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An asymmetric *ent*-kauranoid dimer from *Isodon rubescens* var. *lushanensis*

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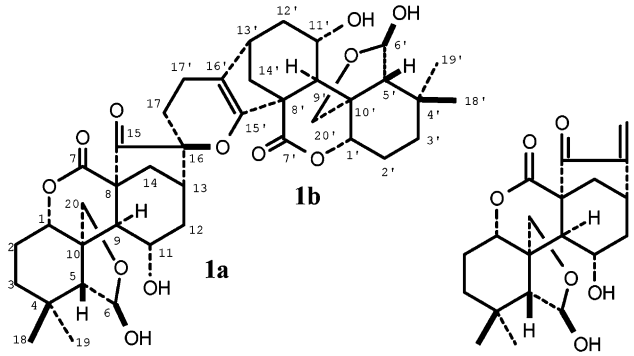
Abstract—A novel asymmetric *ent*-kauranoid dimer, lushanrubescensin J (**1**), was isolated from *Isodon rubescens* var. *lushanensis*. Its structure was elucidated by the spectroscopic evidences. The stereochemistry was confirmed by the single crystal X-ray diffraction of its tetraacetate. Compound (**1**) exhibited potent inhibitory activity against K562 cells with IC_{50} = 0.93 μg/mL.

In our previous reports of the phytochemical investigation of *Isodon rubescens* complex, several novel *ent*-kaurane dimers that possessed a rare linkage of a single carbon bond between two structural subunits were chemically described.[1,2] Their biotransformation from the normal *ent*-kauranoids isolated from the genus *Isodon* was also proposed. They were believed to be interesting and mentioned in 'Hot off the press' of Natural Product Reports.[3] In the proposed biotransformation, the [4+2] cycloaddition, between the α,β-unsaturated ketone group of one diterpene with the olefin group of another one, yielded a six-membered heterocycle, which linked the two monomers together. Our further search for more new bioactive compounds from *I. rubescense* var. *lushanensis* led to the isolation and elucidation of another novel *ent*-kauranoid dimer (**1**).[4] Interestingly, this dimer contained the key six-membered heterocyclic linkage. Its stereochemistry was determined by the single crystal X-ray diffraction of its tetraacetate. This discovery is of important support to the proposed biotransformation. Compound (**1**) was also assayed for its inhibitory effect against K562 cells and obtained IC_{50} of 0.93 μg/mL.



Compound **1**, obtained as amorphous powder, was determined to possess the molecular formula $C_{40}H_{52}O_{12}$ by its negative HRFABMS ($[M-H]^+$, found 723.3400, calcd. 723.3381),[5] corresponding to 15 degrees of unsaturation. It exhibited a single spot on TLC (silica gel) developing with several solvent systems. And its homogeneity was confirmed by the ^{13}C NMR spectrum, in which 40 carbon signals mostly appeared in pairs, due to the asymmetric skeleton of diterpene dimer.

By comparison with the ^{13}C NMR data (Table 1) of a known *ent*-kauranoid epinodosin (**2**), one of the major constituents of this plant,[4] it was revealed that these

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Table 1. The ^{1}H and ^{13}C NMR assignments for compounds **1** and **2** in C_5D_5N

No.	1a		No.	1b		2
	^{1}H	^{13}C		^{1}H	^{13}C	^{13}C
1	4.55 (dd, 6.8, 10.4)	76.8 d	1′	4.82 (dd, 6.5, 11.0)	76.5 d	76.7 d
2	1.76 (2H)[d]	[a]23.8 t	2′	1.84 (2H)[e]	[a]24.0 t	24.0 t
3	1.33[f] and 1.24[g]	37.0 t	3′	1.33[f] and 1.24[g]	37.0 t	36.9 t
4		31.5 s	4′		31.5 s	31.6 s
5	3.06 (s)	53.9 d	5′	2.94 (s)	54.3 d	52.3 d
6	5.70 (br s)	102.0 d	6′	5.65 (d, 6.4)	102.5 d	102.0 d
7		172.2 s	7′		170.3 s	170.8 s
8		57.3 s	8′		53.9 s	56.5 s
9	2.78 (d, 10.2)	54.1 d	9′	2.64 (d, 9.8)	49.7 d	53.9 d
10		51.0 s	10′		50.0 s	51.0 s
11	4.27–4.29 (m)	62.6 d	11′	4.65–4.68 (m)	64.4 d	63.2 d
12	2.88–2.91 (m, Hax) 2.84 (Heq)[h]	32.0 t	12′	1.90–1.94 (m) and 1.24[g]	37.1 t	41.5 t
13	2.25–2.28 (m)	35.4 d	13′	2.41–2.45 (m)	36.0 d	35.2 d
14	2.86[h] and 1.62[i]	35.2 t	14′	2.66–2.68 (m) and 1.63[i]	33.5 t	33.0 t
15		208.5 s	15′		153.9 s	201.5 s
16		85.7 s	16′		119.2 s	150.9 s
17	2.26–2.29 (m, Heq) 1.96 (Hax)[j]	23.3 t	17′	1.96 (2H)[j]	17.6 t	117.6 t
18	0.95 (s, 3H)	[b]33.0 q	18′	0.95 (s, 3H)	[b]32.9 q	33.5 q
19	0.95 (s, 3H)	[c]23.1 q	19′	0.95 (s, 3H)	[c]23.0 q	23.1 q
20	4.34 (d, 9.4) 4.23 (d, 9.4)	73.8 t	20′	4.07 (d, 9.4) 4.02 (d, 9.4)	74.3 t	73.6 t

[a,b,c] Signals which may be exchanged.
[d,e,f,g,h,i,j] Overlapped signals.

two substructures of **1** (**1a** and **1b**), encompassing rings A–C with their associated substituents, were identical with compound **2**. And the α,β-unsaturated ketone group [δ 201.5 (s, C-15), δ 150.9 (s, C-16) and δ 117.6 (t, C-17)] of **2** were replaced by a ketonic carbon [δ 208.5 (s, C-15)], an oxygenated quaternary carbon [δ 85.7 (s, C-16)], an olefinic bond [δ 153.9 (s, C-15′), δ 119.2 (s, C-16′)], and two methylenes [δ 23.3 (t, C-17) and δ 17.6 (t, C-17′)] of **1a** and **1b**. These key changes of these characteristic signals suggested that subunits **1a** and **1b** were linked by a six-membered dihydropyran ring. This was confirmed by the related HMBC and ROESY correlations (Fig. 1).

The configuration of C-16 was deduced to be *S* on the basis of the upfield shift of C-12 ($\Delta\delta$ −9.5) caused by the γ-steric compress effect between Heq-17 and Heq-12,[6] which was supported by the NOE effects of these two protons. Therefore, compound **1** was elucidated to

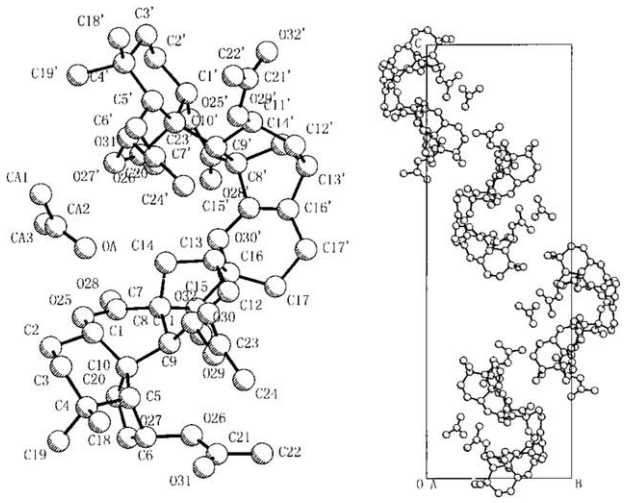

Figure 2. Crystal structure of tetraacetate of **1**.

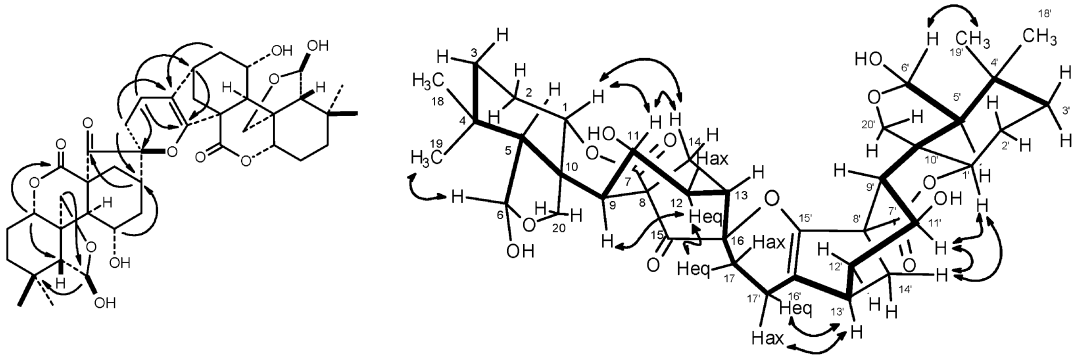

Figure 1. Selected HMBC (from H to C) and ROESY correlations of **1**.

be an asymmetric dimer of **2** with a novel linkage of a six-membered dihydropyran ring as shown in Fig. 1, and named lushanrubescensin J. This dimer exhibited cytotoxic activity against human tumor K562 cells with IC_{50} = 0.93 μg/mL (with cisplatin as the positive control, IC_{50} = 1.14 μg/mL).[4]

However, this elucidation arose some questions about the unusual six-membered heterocycle, since there were several signals overlapped in the ^1H NMR spectrum. Attempts to get its single crystal for X-ray analysis have been taken and gained nothing. Finally, we obtained the crystal of its tetraacetate from its solution of acetone/EtOAc/petroleum ether. The X-ray diffraction completely confirmed its structural assignment including its relative stereochemistry (Fig. 2).[7]

References and notes

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3. Hill, R. A.; Sutherland, A. *Nat. Prod. Rep.* **2004**, *21*, H9.
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5. Compound **1**: white amorphous powder; $[\alpha]_D^{20}$ −72.7 (C_5H_5N, *c* 0.3); IR (KBr) ν_{max}: 3397, 2956, 1773, 1725, 1457, 1259, 1055 cm^{-1}; ^1H NMR (400 MHz, C_5D_5N) and ^{13}C NMR (100 MHz, C_5D_5N) see Table 1; negative FABMS *m/z*: 723 $[M-H]^+$; negative HRFABMS *m/z*: 723.3400 $[M-H]^+$ (calcd 723.3381 for $C_{40}H_{51}O_{12}$).
6. Han, Q. B.; Li, S. H.; Peng, L. Y.; Sun, H. D. *Heterocycles* **2003**, *60*, 933–938.
7. Colorless cube crystals of tetraacetate of lushanrubescensin J, crystallized from acetone/EtOAc/petroleum ether, belong to the orthorhombic space group $P2_12_12_1$. Crystal data: $C_{48}H_{60}O_{16}\cdot(C_3H_6O)_{0.5}$, M = 892.99, a = 9.198(1), b = 13.904(1), c = 42.068(1) Å, β = 104.56(1)°, V = 5380.0(7) $Å^3$, Z = 4, d = 1.138 g/cm^3, Mo Kα radiation, linear absorption coefficient μ = 1.0 cm^{-1}. A colorless cube of dimensions 0.20 × 0.40 × 0.80 mm was used for X-ray measurements on a MAC DIP-2030K diffractometer with a graphite monochromator, maximum 2θ value of 50.0° was set. The total number of independent reflections measured was 6029, 4779 of which were considered to be observed ($|F|^2 \geqslant 3\sigma|F|^2$). The structure was solved by the direct method SHELXS-86 and expanded using difference Fourier techniques, refined by the program and method NOMCSDP and full-matrix least-squares calculations. Hydrogen atoms were fixed at calculated positions. The final indices were R_f = 0.077, R_w = 0.090 ($w = 1/\sigma|F|^2$). The crystal structure has been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 266501.