PERGAMON

Phytochemistry 60 (2002) 55–60

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Diterpenoids from *Isodon enanderianus*

Zhi Na, Wei Xiang, Xue-Mei Niu, Shuang-Xi Mei, Zhong-Wen Lin, Chao-Ming Li, Han-Dong Sun*

Laboratory of Phytochemistry, Kunming Institute of Botany, Academia Sinica, Kunming, 650204, Yunnan, People's Republic of China

Received in revised form 28 January 2002

Abstract

Four *ent*-kauranoids, 6-epiangustifolin and enanderinanins F–H, as well as 11 known *ent*-kaurane diterpenoids, macrocalin B, xerophilusin A, trichorabdal A, trichorabdal B, effusin, angustifolin, longikaurin D, longikaurin F, enanderinanin B, xerophilusin G and shikokianin were isolated from the aerial parts of *Isodon enanderianus*. The new diterpenoids were identified as 6-epiangustifolin (11α-hydroxy-6α-methoxy-6,19-epoxy-6,7-*seco*-*ent*-kaur-16-en-15-one-7,20-olide), enanderinanin F (19-acetoxy-6,20:6,11β-diepoxy-6,7-*seco*-*ent*-kaur-16-en-15-one-1β,7-olide), enanderinanin G (1β,6β,7β-trihydroxy-19-acetoxy-16β-methoxymethyl-7α,20-epoxy-*ent*-kaur-15-one) and enanderinanin H (6β,7β,14β-trihydroxy-1α,11β-acetonide-7α,20-epoxy-*ent*-kaur-16-en-15-one), respectively, on the basis of spectral data, especially by 2D NMR techniques. 6-Epiangustifolin showed significant cytotoxic activity against K562 cell.

Keywords: *Isodon enanderianus*; Labiatae; *ent*-Kaurane diterpenoids; 6-Epiangustifolin; Enanderinanin F–H

1. Introduction

Isodon enanderianus (Hand.-Mazz.) H. W. Li, widely distributed in the southern region of Yunnan Province, PR China, has been used in Chinese traditional folk medicine as anti-inflammatory and detoxification agent (Kunming Institute of Botany, Chinese Academy of Sciences, 1977). Previous phytochemical investigations have shown the presence of a series of *ent*-kaurenoids in this plant (Wang et al., 1998a,b). Reinvestigation of this plant led to the isolation of four new diterpenoids, 6-epiangustifolin (**1**) and enanderinanins F–H (**2–4**), together with 11 known ones, macrocalin B (**5**) (Cheng et al., 1984), xerophilusin A (**6**) (Hou et al., 2000b), trichorabdal A (**7**) (Xu and Wu, 1989), trichorabdal B (**8**) (Zhang et al., 1998), effusin (**9**) (Kubo et al., 1980), angustifolin (**10**) (Sun et al., 1984), longikaurin D (**11**) (Zhang et al., 1992), longikaurin F (**12**) (Sun et al., 1995), enanderinanin B (**13**) (Wang et al., 1998a), xerophilusin G (**14**) (Hou et al., 2000a), and shikokianin (**15**) (Xu et al., 1996). Compounds **1** and **2** were tested for their cytotoxicity toward K562 cell. In this paper, we describe the isolation and structural elucidation of these new compounds by spectral analysis.

2. Results and discussion

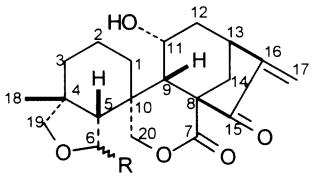
After chromatographic purification on silica gel, the EtOAc soluble portion of the Me_2CO extract of *I. enanderianus* yielded the four new diterpenoids, 6-epiangustifolin (**1**), enanderinanins F–H (**2–4**), as well as the 11 known compounds **5–12**.

6-Epiangustifolin (**1**), colorless needles, was established to have a molecular formula of $C_{21}H_{28}O_6$ by HREIMS ($[M]^+$ m/z 376.1893) and ^{13}C NMR spectroscopic data including DEPT technique. In its IR spectrum, the absorption at 3445 cm^{-1} showed the presence of an hydroxyl group. It was also indicated that the compound contained a five-membered ring with a ketone conjugated to an *exo*-methylene group from the following spectral data: UV (λ_{max} at 233 nm), IR (υ_{max} at 1714 and 1644 cm^{-1}), and NMR [^1H NMR δ 6.12 and 5.45 (each 1H, *s*); ^{13}C NMR δ 200 (*s*), 151.4 (*s*) and 118.1 (*t*)]. In addition to the above-mentioned signals, the ^{13}C NMR and DEPT spectra also showed signals

* Corresponding author. Tel.: +86-871-5223251; Fax: +86-871-5216343.

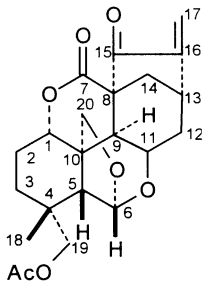
E-mail address: hdsun@mail.kib.ac.cn (H.-D. Sun).

0031-9422/02/$ - see front matter
PII: S0031-9422(02)00073-0

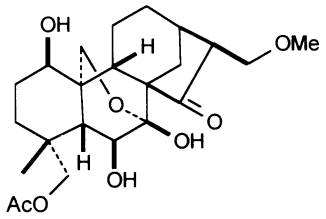


1: **R = α-OCH$_3$**

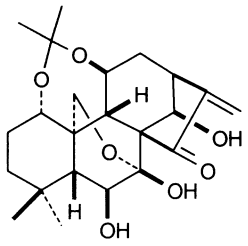
10: **R = β-OCH$_3$**



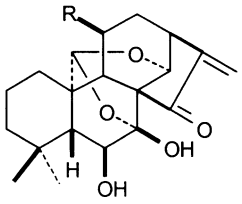
2



3

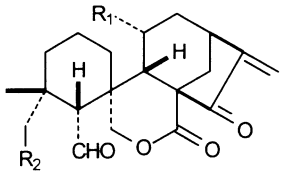


4



5: **R = OH**

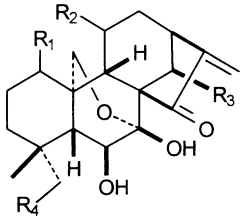
6: **R = OAc**



7: **R$_1$ = OH, R$_2$ = H**

8: **R$_1$ = OH, R$_2$ = OAc**

9: **R$_1$ = H, R$_2$ = OAc**



11: **R$_1$ = H, R$_2$ = α-OH, R$_3$ = H, R$_4$ = OAc**

12: **R$_1$ = H, R$_2$ = α-OAc, R$_3$ = H, R$_4$ = OAc**

13: **R$_1$ = β-OH, R$_2$ = α-OH, R$_3$ = H, R$_4$ = OAc**

14: **R$_1$ = β-OH, R$_2$ = H, R$_3$ = OH, R$_4$ = OAc**

15: **R$_1$ = α-OAc, R$_2$ = α-OAc, R$_3$ = H, R$_4$ = H**

due to one methoxyl group, one methyl, seven methylenes (including two oxygenated ones), five methines (including two oxygen-bearing ones) and four quaternary carbons (including one lactonic carbonyl). The ^{1}H and ^{13}C NMR spectra of **1** were very similar to those of angustifolin (**10**), so it was assumed that **1** had the same skeleton as that of angustifolin, 6,7-*seco*-spirolactone-*ent*-kauranoid, which was verified by 2D NMR spectroscopic experiments. In the HMBC spectrum, correlations were clearly observed among H-5 (with C-6 and C-9), H-6 (with C-4, C-10 and C-19), H-13 (with C-8, C-11, C-15 and C-16), H-19a and H-19b (with C-3, C-5 and C-6), H-20a and H-20b (with C-7, C-9 and C-10). Meanwhile, according to the cross-peaks in the HMBC and ^{1}H–^{1}H COSY spectra, one methoxyl group and one hydroxyl group were obviously located at C-6 and C-11, respectively. The relative configuration of the substituents was revealed by NOE experiments (Fig. 1). The significant difference between **1** and angustifolin (**10**) in the ROESY spectrum was that there were correlations

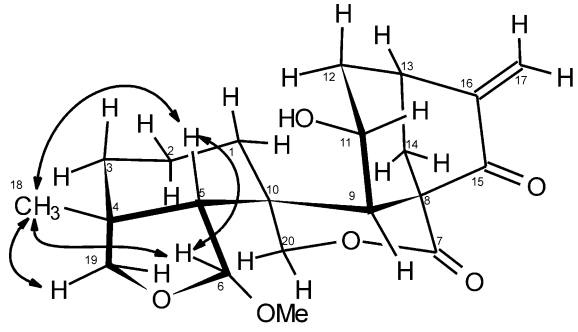
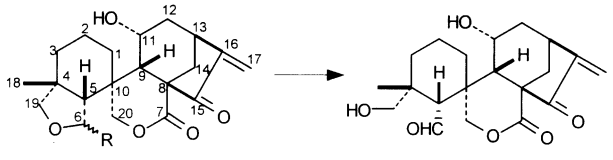


Fig. 1. Key ROESY correlations of compound **1**.

of H-6 with Me-18 and H-5β in **1**. Thus, the methoxyl group at C-6 in **1** was in the α-orientation, and **1** was the C-6 epimer of **10**. According to the procedure in literature (Node et al., 1989), acid-catalyzed hydrolysis of **1** and **10** yielded the same compound (**16**) (Fig. 2), which confirmed that **1** and **10** were a pair of epimers. There-



1: R = α-OCH_3 16

10: R = β-OCH_3

Fig. 2. Acid-catalyzed hydrolysis of compounds **1** and **10**.

fore, **1** was determined as **11**α-hydroxy-6α-methoxy-6,19-epoxy-6,7-*seco*-*ent*-kaur-16-en-15-one-7,20-olide.

Enanderinanin F (**2**), isolated as an amorphous powder, had a molecular formula of $C_{22}H_{26}O_7$ deduced from the HREIMS ($[M]^+$ *m*/*z* 402.1681), ^{13}C NMR and DEPT data, suggesting 10 degrees of unsaturation. Its UV (λ_{max} at 230.5 nm), IR (υ_{max} at 1696 and 1642 cm^{-1}), and NMR [1H NMR δ 6.03 and 5.36 (each 1H, s); ^{13}C NMR δ 202.2 (*s*), 150.6 (*s*), 118.8 (*t*)] spectra exhibited an *exo*-methylene group conjugated with a carbonyl group on a five-membered ring. Furthermore, an acetoxyl group, a methyl, six methylenes including two oxygen-bearing ones, six methines including three oxygenated ones and four quaternary carbons including a lactonic carbonyl were also shown in the ^{13}C NMR spectrum of **2**. The above data coupled with a consideration of the structures of diterpenoids isolated so far from the *Isoson* genus (Wang et al., 1998a,b), suggested that **2** was an *ent*-kauranoid. The absence of HMBC cross-peaks between H-5 or H-6 and C-7 indicated a 6,7-*seco* structure, and lactone formation of C-7 to C-1 was supported by an H-1/C-7 correlation in the HMBC spectrum. compound **2** was determined to possess an enmein-type skeleton. However, the extra degree of unsaturation required by the molecular formula indicated the presence of an additional ring. The correlation between H-6 (δ 5.84, *br s*) and C-11 (δ 61.2, *d*) in the HMBC spectrum unambiguously proved that the additional ring was an ether bridge from C-6 to C-11. There were cross-peaks of an acetoxyl (δ 170.8) with H-19a (δ 4.87) and H-19b (δ 4.83) in the HMBC spectrum, so the acetoxyl group had to be attached to C-19. NOE cross peaks of H-6 with Me-18, H-5β, H-19b; H-11 with H-9α, H-12α were observed, which indicated the β-orientation of H-6 and the α-orientation of H-11, respectively. The β-orientation of H-1 (δ 5.35) was deduced by the coupling constants of H-1β with H-2α ($J = 10.6$ Hz) and H-2β ($J = 6.0$ Hz) (Wang et al., 1997). In conclusion, the structure of enanderinanin F (**2**) should be 19-acetoxy-6,20:6,11β-diepoxy-6,7-*seco*-*ent*-kaur-16-en-15-one-1β,7-olide.

Enanderinanin G (**3**), obtained as amorphous powder, possessed the molecular formula of $C_{23}H_{34}O_8$ as inferred from its HREIMS ($[M]^+$ *m*/*z* 438.2256), 13 C NMR and DEPT data. The ^{13}C NMR spectrum of **3** revealed the presence of one acetoxyl, one methyl, one methoxyl group, eight methylenes (including three oxygenated ones), six methines (including two oxygen-bearing ones), four quaternary carbons (including a ketalic group) and a ketone group carbon. With consideration of the types of diterpenoids isolated from this plant (Wang et al., 1998a,b), the signals due to an acetal carbon at δ 95.9 (C-7, s) and one oxygenated methyl group at δ 66.2 (C-20, t) suggested that **3** had 7β- hydroxy-7α, 20-epoxy-*ent*-kaur-15-one as the basic skeleton, which was substituted by two hydroxyls and one acetoxyl. Due to the absence of the characteristic UV absorption at about 230 nm for an α,β-unsaturated *exo*-methylene ketone, it was obvious that the *exo*-methylene group and the ketone were not conjugated. The *exo*-methylene at C-16 was replaced by a methoxymethyl group judging from the following evidence: 1H NMR δ 3.67 (1H, *dd*, $J = 4.8$, 10.0 Hz, H-17a), 3.48 (1H, t, $J = 10.0$ Hz, H-17b), 3.17 (3H, *s*, OMe) and 2.92 (1H, *m*, H-16α); ^{13}C NMR δ 223.8 (C-15, *s*), 57.3 (C-16, *d*), 68.7 (C-17, *t*) and 58.3 (OMe, q). The signals at δ 3.69 (1H, *br s*), δ 63.9 (*d*) and the downfield shift of C-10 (δ 40.8 ppm) in the NMR spectra suggested one hydroxyl group at C-1. The peak form of H-1, a broad singlet, resulted in the β-orientation of the hydroxyl at C-1 position (Wang et al., 1997). On the basis of the ^1H–^1H COSY spectrum, another hydroxyl was assigned to C-6. The acetoxyl was presumed to be at C-19 because only one methyl at δ 26.7 (Me-18) was observed. The methylene at δ 66.6 (C-19) and the correlation between H_2–19 and the ester carbonyl at δ 170.6 in HMBC spectrum proved this assumption. Furthermore, according to the NOE correlations of H-6 with H_2–19, the hydroxyl at C-6 had a β-orientation. The β-orientation of methoxymethyl group was deduced from the upfield shift of C-12 (δ 19.6 ppm) caused by a γ-steric compression effect between 16β-methoxymethyl group and H-12β. Therefore, enander-

Table 1
Cytotoxic activity of compounds **1** and **2** for K562 cells

Compound	M_r	Concentration	Inhibition (%)	IC_{50} (μg/ml)
1	376	10^{-8} mol/l	16	0.0865
		10^{-7} mol/l	25	
		10^{-6} mol/l	34	
		10^{-5} mol/l	90	
		10^{-4} mol/l	105	
2	402	10^{-8} mol/l	18	1.56×10^5
		10^{-7} mol/l	14	
		10^{-6} mol/l	23	
		10^{-5} mol/l	19	
		10^{-4} mol/l	37	
*Cis-p*latin		0.1 μg/ml	16	2.018
		1.0 μg/ml	28	
		10 μg/ml	78	

inanin G (**3**) was elucidated as 1β,6β,7β-trihydroxy-19-acetoxy-16β-methoxymethyl-7α,20-epoxy-*ent*-kaur-15-one.

Enanderinanin H (**4**), amorphous powder, showed an EIMS molecular ion peak at *m/z* 420 in accordance with the formula $C_{23}H_{32}O_7$, which was confirmed by HREIMS and analysis of its ^{13}C NMR and DEPT spectra. The comparison of its ^1H and ^{13}C NMR spectral data with those of rosthorin A (Li and Wang, 1984) indicated that **4** had the skeleton of 6β,7β,11β,14β-tetrahydroxy-7α,20-epoxy-*ent*-kaur-16-en-15-one. The signals at δ 100.7 (*s*), 24.3 (*q*), 24.0 (*q*) ppm and δ 1.05, 1.03 ppm (each 3H, *s*) proved the presence of an acetonide group. Another carbon signal at δ 72.9 ppm (*d*) assigned to C-1 was supported by the downfield shifts of C-10 (δ 40.0 ppm) and C-2 (δ 26.3 ppm). Meanwhile, the proton signals at δ 4.32 (1H, *m*, H-11α), 3.34 (1H, *dd*, $J=4.6$, 12.3 Hz, H-1β) and their cross-peaks with the acetonic carbon δ 100.7 ppm (*s*) in the HMBC spectrum demonstrated the existence of an 1α,11β-acetonide. From all the evidence mentioned above, enanderinanin H (**4**) was identified as 6β,7β,14β-trihydroxy-1α,11β-acetonide-7α,20-epoxy-ent-kaur-16-*en*-15-one. To date, three ent-kauranoids with 1α,11β-acetonide group have been reported (Wu et al., 1993; Xu and Kubo, 1993). Such kind of *ent*-kauranoids may be artifacts, being most likely generated from reaction with acetone used in the isolation process. In our experiment, 6β,7β,14β-trihydroxy-1α,11β-dihydroxy-7α,20-epoxy-*ent*-kaur-16-en-15-one, the possible biosynthetic origin for **4**, was not obtained.

Compounds **1** and **2** were tested for their ability to inhibit K562 human leukemia cells, with *cis*-platin used as the positive reference substance; the results are shown in Table 1. Compound **1** displayed significant cytotoxic activity against K562 cell with an IC_{50} value of 0.0865 μg/ml, which is better than the IC_{50} value of 2.018 μg/ml of *cis*-platin.

3. Experimental

3.1. General

Melting points were measured on an XRC-1 micro melting point apparatus and are uncorrected. IR spectra were obtained on a Bio-Rad FTS-135 infrared spectrometer with KBr pellets. UV spectra were recorded on a Shimadzu double-beam 210A spectrophotometer in MeOH. Optical rotations were taken on an SEPA-300 polarimeter. The MS data were carried out on a VG Auto Spec-3000 spectrometer, 70 eV for EIMS. ^1H NMR, ^{13}C NMR and 2D NMR spectra were run on Bruker AM-400 and DRX-500 instruments with TMS as internal standard. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Column chromatography was performed either on silica gel (200–300 mesh, Qingdao Marine Chemical Inc., China), silica gel H (10–40 μ, Qingdao Marine Chemical Inc., China), Lichroprep RP_{18} gel (40–63 μm, Merck, Darmstadt, Germany), or on MCI-gel CHP-20P (70–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 20% aq. H_2SO_4.

3.2. Plant material

The aerial parts of *I. enanderianus* were collected from Shiping county, Yunnan Province, China, in 1997 and identified by Professor Zhong-Wen Lin. A voucher specimen (KIB 99–10–15) is deposited in the Laboratory of Phytochemistry, Kunming Institute of Botany, Academia Sinica.

3.3. Extraction and isolation

The air-dried and powdered plants (7.8 kg) were extracted with 70% aq. acetone (3×20 l) at room temperature for three 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 silica gel column and eluted with $CHCl_3$ containing increasing amounts of Me_2CO to give seven fractions (I–VII). Then, Fraction II–VII were decolorized on MCI-gel CHP-20P with MeOH–H_2O (4:1) and further purified by repeated CC on silica gel with petroleum ether–EtOAc (4:1, 3:1, 2:1), cyclohexane–isopropyl alcohol (15:1, 10:1, 6:1), petroleum ether–Me_2CO (7:1, 7:2, 7:3) and RP_{18} gel eluted with MeOH–H_2O (1:1, 6:4 at 7:3) to afford compounds **1** (15 mg), **2** (10 mg), **3** (25 mg), **4** (18 mg), **5** (78 mg), **6** (56 mg), **7** (71 mg), **8** (3.2 g), **9** (89 mg), **10** (93 mg), **11** (106 mg), **12** (132 mg), **13** (96 mg), **14** (121 mg) and **15** (116 mg), respectively.

3.4. 6-Epiangustifolin (*1*)

Colorless needles (MeOH): mp 240–242 °C; $[\alpha]_D^{17}$ −94.7 (MeOH, *c* 0.41); UV (MeOH) λ_{max} (log ε): 233.0 (3.67); IR (KBr) υ_{max} cm^{-1}: 3445, 2937, 1745, 1714, 1644, 1462, 1396, 1273, 1121, 1036; EIMS (70eV) *m/z* (rel. int.): 376 $[M]^+$ (5), 344 (20), 327 (18), 316 (55), 298 (26), 286 (22), 234 (24), 136 (86); HREIMS *m/z*: 376.1893 $[M]^+$ (calculated for $C_{21}H_{28}O_6$, 376.1886); ^1H NMR (400 MHz, C_5D_5N) δ 6.89 (1H, *d*, $J=3.6$ Hz, OH-11α), 6.12 and 5.45 (each 1H, *br s*, H_2-17), 5.29 (1H, AB*d*, $J=10.5$ Hz, H-20a), 5.24 (1H, *d*, $J=4.6$ Hz, H-6β), 4.51 (1H, *t*, $J=3.6$ Hz, H-11β), 4.34 (1H, ABdd, $J=1.9$, 10.5 Hz, H-20b), 3.72 and 3.63 (each 1H, AB*d*, $J=8.1$ Hz, H_2-19), 3.70 (1H, *d*, $J=11.3$ Hz, H-14α), 3.10 (1H, *dd*, $J=4.5$, 9.0 Hz, H-13α), 2.46 (1H, *dd*, $J=9.0$, 14.5 Hz,

H-12α), 2.29 (1H, *d*, *J* = 4.6 Hz, H-5β), 2.24 (1H, *brs*, H-9β), 2.19 (1H, *dd*, *J* = 4.5, 11.3 Hz, H-14β), 1.79 (1H, *overlap*, H-12β), 1.04 (3H, *s*, Me-18), 3.24 (3H, *s*, OMe); ^{13}C NMR spectral data see Table 2.

3.5. Enanderinanin F (**2**)

White amorphous powder: $[\alpha]_D^{26}$ + 24.6 (MeOH, *c* 0.20); UV (MeOH) λ_{max} (log ε): 230.5 (3.82); IR (KBr) ν_{max} cm^{-1}: 3469–3414 (*br*), 2894, 1732, 1696, 1642, 1450, 1374, 1253, 1148, 1126, 1036, 975; EIMS *m*/*z* (rel. int.): 402 $[M]^+$ (14), 372 (21), 360 (16), 342 $[M\text{-}AcOH]^+$ (32), 330 (35), 311 (24), 299 (29), 283 (43), 263 (83), 253 (20), 233 (28), 211 (20), 159 (25), 145 (31), 119 (52), 105 (89), 91 (100), 79 (73); HREIMS *m*/*z*: 402.1681 $[M]^+$ (calculated for $C_{22}H_{26}O_7$, 402.1679); ^{1}H NMR (400 MHz, C_5D_5N) δ 6.03 and 5.36 (each 1H, *br s*, H_2-17), 5.84 (1H, *br s*, H-6β), 5.35 (1H, *dd*, *J* = 6.0, 10.6 Hz, H-1β), 5.13 (1H, AB*d*, *J* = 12.5 Hz, H-20a), 4.88 (1H, *d*, *J* = 3.5 Hz, H-11α), 4.87 and 4.83 (2H, AB*d*, *J* = 12.0 Hz, H_2-19), 4.52 (1H, AB*d*, *J* = 12.5 Hz, H-20b), 3.25 (1H, *s*, H-9α), 3.22 (1H, *d*, *J* = 11.8 Hz, H-14β), 3.03 (1H, *m*, H-13β), 2.70 (1H, *dd*, *J* = 5.0, 11.8 Hz, H-14α), 2.33 (1H, *m*, H-2α), 2.22 (1H, *d*, *J* = 15.0, 8.4 Hz, H-12β), 2.14 (1H, *d*, *J* = 2.2 Hz, H-5β), 1.80 (1H, *m*, H-2β), 1.72 (2H, *m*, H_2-3), 1.64 (1H, *m*, H-12α), 2.02 (3H, *s*, OAc), 1.06 (3H, *s*, Me-18); ^{13}C NMR spectral data see Table 2.

Table 2
^{13}C NMR spectroscopic data for compounds **1–4** in C_5D_5N (100 MHz, δ in ppm)

Carbon	1	2	3	4
1	26.8 (*t*)	77.7 (*d*)	63.9 (*d*)	72.9 (*d*)
2	19.5 (*t*)	23.9 (*t*)	28.3 (*t*)	26.3 (*t*)
3	35.3 (*t*)	28.8 (*t*)	28.9 (*t*)	39.7 (*t*)
4	38.7 (*s*)	36.7 (*s*)	37.6 (*s*)	33.5 (*s*)
5	49.7 (*d*)	46.2 (*d*)	56.8 (*d*)	59.3 (*d*)
6	107.5 (*d*)	91.5 (*d*)	73.3 (*d*)	74.0 (*d*)
7	171.6 (*s*)	171.0 (*s*)	95.9 (*s*)	98.2 (*s*)
8	54.4 (*s*)	55.4 (*s*)	60.3 (*s*)	61.6 (*s*)
9	44.2 (*d*)	38.7 (*d*)	45.7 (*d*)	55.8 (*d*)
10	38.2 (*s*)	41.4 (*s*)	40.8 (*s*)	40.0 (*s*)
11	65.6 (*d*)	61.2 (*d*)	15.7 (*t*)	62.1 (*d*)
12	42.3 (*t*)	38.0 (*t*)	19.6 (*t*)	37.5 (*t*)
13	35.1 (*d*)	35.4 (*d*)	29.5 (*d*)	43.5 (*d*)
14	34.4 (*t*)	31.6 (*t*)	27.0 (*t*)	71.9 (*d*)
15	200.0 (*s*)	202.2 (*s*)	223.8 (*s*)	207.3 (*s*)
16	151.4 (*s*)	150.6 (*s*)	57.3 (*d*)	152.0 (*s*)
17	118.1 (*t*)	118.8 (*t*)	68.7 (*t*)	119.9 (*t*)
18	25.0 (*q*)	27.0 (*q*)	26.7 (*q*)	32.7 (*q*)
19	82.1 (*t*)	67.0 (*t*)	66.6 (*t*)	22.2 (*q*)
20	74.2 (*t*)	61.7 (*t*)	66.2 (*t*)	64.3 (*t*)
OAc		170.8 (*s*)	170.6 (*s*)	
		20.8 (*q*)	20.4 (*q*)	
OMe	54.4 (*q*)		58.3 (*q*)	
Acetonide				100.7 (*s*)
				24.0 (*q*)
				24.3 (*q*)

3.6. Enanderinanin G (**3**)

White amorphous powder: $[\alpha]_D^{26}$ −108.2 (MeOH, *c* 0.50); UV: no absorption; IR (KBr) ν_{max} cm^{-1}: 3304, 2937, 2887, 1735, 1458, 1391, 1243, 1191, 1069, 974, 950; EIMS *m*/*z* (rel. int.): 438 $[M]^+$ (13), 420 $[M\text{-}H_2O]^+$ (1), 406 (56), 388 (6), 378 (10), 346 (100), 328 (17), 297 (28), 269 (22), 241 (35), 199 (34), 149 (22), 135 (31), 119 (28), 105 (47), 91 (53), 79 (51); HREIMS *m*/*z*: 438.2256 $[M]^+$ (calculated for $C_{23}H_{34}O_8$, 438.2254); ^{1}H NMR (400 MHz, C_5D_5N) δ 6.79 (1H, *d*, *J* = 10.5 Hz, OH-6β), 4.76 and 4.43 (each 1H, AB*d*, *J* = 11.0 Hz, H_2-19), 4.40 (1H, *dd*, *J* = 5.2, 10.5 Hz, H-6α), 4.16 (1H, AB*d*, *J* = 10.2 Hz, H-20a), 4.01 (1H, AB*d*, *J* = 10.2 Hz, H-20b), 3.69 (1H, *br s*, H-1α), 3.67 (1H, *dd*, *J* = 4.8, 10.0 Hz, H-17a), 3.48 (1H, *t*, *J* = 10.0 Hz, H-17b), 3.17 (3H, *s*, OMe), 2.92 (1H, *m*, H-16α), 2.68 (1H, *m*, H-13α), 2.37 (1H, *dd*, *J* = 5.3, 11.4 Hz, H-9β), 2.33 (1H, *d*, *J* = 5.2 Hz, H-5β), 1.96 (3H, *s*, OAc), 1.39 (3H, *s*, Me-18); ^{13}C NMR spectral data see Table 2.

3.7. Enanderinanin H (**4**)

White amorphous powder: $[\alpha]_D^{26}$ (MeOH, *c* 0.37); UV (MeOH) λ_{max} (log ε): 236.0 (3.80); IR (KBr) ν_{max} cm^{-1}: 3372, 2977, 2940, 1706, 1642, 1551, 1455, 1307, 1266, 1220, 1207, 1169, 1078, 1058, 978; EIMS *m*/*z* (rel. int.): 420 $[M]^+$ (100), 402 $[M\text{-}H_2O]^+$ (18), 392 (10), 362 (6), 348 (13), 334 (10), 288 (11), 272 (7), 258 (15), 245 (13), 185 (7), 85 (18), 69 (21), 59 (76); HREIMS *m*/*z*: 420.2149 $[M]^+$ (calculated for $C_{23}H_{32}O_7$, 420.2148); ^{1}H NMR (400 MHz, C_5D_5N) δ 6.53 (1H, *d*, *J* = 10.4 Hz, OH-6β), 5.95 and 5.20 (each 1H, *br s*, H_2-17), 5.00 (1H, *s*, H-14α), 4.32 (1H, *m*, H-11α), 4.19 (1H, AB*d*, *J* = 10.3 Hz, H-20a), 4.02 (1H, AB*dd*, *J* = 1.6, 10.3 Hz, H-20b), 3.84 (1H, *dd*, *J* = 6.2, 10.4 Hz, H-6α), 3.34 (1H, *dd*, *J* = 4.6, 12.3 Hz, H-1β), 2.92 (1H, *d*, *J* = 9.4 Hz, H-13α), 2.36 (1H, *dt*, *J* = 9.4, 13.7 Hz, H-12α), 1.05 and 1.03 (each 3H, *s*, acetonide, 2×Me), 0.86 (3H, *s*, Me-18), 0.71 (3H, *s*, Me-19); ^{13}C NMR spectral data see Table 2.

3.8. Cytotoxicity against K562 human leukemia cells

The cytotoxicity assay was performed in a method of MTT, the experimental details of which have been reported previously (Hou et al., 2000b).

Acknowledgements

The authors are grateful to Professor Ma-Lin Li, Yunnan Key Laboratory of Pharmacology for Natural Products, Kunming Medical College, PR China, for the experiments of cytotoxicity against human tumor cells of K562.

References

Cheng, P.Y., Lin, Y.L., Xu, G.Y., 1984. New diterpenoids of *Rabdosia macrocalyx*: the structure of macrocalin A and macrocalin B. Acta Pharm Sin. 19, 593–598.

Hou, A.J., Jiang, B., Yang, H., Zhao, Q.S., Lin, Z.W., Sun, H.D., 2000a. Three new *ent*-kaurane diterpenoids from *Isodon xerophilus*. Acta Botanica Yunnanica 22, 197–200.

Hou, A.J., Li, M.L., Jiang, B., Lin, Z.W., Ji, S.Y., Zhou, Y.P., Sun, H.D., 2000b. New 7,20:14,20-diepoxy *ent*-kauranoids from *Isodon xerophilus*. J. Nat. Prod. 63, 599–601.

Kubo, I., Kamikawa, T., Isobe, T., Kubota, T., 1980. Stucture of effusin. J. Chem. Soc. Chem. Commun., 1206–1207.

Kunming Institute of Botany, Chinese Academy of Sciences, 1977. Flora Yunnanica, Vol. 1. Science Press, China, 764.

Li, G.Y., Wang, Y.L., 1984. Studies on the diterpenoids of *Rabdosia rosthornii (*Diels) Hara. Acta Pharm. Sin. 19 (8), 590–592.

Node, M., Sai, Midori., Fujita, E., Fuji, K., 1989. Terpenoids LII. The stuctures of trichorabdal F. trichorabdal G acetate, and trichorabdal H. A comment on the structure of shikodonin. Chem. Pharm. Bull. 37(6), 1470–1471.

Sun, H.D., Lin, Z.W., Niu, F.D., Lin, L.Z., Chai, H.B., Pezzuto, J.M., Cordell, G.A., 1995. Cytotoxic *ent*-kaurene diterpenoids from three *Isodon* species. Phytochemistry 38, 437–442.

Sun, H.D., Lin, Z.W., Minari, Y., Marunaka, T., 1984. Study on the diterpenoids from *Rabdosia angustifolia*. Acta Pharm. Sin. 19, 633–635.

Wang, Y.H., Chen, Y.Z., Lin, Z.W., Sun, H.D., Fan, J.S., 1998a. 7,20-Epoxy-*ent*-kaurenoids from *Isodon enanderianus*. Phytochemistry 48, 1267–1269.

Wang, Y.H., Chen, Y.Z., Lin, Z.W., Sun, H.D., 1998b. A new 1β-hydroxy-*ent*-kaurenoid from *Isodon enanderianus*. Chin. Chem. Lett. 9, 733–734.

Wang, J., Lin, Z.W., Shingu, T., Sun, H.D., 1997. Four new compounds from *Isodon eriocalyx*. Chin. Chem. Lett. 8, 603–606.

Xu, F.N., Hu, H.P., Wang, Z.Q., 1996. Diterpenoid constituents of *Isodon japonica*. Zhongguo Zhongyao Zazhi 21, 678–679.

Xu, Y.L., Wu, M., 1989. Diterpenoid constituents from *Rabdosia weisiensis*. Phytochemistry 28, 1978–1979.

Zhang, K., Wang, Y.H., Chen, Y.Z., Lin, Z.W., Sun, H.D., 1998. The constituents of diterpenoids from *Isodon enanderianus*. Acta Scientiarum Naturalium Universitatis Sunyatseni 37, 49–51.

Zhang, R.P., Zhang, H.J., Lin, Z.W., Zhen, Y.L., Sun, H.D., 1992. Diterpenoids from *Isodon adenoloma*. Phytochemistry 31, 4237–4240.

Wu, S.H., Zhang, H.J., Chen, Y.P., Lin, Z.W., Sun, H.D., 1993. Diterpenoids from *Isodon wikstroemioides*. Phytochemistry 34, 1099–1102.

Xu Y.L., Kubo, I., 1993, Diterpenoid constituents from *Rabdosia coetsa*. Phytochemistry 34, 576–578.