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7,20-EPOXY-*ENT*-KAURENOIDS FROM *ISODON ENANDERIANUS*

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Key Word Index—*Isodon enanderianus*, Labiatae, *ent*-Kauren, diterpenoid.

Abstract—Two new 7,20-epoxy-*ent*-kaurenoids, enanderianin **A** and **B**, were isolated from the leaves of *Isodon enanderianus* and identified on the basis of spectroscopic methods. The structures of the new compounds were established at 1β,6β,7β-trihydroxy-11α,19-diacetoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one and 1β,6β,7β,11α-tetrahydroxy-19-acetoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one. © 1998 Elsevier Science Ltd. All rights reserved

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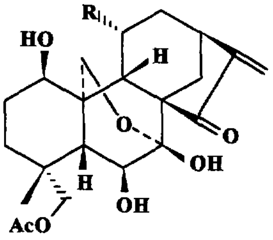
INTRODUCTION

Isodon enanderianus is mainly distributed in the southeast of Yunnan, P.R. China. It has been used as a traditional herb to treat eczema and stomatitis [1], but its chemical constituents have not been analysed previously. As part of a study on the biologically active constituents of *Isodon* species [2–4], two new *ent*-kauren diterpenoids, enanderianin **A** (**1**) and **B** (**2**), have been isolated from *I. enanderianus*. In this paper, we deal with the structural elucidation of the new compounds.



1 R=OAc

2 R=OH

RESULTS AND DISCUSSION

The molecular formula $C_{24}H_{32}O_9$ ($[M+1]^+$ m/z 465) of enanderianin **A** (**1**) was determined by its FAB-mass spectrum, and ^1H and ^{13}C NMR spectral data. In the ^{13}C NMR and DEPT spectra (see Tables 1 and 2), the presence of one methyl carbon, six methylenes, six methines, four quaternary carbons, two olefinic carbons, one ketonic carbon and two acetoxy groups were observed. It had a five-member ring with a ketone conjugated with an exo-methylene group on the basis of the following spectral data: UV λ_{max}^{MeOH} 237 (3.83) nm; IR ν_{max}^{KBr} 1739 and 1642 cm^{-1}; ^1H NMR δ 5.95 and 5.27 (each 1H, *br s*); ^{13}C NMR δ 153.2 (*s*), 117.2 (*t*) (exo-methylene), 210.1 (*s*) (ketone) [5]. In addition, the signals due to an acetal carbon at δ 96.5 (*s*, C-7), two oxygenated methyl groups at δ 67.9 (*t*, C-19) and δ 68.4 (*t*, C-20), and one tertiary methyl group at δ 29.0 (*q*, C-18) were also observed in the ^{13}C NMR and DEPT spectra of **1**. These spectroscopic data suggested that **1** had 7β-hydroxy-19,20-dioxy-7α,20-epoxy-*ent*-kaur-16-en-15-one as the basic skeleton [6].

All proton signals of **1** were assigned on the basis of ^1H–^1H COSY and ^1H–^{13}C COSY spectra. A triplet signal at δ 3.90 (1H, *t*, $J = 2.6$ Hz, H-1α) coupled with a multiplet signal at δ 1.76 (H_2-2), which coupled with two multiplet signals at δ 2.16 (H-3α) and 1.67 (H-3β). A doublet of doublet signal at δ 4.53 (1H, *dd*, $J = 7.3$, 10.8 Hz, H-6α) coupled with two doublet signals at δ 2.24 (H-5β) and 6.77 (OH-6β). A triplet signal at δ 6.30 (1H, *t*, $J = 4.6$ Hz, H-11β) coupled with a multiplet signal at δ 1.81 (H-12β) and a broad doublet signal at δ 2.73 (H-9β). The H-12β signal coupled with a doublet of doublets signal at δ 2.33 (H-12α), which coupled with a multiplet signal at δ 3.07 (H-13α). The H-13α signal coupled with a doublet of doublets signal at δ 2.64 (H-14β), which coupled with a doublet signal at δ 3.10 (H-14α). In addition, the coupling correlation of two H-17 protons (δ 5.95

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Table 1. ^{1}H NMR Data of Compounds **1** and **2** (400.13 MHz, δ in ppm with reference to the signal of C_5D_5N)

H	1	2
H-1α	3.90 (*t*, 2.6)	3.75 (*t*, 2.8)
H_2-2	1.76 (overlap)	1.76 (overlap)
H-3α	2.16 (overlap)	2.20 (overlap)
H-3β	1.67 (overlap)	1.71 (overlap)
H-5β	2.24 (*d*, 7.3)	2.39 (*d*, 6.8)
H-6α	4.53 (*dd*, 7.3, 10.8)	4.57 (*dd*, 6.8, 10.4)
H-9β	2.73 (*d*, 4.2)	2.60 (*d*, 4.0)
H-11β	6.30 (*br t*, 4.6)	5.31 (*br t*, 4.4)
H-12α	2.33 (*dd*, 9.4, 15.9)	2.51 (*dd*, 9.6, 15.6)
H-12β	1.81 (overlap)	1.80 (overlap)
H-13α	3.07 (*m*)	3.15 (*m*)
H-14α	3.10 (*d*, 12.2)	3.20 (*d*, 11.6)
H-14β	2.64 (*dd*, 4.4, 12.2)	2.67 (*dd*, 4.4, 11.6)
Ha-17	5.95 (*s*)	5.93 (*s*)
Hb-17	5.27 (*s*)	5.26 (*s*)
Me-18	1.49 (*s*)	1.50 (*s*)
Ha-19	4.77 (*d*, 11.0)	4.85 (*d*, 10.8)
Hb-19	4.43 (*d*, 11.0)	4.54 (*d*, 10.8)
Ha-20	4.65 (*d*, 9.2)	4.60 (*d*, 8.8)
Hb-20	4.23 (*d*, 9.2)	4.24 (*d*, 8.8)
OH-6β	6.77 (*d*, 10.8)	7.13 (*d*, 10.4)
OAc	2.10 (*s*), 2.02 (*s*)	1.97 (*s*)

Table 2. ^{13}C NMR Data of Compounds **1** and **2** (100.6 MHz, δ in ppm with reference to the signal pyridine-d_5)

C	1	2
1	66.0 *d*	66.1 *d*
2	27.6 *t*	27.5 *t*
3	28.1 *t*	28.4 *t*
4	37.3 *s*	37.7 *s*
5	56.7 *d*	57.3 *d*
6	74.3 *d*	74.5 *d*
7	96.5 *s*	96.7 *s*
8	58.7 *s*	59.6 *s*
9	48.7 *d*	50.0 *d*
10	42.1 *s*	42.7 *s*
11	69.7 *d*	66.4 *d*
12	38.4 *t*	41.8 *t*
13	34.2 *d*	35.1 *d*
14	27.9 *t*	28.0 *t*
15	210.1 *s*	211.7 *s*
16	153.2 *s*	154.6 *s*
17	117.2 *t*	115.3 *t*
18	29.0 *q*	28.5 *q*
19	67.9 *t*	67.8 *t*
20	68.4 *t*	68.9 *t*
OAc	170.8 *s*, 20.8 *q* 170.0 *s*, 21.6 *q*	170.8 *s*, 20.8 *q*

and 5.27), two H-19 protons (δ 4.77 and 4.43), two H-20 protons (δ 4.65 and 4.23) and two H-3 protons (δ 2.16 and 1.67) were also observed in **1**. The series of coupling correlations from H-9β to H-14β and the loss of correlated signals between δ 2.33 (H-12α) and 6.30 (H-11β), between δ 1.81 (H-12β) and 3.07 (H-13α), as well as between δ 3.07 (H-13α) and 3.10 (H-14α) suggested that the C-ring had a hemichair conformation, and the dihedral angles between them was about 90°.

In the NOESY spectrum of **1**, several cross-signals were observed. The proton signal at δ 4.77 (Ha-19) coupled with the proton signals at δ 1.71 (H-2α), 2.16 (H-3α) and 4.65 (Ha-20). The δ 4.53 (H-6α) signal coupled with the proton signal at δ 1.49 (18-Me). The proton signal at δ 3.10 (H-14α) coupled with the proton signal at δ 4.23 (Hb-20), and the proton signal of δ 3.90 (H-1α) correlated to the signal at δ 6.30 (H-11β). Based on the above evidence, the A-, B- and C-ring conformations of **1** were established as chair, boat and hemichair, respectively [7].

The ^{1}H and ^{13}C NMR spectra of **1** (see Tables 1 and 2) showed the presence of two secondary hydroxy groups and two acetoxyl groups, which were previously assigned to OH-1β, OH-6β, OAc-11α and OAc-19. In the HMBC spectrum of **1**, the correlations of signals between δ 170.8 (OAc) to δ 4.77 (Ha-19) and 4.43 (Hb-19), and between δ 170.0 (OAc) to δ 6.30 (H-11β) suggested that the two acetoxyl groups were located at C-19 and C-11. All the above data identified enanderianin **A** (**1**) as 1β,6β,7β-trihydroxy-11α,19-diacetoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one.

Enanderianin **B** (**2**), colourless needles, $C_{22}H_{30}O_9$ ($[M+1]^+$ m/z 423), showed similarities in the ^{1}H and ^{13}C NMR spectra, but with one less acetyl signal, than the spectra of **1**. The upfield shift of C-11 and downshift of C-9 and C-12 in the ^{13}C NMR spectrum of **2** suggested that **2** was deacetylated as the C-11 position compound to **1**. Thus, enanderianin **B** (**2**) was elucidated at 1β,6β,7β,11α-tetrahydroxy-19-acetoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one.

EXPERIMENTAL

General

Mps: uncorr; IR: KBr; UV: MeOH; ^{1}H NMR (400.13 MHz); ^{13}C NMR (100.6 MHz, broad band and DEPT), pyridine-d_5 as solvent, TMS as int. standard.

Plant material

The leaves of *Isodon enanderianus* was collected in October 1993 in Shiping county, Yunnan Province, P.R. China, and identified by Prof. H.-W. Li. A voucher specimen is deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Academia Sinica, Kunming, P.R. China.

Extraction and isolation

Dried and powdered leaves (4.6 kg) were continuously extracted with boiling 95% EtOH and the extracts concd *in vacuo*. The residue (570 g) was suspended in H_2O (100 ml) and the mixture was successively extracted with petrol and EtOAc. The combined EtOAc layers were concd to give an EtOAc fraction (185 g), which was subjected to CC (silica gel, 600 g) with petrol–Me_2CO (9:1–6:4) as eluant. The fractions (8:2 and 7:3 parts) were further purified by silica gel CC and recrystallization yielding enanderianin **A** (**1**, 53 mg) and **B** (**2**, 45 mg).

Enanderianin **A** (**1**), colourless needles, mp 225–227°; $[\alpha]_D^{22}$ −76.9° (MeOH, *c* 0.52); UV λ_{max}^{MeOH} nm (log ε): 237 (3.83). IR ν_{max}^{KBr} cm^{-1}: 3462, 3281, 3202, 1739, 1707, 1642, 1462, 1241, 1023. FAB-MS *m/z* (rel. int.) 465 $[M+1]^+$ (75), 447 (24), 404 (32), 387 (86), 363 (40), 345 (42), 327 (78), 309 (100), 279 (86). ^1H NMR and ^{13}C NMR: see Tables 1 and 2.

Enanderianin **B** (**2**), colourless needles, mp 208–210°C; $[\alpha]_D^{22}$ −71.4° (MeOH, *c* 0.63); UV λ_{max}^{MeOH} nm (log ε): 237 (3.81). IR ν_{max}^{KBr} cm^{-1}: 3507, 3209, 1712, 1641, 1470, 1264, 1057, 1032. FAB-MS *m/z* (rel. int.): 423 $[M+1]^+$ (27), 405 (36), 363 (67), 345 (48), 327 (55), 307 (58), 279 (100); ^1H NMR and ^{13}C NMR see Tables 1 and 2.

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REFERENCES

1. Wu, C. Y. and Li, H. W., *Flora Reipublicae Popularis Sinicae*, Vol. 66(2). Beijing Academic Press, Beijing, 1977, p. 443.
2. Wu, S. H., Zhang, H. J., Ling, Z. W. and Sun, H. D., *Phytochemistry*, 1993, **34**, 1176.
3. Wu, S. H., Zhang, H. J., Chen, Y. P., Lin, Z. W. and Sun, H. D., *Phytochemistry*, 1993, **34**, 1099.
4. Sun, H. D., Lin, Z. W., Shen, X. Y., Takeda, Y. and Fujita, T., *Phytochemistry*, 1991, **30**, 603.
5. Xu, Y. L., Sun, X. C. and Sun, H. D., *Acta Bot. Yunn.*, 1981, **3**, 283.
6. Shen, X. Y., Isogai, A., Furihata, K., Kaniwa, H., Sun, H. D. and Swzuki, A., *Phytochemistry*, 1989, **28**, 855.
7. Zhang, R. P., Zhang, H. J., Lin, Z. W., Zhen, Y. L. and Sun, H. D., *Phytochemistry*, 1992, **31**, 4237.