Pergamon

Tetrahedron Letters 43 (2002) 661–664

TETRAHEDRON LETTERS

Two novel *ent*-kaurane diterpenoids isolated from *Isodon eriocalyx* var. *laxiflora*

Xuemei Niu,[a] Shenghong Li,[a] Qinshi Zhao,[a] Zhongwen Lin,[a] Handong Sun,[a,*] Yang Lu,[b] Cheng Wang[b] and Qitai Zheng[b]

[a]*Laboratory of Phytochemistry, Kunming Institute of Botany, The Chinese Academy of Sciences, Kunming 650204, PR China*
[b]*Institute of Materia Medica, The Chinese Academy of Medical Sciences, Beijing 100050, PR China*

Received 13 September 2001; revised 12 November 2001; accepted 22 November 2001

Abstract—Two novel 3,6-epoxy-6,7:8,15-*seco*-7,20-olide-*ent*-kaurane diterpenoids laxiflorin F (**1**) and laxiflorin G (**2**) have been isolated from the leaves of *Isodon eriocalyx* var. *laxiflora*. The structure elucidation of **1** and **2** was accomplished by spectroscopic methods including 2D NMR analysis. The relative stereochemistry of **1** was established by single crystal X-ray crystallography, which also confirmed the novel carbon skeleton of the new *ent*-kaurane diterpenoid. Both compounds were tested for their cytotoxicity toward human leukemia K562 cells.

Isodon eriocalyx (Dunn) Hara var. *laxiflora* Wu and Li, a perennial shrub native to Yunnan province, has antitumor, antibacterial and anti-inflammatory activities.[1] In a previous study on the leaves of *I. eriocalyx* var. *laxiflora*, a series of *ent*-kaurane diterpenoids from the plant were isolated.[2] Among them, we found that eriocalyxin B was one of the major constituents in the ethyl actetate extracts of the leaves of *I. eriocalyx* and had remarkable cytotoxicity, antibacterial and anticancer effects through bioassay experiments. Our recent investigation on the bioactive diterpenoids from the plant led to the isolation of two 3,6-epoxy-6,7:8,15-*seco*-7,20-olide-*ent*-kauranoids, **1** and **2**, which represented a novel carbon skeleton. Their structures were identified by extensive 2D NMR spectroscopic means including ^{1}H–^{1}H COSY, HMQC, HMBC and ROESY techniques. The relative stereochemistry of **1** was also determined by X-ray analysis. Both compounds have been evaluated for their cytotoxicity against human leukemia K562 cells. In this paper, we wish to report the isolation and structure elucidation of compounds **1** and **2**.

The 70% Me_2CO extracts of the air-dried and powdered leaves[3] (25 kg) were partitioned with EtOAc to afford the EtOAc extract (1000 g), which was subjected to silica gel column chromatography using $CHCl_3$, $CHCl_3$–Me_2CO (9:1, 8:2, 7:3, 6:4) and Me_2CO as elu-

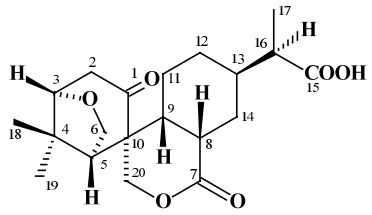
* Corresponding author. Tel.: (86) 871-5223251; fax: (86) 871-5216343; e-mail: hdsun@mail.kib.ac.cn, han_dongsun@hotmail.com

ents. Compounds **1** and **2** were purified from the $CHCl_3$–Me_2CO (6:4) fraction after repeated medium-pressure column chromatographic separations followed by recrystallization in CH_3OH:Me_2CO:$CHCl_3$ (3:1:1).

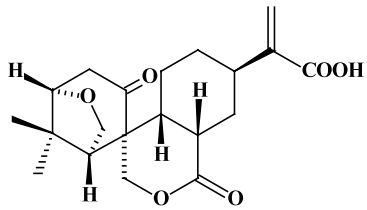
Compound **1**, obtained as colorless lumpish crystals and giving a molecular ion peak at m/z 364 in the EIMS spectrum, was deduced to have a molecular formula $C_{20}H_{28}O_6$ by HREIMS[4] and NMR spectra (Table 1). The fact that no absorption was recorded in the UV spectrum indicated the absence of any conjugated systems in the molecule. Observed in the ^{13}C NMR (DEPT) spectra of **1** were three carbonyl carbons, two quaternary carbons, six methines including an oxygenated one, six methylenes including two oxygen-bearing ones, two tertiary methyls and one secondary methyl, which obviously indicated a diterpenoid skeleton. Considering the structures of the compounds previously isolated from this plant, along with the characteristic lactone carbonyl signal at δ_C 174.4 (s) due to C-7 and the two noticeable oxymethylenes [one emerged at δ_C 73.4 (t) and δ_H 4.65/3.93 (J=11.7 Hz) assignable to C-20/H-20, and the other resonated at δ_C 67.8 (t) and δ_H 3.97/3.73 (J=9.9 Hz) attributable to C-6/H-6], we initially presumed that **1** should be a 6,7-*seco*-7,20-olide-skeletal *ent*-kaurane diterpenoid similar to laxiflorin C (**3**),[2] which was also isolated as one of the major constituents. However, through careful observations we found that **1** was obviously different from normal *ent*-kaurane diterpenoids in having one less quaternary carbon (three quaternary carbons

0040-4039/02/$ - see front matter
PII: S0040-4039(01)02229-8

Table 1. NMR spectral data and HMBC correlations for **1** and **2**[a]



1



2

No.	**1**		**2**		HMBC[b]	**3**
	^{1}H	^{13}C[c]	^{1}H	^{13}C	H–C	^{13}C
1		212.2 s		212.2 s		200.9 s
2	2.66 br. s	47.9 t	2.68 br. s	47.9 t	1, 3, 4, 10	124.5 d
3	3.85 br. s	82.0 d	3.87 br. s	82.1 d	1, 4, 5, 6, 18, 19	158.9 d
4		43.1 s		43.2 s		36.7 s
5	2.20 d, 4.6	51.6 d	2.23 d, 4.6	51.7 d	1, 3, 4, 6, 9, 10, 18, 19, 20	47.6 d
6	3.97 dd, 9.9, 4.8 3.73 d, 9.9	67.8 t	3.98 dd, 10.0, 4.8 3.75 d, 10.0	67.8 t	3, 4, 5, 10	58.3 t
7		174.4 s		174.3 s		170.4 s
8	3.21 br. s	38.5 d	3.26 br. s	39.0 d	7, 9, 14	60.0 s
9	2.47 overlap	43.9 d	2.57 dd, 4.4, 9.0	43.8 d	5, 7, 8, 10, 11, 12	41.9 d
10		55.2 s		55.3 s		52.3 s
11	1.45 overlap	29.2 t	1.58 m 1.47 m	29.7 t	8, 9, 13	20.3 t
12	1.91 br. d, 12.8 1.38 overlap	31.2 t	2.04 br. d, 12.6 1.27 overlap	33.2 t	9, 14	18.1 t
13	2.44 overlap	35.8 d	3.70 m	35.0 d	12, 16	32.7 d
14	2.62 br. d, 13.0	30.4 t	2.66 overlap	31.8 t	7, 8, 9, 12, 13	32.8 t
15	1.41 overlap	178.4 s	1.69 dt, 4.4, 12.9	169.9 s		217.4 s
16	2.44 overlap	46.4 d		147.5 s		48.8 d
17	1.30 d, 6.5 (3H)	15.4 q	6.48 s 5.60 s	122.7 t	13, 15, 16	11.8 q
18	1.16 s (3H)	26.8 q	1.17 s (3H)	26.9 q	3, 4, 5, 19	31.8 q
19	1.26 s (3H)	22.2 q	1.31 s (3H)	22.3 q	3, 4, 5, 18	23.8 q
20	4.65 d, 11.7 3.93 d, 11.7	73.4 t	4.68 d, 11.8 3.94 d, 11.8	73.5 t	1, 5, 7, 9, 10	70.8 t

[a] ^{1}H NMR, 500 MHz; ^{13}C NMR, 125 MHz, pyridine-d_5: data in ppm (J in Hz).
[b] HMBC correlation for compounds **1** and **2**.
[c] Multiplicity was determined from DEPT data.

arising from C-4, 8 and 10 are necessary in usual *ent*-kauranoids) in the high field region of the ^{13}C NMR (DEPT) spectra. By detailed comparison of the ^{13}C NMR data of **1** with those of **3**, it is easily recognized that, in the high field region, the quaternary carbon signal for C-8 in **3** was replaced by a methine signal at δ_C 38.5 (d) in **1**. A carboxylic carbonyl signal at δ_C 178.4 (s) in **1** took the place of the keto carbonyl carbon signal at δ_C 217.4 (s) due to C-15 in **3** in the low field region of the ^{13}C NMR spectra. Thus, it could be deduced that the D-ring in **3** was split between C-8 and C-15 in **1**. There were other differences between the A-rings of these two compounds. A methylene signal at $\delta_{C/H}$ 47.9/2.66 and an oxygenated methine signal at $\delta_{C/H}$ 82.0/3.85 in **1** substituted for those of the $\Delta^{2,3}$ double bond in **3**, and the keto carbonyl carbon due to C-1 was shifted downfield greatly from δ_C 200.9 (s) in **3** to 212.2 (s) in **1**, which suggested the reduction of the double bond of the α,β-unsaturated ketone moiety in **1**. The presence of a cross peak between H-3 and the oxymethylene signal at δ_C 67.8 (C-6) and cross peaks between H_2-6 (δ_H 3.97/3.73) and C-3 (δ_C 82.0) in the HMBC spectrum indicated undoubtedly that there was an oxygen-bridge between C-3 and C-6. Until now, such types of linkages were unprecedented in natural *ent*-kaurane diterpenoids.

The stereochemistry of H-8 was established as β by the correlation of H-5β with H-8 in the ROESY spectrum. In the same manner, the NOE between the signal at δ_H 3.73 (H-6) and H-20 provided the hint that the above-mentioned oxygen bridge occurred in the α-direction, and therefore it was concluded that H-3 was of β-orientation. The stereochemistry of C-16 could not be determined by the ROESY experiment. Fortunately, **1** was obtained as colorless lumpish crystals after several recrystallizations. The analysis of the single crystal X-ray diffraction[5] of **1** established C-16 as having the *R* configuration and also confirmed the novel carbon skeleton proposed (Fig. 1). Thus, **1** was characterized as

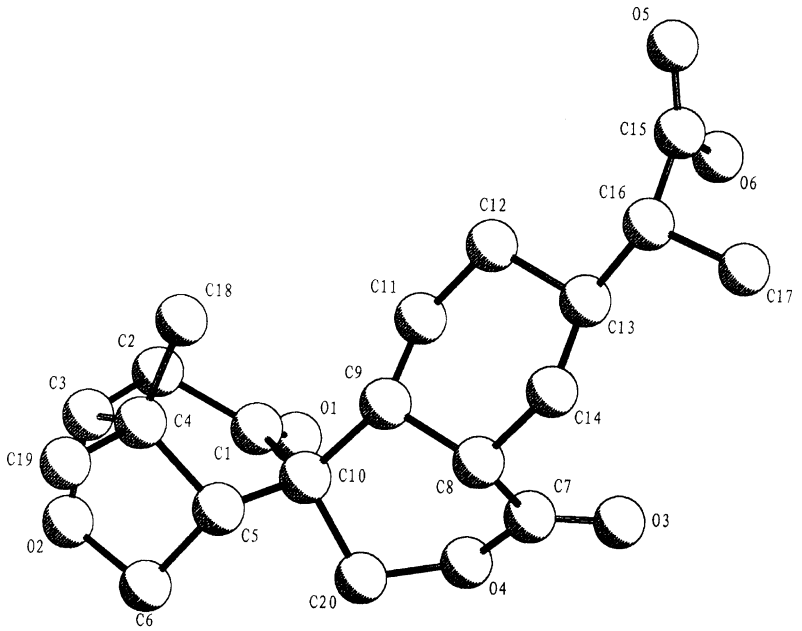


Figure 1. X-Ray crystal structure of **1**.

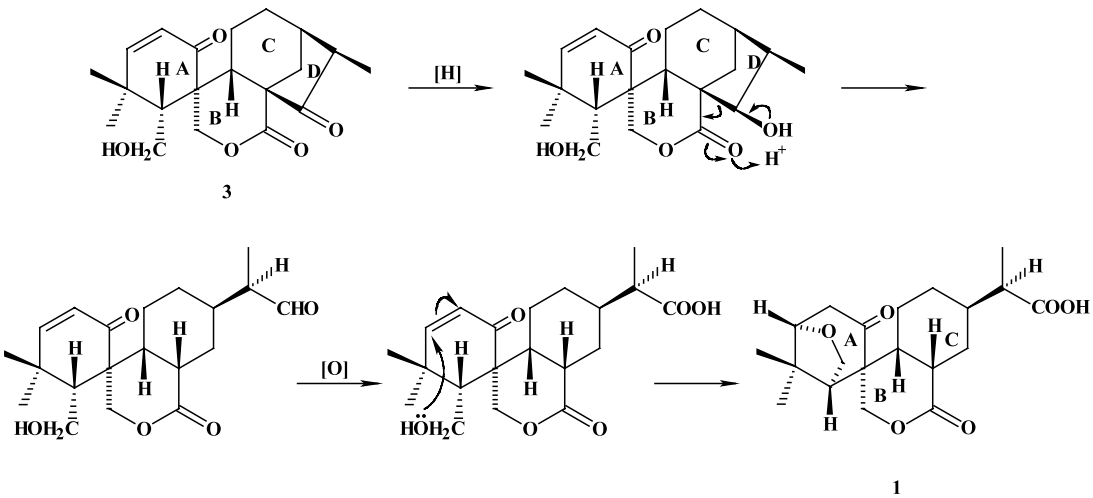


Figure 2. Postulated biogenesis of **1**.

16-(*R*)-methyl-3,6-epoxy-6,7:8,15-*seco*-7,20-olide-*ent*-kaur-15-oic acid, and given the trivial name laxiflorin F.

Compound **2**, isolated as colorless needles and exhibiting a molecular ion peak at m/z 362 in the EIMS spectrum, was concluded to have the molecular formula $C_{20}H_{26}O_6$ by HREIMS and NMR spectra, two hydrogen atoms less than **1**, and thus with one degree of unsaturation more than **1**. Comparison of the NMR spectra of **1** and **2** revealed that the two compounds were very similar. The only difference between them was at the side-chain attached to C-13. The C-16 methine signals at $\delta_{C/H}$ 46.4/2.44 and the C-17 methyl signals at $\delta_{C/H}$ 15.4/1.30 in **1** were replaced by double bond signals [δ_C 147.5 (s), 122.7 (t) and δ_H 6.48 (s), 5.60 (s)] in **2**. The C-15 signal also moved upfield from δ_C 178.4 (s) to δ_C 169.9 (s), which suggested the existence of an α,β-unsaturated carboxylic acid system in **2**. This was also supported by the UV absorption at 207 nm. Further evidence came from the HMBC spectrum, in which long-range ^1H–^{13}C correlations between H_2-17 and C-15 were clearly displayed. The relative stereochemistry of the asymmetric carbons in **2** were shown to be the same as in **1** on the basis of their similar ROESY spectra, as well as their similar coupling patterns. The full assignments of the protons and carbons of **2** were accomplished by using ^1H–^1H COSY, HMQC and HMBC experiments. Therefore, **2** was identified as 3,6-epoxy-6,7:8,15-*seco*-7,20-olide-*ent*-kaur-16-en-15-oic acid, and named laxiflorin G.

Compounds **1**, **2** and **3** were tested for cytotoxicity towards human leukemia K562 cells.[8] *cis*-Platin was used as the positive reference substance in the bioassays. Compound **3** had a remarkable inhibitory activity with an IC_{50} value 0.569 μg/mL. No desirable results were obtained for compounds **1** and **2**.

The possibility that compound **1** is an artifact produced during the separation can be excluded because the isolation conditions did not involve the use of temperatures above 60°C or of acid and alkali. Based on the structures of the compounds isolated from *I. eriocalyx* var. *laxiflora* so far, as well as the fact that compound **3** with the nucleus of a 6,7-*seco*-7,20-olide-*ent*-kaurane was separated in large amounts, a plausible biosynthetic origin for the skeleton of **1** is proposed in which the carbon skeleton of compound **1** is biosynthesized from **3**, as shown in Fig. 2. The possible mechanism for the cleavage reaction of the bond between C-8 and C-15 would be a reverse aldol reaction.[9–11]

References

1. Wu, C. Y.; Li, H. W. *Flora Republicae Popularis Sinicae*; Beijing Academic Press: Beijing, 1987; Vol. 66, p. 494.
2. Sun, H. D.; Lin, Z. W.; Niu, F. D.; Shen, P. Q.; Pan, L. T.; Lin, L. Z.; Geoffrey, A. C. *Phytochemistry* **1995**, *38*, 1451–1455.
3. The leaves of *I. eriocalyx* (Dunn) Hara var. *laxiflora* Wu and Li were collected in Xishuangbana prefecture, Yunnan Province, People's Republic of China, in November 1999. It was identified by Professor G. D. Tao, Xishuangbana Botanic Garden. A voucher specimen has been deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.
4. Compound **1**: Colorless lumpish crystals. Mp 263.5–265.0°C; $[\alpha]_D^{13.7}$ +69.35 (*c* 0.16, C_5H_5N); UV (H_2O) λ_{max}: no absorption; IR (KBr) ν_{max}: 3411, 2931, 2631, 1742, 1705, 1461, 1287, 1226, 1182, 1128, 1049, 1024, 1001, 986, 950, 911, 843, 805, 726 cm^{-1}; ^1H and ^{13}C NMR, data see Table 1; EIMS (70 eV) m/z $[M]^+$ 364 (10), 346 (3), 333 (12), 318 (11), 251 (9), 233 (13), 207 (21), 205 (23), 180 (12), 167 (100), 152 (22), 136 (58), 121 (47), 107 (36), 95 (45), 79 (48), 69 (52), 55 (72); HREIMS m/z $[M]^+$ 364.1859 (calcd for $C_{20}H_{28}O_6$ 364.1886). Compound **2**: colorless needle crystals. Mp 253.5–255.0°C; $[\alpha]_D^{14.7}$ +68.57 (*c* 0.18, MeOH); UV (MeOH) λ_{max}: 207 nm; IR (KBr) ν_{max}: 3415, 2964, 2958, 2884, 2805, 1740, 1700, 1631, 1495, 1408, 1368, 1315, 1292, 1247, 1227, 1186, 1128, 1050, 1026, 992, 952, 911, 843, 821 cm^{-1}; ^1H and ^{13}C NMR, data see Table 1; EIMS m/z $[M]^+$ 362 (100), 344 (42), 332 (15), 316 (18), 300 (10), 285 (10), 276 (19), 259 (15), 249 (21), 231 (32), 213 (12), 203 (12), 191 (30), 167 (20), 149 (15), 133 (20), 123 (36), 105 (35), 91 (45), 79 (40), 69 (33), 55 (53); HREIMS m/z $[M]^+$ 362.1703 (calcd for $C_{20}H_{26}O_6$ 362.1729).
5. X-Ray crystal structure analysis of compound **1**: Crystal data: $C_{20}H_{28}O_6 \cdot H_2O$, $M=364.44$, orthorhombic system, space group: $P2_12_12_1$, $a=6.7260(5)$, $b=11.5340(4)$, $c=24.6770(14)$ Å, $V=1914.38(19)$ $Å^3$, $Z=4$, $d=1.332$ g cm^{-3}, Mo Kα radiation, linear absorption coefficient $\mu=1.0$ cm^{-1}. A colorless lumpish crystal of dimensions 0.10×0.10×0.50 mm was used for X-ray measurements on a MAC DIP-2030 diffractometer with a graphite monochromator. The maximum 2θ value was set at 50.0°. The total number of independent reflections measured was 1737, of which 1736 were considered to be observed ($|F|^2 \geq 8\sigma|F|^2$). The structure was solved by the direct method SHELX-86[6] and expanded using difference Fourier techniques, refined by the program and method NOMCSDP[7] and full-matrix least-squares calculations. Hydrogen atoms were fixed at calculated positions. The final indices were $R_f=0.073$, $R_w=0.069$ ($w=1/\sigma|F|^2$), $S=7.084$, $(\Delta/\sigma)_{max}=0.135$, $(\Delta\rho)_{min}=-0.390$ e $Å^{-3}$, $(\Delta\rho)_{max}=0.340$ e $Å^{-3}$.
6. Sheldrick, G. M. University of Göttingen, Federal Republic of Germany, 1985.
7. Lu, Y.; Wu, B. M. *Chin. Chem. Lett*. **1992**, *3*, 637–640.
8. Hou, A. J.; Li, M. L.; Lin, Z. W.; Ji, S. Y.; Zhou, Y. P.; Sun, H. D. *J. Nat. Prod*. **2000**, *63*, 599–601.
9. Isobe, T.; Kamikawa, T.; Kubo, I.; Kubota, T. *Bull. Chem. Soc. Jpn*. **1973**, *46*, 583–586.
10. Fujita, E.; Fujita, T.; Katayama, H.; Shibuya, M. *J. Chem. Soc. (C)* **1970**, 1674–1681.
11. Fujita, E.; Fujita, T.; Shibuya, M. *Tetrahedron* **1969**, *25*, 2517–2530.