta$), 2.62 (overlap, 1H, H-12$\alpha$), 2.44 (overlap, 2H, H-17), 1.98—2.02 (m, 1H, H-12$\beta$), 1.83 (s, 3H, OAc-11), 1.73 (s, 1H, H-5$\beta$), 1.40—1.50 (m, 2H, $H_2$-2), 1.29—1.34 (m, 2H, $H_2$-3), 1.10 (s, 3H, Me-18), 0.84 (s, 3H, Me-19); 5.61 (d, $J$=7.2 Hz, 1H, H-11′$\alpha$), 5.37 (s, 1H, H-20′), 4.96 (d, $J$=5.6 Hz, 1H, H-14′$\beta$), 4.02 (brs, 1H, H-6′$\alpha$), 2.66 (s, 1H, H-9′$\beta$), 2.61 (overlap, 1H, H-13′$\alpha$), 2.39 (overlap, 1H, H-12′$\alpha$), 2.20 (s, 3H, OAc-11′), 2.23 (overlap, 2H, H-17′), 1.66—1.73 (m, 1H, H-12′$\beta$), 1.60 (s, 1H, H-5′$\beta$), 1.40—1.50 (m, 2H, $H_2$-2′), 1.09—1.16 (m, 2H, $H_2$-3′), 0.89 (s, 3H, Me-18′), 0.83 (s, 3H, Me-19′); $^{13}$C NMR see Table 1; IR (KBr) $\nu$: 3442, 2924, 2961, 1738, 1678, 1633, 1436, 1371, 1245, 1148, 1088, 1018, 992 cm$^{-1}$; EIMS (70 eV) $m/z$ (%): 808 ($M^+$, 1), 404 (10), 386 (3), 345 (7), 327 (7), 298 (77), 269 (77), 229 (16), 188 (35), 159 (38), 135 (49), 109 (100), 91 (63); HREIMS calcd for $C_{44}H_{56}O_{14}$ 808.3670, found 808.3694.

## References


1 *Flora Yunnannica*, Vol. 1, Kunming Institute of Botany, Chinese Academy of Sciences, Science Press, Beijing, **1977**, p. 764.

2 Wang, Y. H.; Chen, Y. Z.; Lin, Z. W.; Sun, H. D.; Fan, J. S. *Phytochemistry* **1998**, *48*, 1267.

3 Wang, Y. H.; Chen, Y. Z.; Lin, Z. W.; Sun, H. D. *Chin. Chem. Lett.* **1998**, *9*, 733.

4 Hou, A. J.; Li, M. L.; Lin, Z. W.; Ji, S. Y.; Zhou, Y. P.; Sun, H. D. *J. Nat. Prod.* **2000**, *35*, 725.

5 Shen, X. Y.; Isogai, A.; Furihata, K.; Sun, H. D.; Suzuki, A. *Phytochemistry* **1994**, *35*, 725.

6 Wang, Z. M.; Cheng, P. Y. *Acta Pharmaceutica Sinica* **1994**, *29*, 532.


(E0111277 SONG, J. P.; HUANG, W. Q.)