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DITERPENOIDS FROM *ISODON GLUTINOSUS*

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Key Word Index—*Isodon glutinosus*; Labiatae; diterpenoid; isodoglutinosin A-B.

Abstract—Two novel diterpenoids were isolated from a diethylether extract of the leaves of *Isodon glutinosus*. Their structures were elucidated by 1D and 2D NMR experiments, including, ^1H–^1H correlation spectroscopy (COSY, NOESY) and ^1H–^{13}C heteronuclear correlation (COSY, COLOC).

INTRODUCTION

Since the active *ent*-kaurene diterpenoid, enmein, was first isolated from the Japanese folk medicine 'eimeso', a considerable number of diterpenoids have been found in a variety of *Isodon* species [1,2]. Most of these diterpenoids possess the *ent*-kaurene skeleton, and whose main physiological properties are antitumor, antibacterial activities as well as inhibitory activities on the respiration of rat mitochondria and for insect growth [3].

Our earlier work on *Isodon glutinosus* C. Y. Wu et H. W. Li, in the context of an intensive study on the bioactive diterpenoids from *Isodon* plants, yielded three diterpenoids: glutinosin, *ent*-kauran-16α,17-diol, and pisiferic acid [4,5]. Recently, we studied the same plant collected in Lijiang county, Yunnan Province, China, to afford two novel diterpenoids, isodoglutinosin A and B (**1** and **2**).

In this paper, we present the isolation and the structure elucidation of isodoglutinosin A and B (**1** and **2**), through a series of one- and two-dimensional NMR techniques, including DEPT, COSY, NOESY and COLOC experiments.

RESULTS AND DISCUSSION

An ethereal extract from the leave of *Isodon glutinosus* was subjected to column chromatography on silica gel, followed by further repeated column chromatography and recrystallization to give isodoglutinosin A and B (**1** and **2**).

Isodoglutinosin A (**1**), a colourless crystal, displayed a $[M]^+$ ion at m/z 390 in agreement with the molecular formula $C_{22}H_{30}O_6$. The existence of a five-membered ketone conjugated with an *exo*-methylene in **1** was evident from the following data: λ_{max} 231 nm (log ε 4.15); ν_{max} 1720 and 1640 cm^{-1}, δ_H 6.24 and 5.32 (each 1H, *s*) as well as δ_C 149.0 (*s*), 116.9 (*t*) and 202.1 (*s*). The ^{13}C NMR spectrum of isodoglutinosin A (**1**) showed, in addition to the signals of one acetoxy group (δ 171.0*s* and 21.0*q*), 20 carbons being divided by DEPT experiments into Me × 3, CH_2 × 5, CH × 6, and C × 6, suggesting a tetracyclic *ent*-kaur-15-oxo-16-ene nucleus typically found in *Isodon* plants [2]. This nucleus possesses five quaternary skeletal carbons, of which three were assigned to C-4 (34.2*s*), C-8 (70.9*s*) and C-10 (47.0*s*), respectively. Oxygenated substituents at C-1, C-14, and C-7 were indicated by the significant downfield shifts exhibited by C-10 and C-8 in compound **1**, relative to the corresponding values in the model compounds [6]. This deduction was unambiguously confirmed by the proton–carbon long-range chemical shift correlation 2D NMR technique (COLOC) (see Fig. 1).

In the COLOC spectrum of **1**, a two-bond coupling was observed from C-10 (δ 47.0) to the double doublet proton of δ 3.58 (*dd*, J = 9.2, 4.4 Hz) placing it at C-1, and from C-8 (δ 70.9) to a singlet proton at δ 5.17 (*s*) to locate it at C-14, which in turn was associated with the carbon resonating at δ 199.3 (*s*), thus suggesting that the additional ketone carbonyl group was at C-7. Correlations were also observed from C-8 and ester carbonyl signal (δ 171.0) to a proton signal at δ

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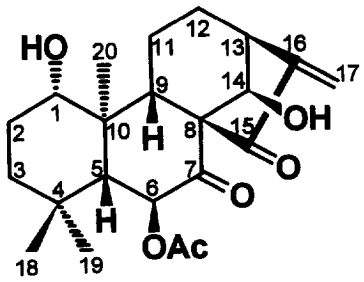

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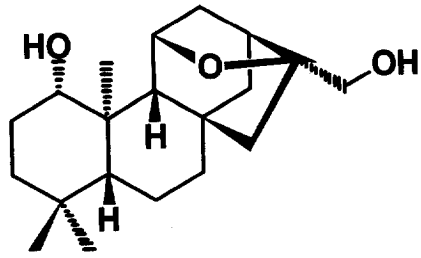

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6.10 (*d*, $J = 12.4$ Hz) which in turn was correlated with the signal at δ 76.1 (*d*) in the ^1H–^{13}C COSY spectrum, so we assigned the δ 6.10 doublet to the signal of H-6.

The relative sterochemistry of **1** was elucidated on the basis of NOESY correlations (Fig. 2). The NOESY spectrum of isodoglutinosin A (**1**) showed cross-peaks of H-1/H-5, H-1/H-9, H-5/H-9, H-6/Me-19, and H-14/Me-20, establishing the 1-H, 6-H and 14-H as β-, α- and α-oriented, respectively. In accordance with the data mentioned above, isodoglutinosin A (**1**) was identified as 1α,14β-dihydroxy-6β-acetoxy-*ent*-kaur-7,15-dioxo-16-ene. The unambiguous assignments for all of the carbons and the key protons are as shown in Tables 1 and 2.

Isodoglutinosin B (**2**), a colourless crystal, was shown to have the molecular formula, $C_{20}H_{32}O_3$, by EI-mass spectrometry, indicating five degrees of unsaturation. The ^{13}C NMR spectrum of isodoglutinosin B (**2**) clearly revealed 20 carbons, DEPT analysis showed the presence of Me × 3, CH_2 × 8, CH × 5 and C × 4. Only the absorptions for a hydroxyl group (3585, 3300 cm^{-1}) and an ether bond (1020 cm^{-1}) were observed in its IR spectrum. These findings suggested that **2** possessed a basic *ent*-kaurane skeleton in which the unsaturated functionalities of ring D (=CH_2 and C=O) were reduced. Meanwhile, the further degree of unsaturation required by the molecular formula indicated the presence of an additional ring.

The δ 60–90 region of the ^{13}C NMR spectrum of **2** exhibited four signals at δ 89.5 (*s*), 80.9 (*d*), 80.1 (*d*) and 65.9 (*t*), suggesting that one of the three oxygens in **2** was connected with two carbons to form an epoxy unit. The linkage of this additional ring through an ether bridge (oxygen atom) from C-11 to C-16 was

Table 1. ^1H NMR data for compounds **1** and **2** in pyridine-d_5

H	1	2
1β	3.58*dd*, 9.2, 4.4	3.59*dd*, 10.0, 4.0
2α	1.88*m*	1.90*m*
2β	1.74*m*	1.74*m*
3α	1.51*dt*, 9.8, 3.0	1.41*dt*, 14.1, 2.6
3β	1.30*m*	1.31*m*
5β	1.80*d*, 12.4	0.76*dd*, 10.6, 2.0
6α	6.10*d*, 12.4	1.35–1.40*
6β	—	1.35–1.40*
7α	—	1.51*dd*, 12.0, 3.0
7β	—	1.48*
9β	2.50*dd*, 5.5, 2.5	1.94*br.s*
11α	3.41*dd*, 15.5, 5.5	5.91*br.s*
11β	1.59*m*	—
12α	2.16*m*	2.06*dd*, 11.0, 3.5
12β	1.75*	1.27*m*
13α	3.29*br.s*	2.72*t*, 6.0
14α	5.17*s*	2.28*d*, 11.0
14β	—	2.06*dd*, 11.0, 4.0
15α	—	1.68*d*, 10.2
15β	—	1.81*dd*, 10.2, 2.0
17	6.24*s*	4.10*d*, 11.2
17	5.32*s*	3.98*d*, 11.2
18	1.12*s*	0.83*s*
19	1.03*s*	0.84*s*
20	1.62*s*	1.34*s*
OAc	2.10*s*	—

*Ambiguous due to signal overlapping.

Table 2. ^{13}C NMR data for compounds **1** and **2** in pyridine-d_5

C	1	2
1	78.8*d*	80.9*d*
2	30.1*t*	29.7*t*
3	39.7*t*	40.1*t*
4	34.2*s*	33.1*s*
5	53.7*d*	56.0*d*
6	76.1*d*	20.4*t*
7	199.3*s*	38.9*t*
8	70.9*s*	43.8*s*
9	57.0*d*	61.1*d*
10	47.0*s*	46.0*s*
11	19.5*t*	80.1*d*
12	32.3*t*	44.2*t*
13	47.1*d*	43.0*d*
14	74.7*d*	40.8*t*
15	202.1*s*	53.9*t*
16	149.0*s*	89.5*s*
17	116.9*t*	65.9*t*
18	35.0*q*	33.7*q*
19	22.3*q*	22.1*q*
20	15.1*q*	13.5*q*
Ac	171.0*s*	
	21.0*q*	

Table 3. 2D ^{1}H–^{1}H COSY data for compounds **1** and **2** in pyridine-d_5

	Correlated proton	
Proton	**1**	**2**
H-1β	H-2	H-2α,2
H-2α	H-2,3	H-2β,3
H-2β	H-2,3	H-2α,3
H-3α	H-3,2	H-2
H-3β	H-3,2	H-2
H-5β	H-6β	H-6
H-6α	H-5β	H-5β
H-7α		H-6
H-9β	H-11	H-11α
H-11α	H-11,12	H-9β,12α
H-11β	H-9β,11	
H-12α	H-12,11α	H-12 β,13α, 11α
H-12β	H-12,13α	H-12α,13α
H-13α	H-12β	H-12β,14β
H-14α		H-14β
H-14β	H-14α,13α,	15β
H-15β		H-14β
H-17a	H-17b	H-17b
H-17b	H-17a	H-17a

Table 4. 2D ^{1}H—^{1}H NOESY data for compounds **1** and **2** in pyridine-d_5

	Correlated proton	
Proton	**1**	**2**
H-1β	H-5β,9β	H-5β,9β
H-5β	H-1β,9β,18	H-1β,9β
H-6α	H-19,20	
H-9β	H-1β,5β, 117-β	H-1β,5β
H-11α		H-9β,12
H-13α	H-14α,12,17a	H-12β,14β, 17
H-14α	H-20,13α	H-12α,20
H-17a	H-17b	
H-17b	H-17a	
CH_3-18	H-5β	
CH_3-19	H-20,6α	H-20
CH_3-20	H-11α,19, 14α	H-19

established unambiguously by analysis of the ^2J and ^3J heteronuclear couplings visualized through a COLOC experiment, i.e. H-11 (5.91 *brs*) was coupled to C-16 (89.5*s*) in its COLOC spectrum, the remainder of the COLOC correlations were given in Table 5 and shown as Fig. 3.

Characterization of the 1α-hydroxyl was accomplished by essentially the same evidence as that for isodoglutinosin A (**1**), and the C-17 position was finally determined as the site of the remaining hydroxyl group from the ^{1}H NMR data and NOESY experiments (see Tables 1 and 4). Consequently, isodoglutinosin B (**2**) was established to be 1α,17-dihydroxy-11β,16β-epoxy-*ent*-kaurane.

EXPERIMENTAL

General. Mps determined on a Kofler hot-stage apparatus and are uncorr. UV spectra measured on Beckman DU-7 spectrophotometers; IR spectra taken on a Perkin-Elmer 577 instrument and recorded in KBr pellets. MS were obtained from ZAB-HS mass spectrometer in the EI-mode. All NMR spectra were recorded with a Bruker AM-400 NMR spectrometer; pyridine-d_5 as solvent and TMS as int. standard.

Plant material. The plant material of *I. glutinosus* C. Y. Wu et H. W. Li was collected from Lijiang County, Yunnan Province, P. R. China, and identified by Prof. H. W. Li. The voucher specimen of *I. glutinosus* is deposited in the Herbarioum of the Department of Taxonomy, Kunming Institute of Botany, Academia Sinica, Kunming, P. R. China.

Extraction and isolation. Powdered air-dried leaves (3.0 kg) of *I. glutinosus* were extracted with Et_2O and the solvent removed under vacuum. The residue (370 g) was treated with activated C (2 × 20 g) in MeOH. The soln. was filtered and the solvent evaporated to yield 260 g yellow gum which was subjected to CC on silica gel, eluted with petrol, $CHCl_3$, and $CHCl_3$—Me_2CO with increasing proportions of Me_2CO. Frs were collected, and combined after monitoring with TLC, followed by recrystallization to afford iso-

Table 5. 2D COLOC data for compounds **1** and **2** in pyridine-d_5

	Correlated proton	
Carbon	**1**	**2**
C-1	H-9β,2,3	—
C-2	H-3	—
C-3	H-18,19	H-5β
C-4	H-5β,3,18,19	H-5β,2
C-5	H-6α,20,18,19	H-18,19,20
C-6	H-5β	H-7
C-7	H-5β,6α	H-6
C-8	H-9β,13α,11α	H-9β,5,7
C-9	H-11	H-20
C-10	H-20,5β,9β	H-11α,9β
C-11	H-13α	H-9β
C-12	—	H-9β
C-13	H-17a,17b	—
C-14	—	—
C-15	H-14α,17a	H-14
C-16	H-13α,14α,17b	H-11α,12,14
C-17	—	—
C-18	H-5β,19	H-5β
C-19	H-8,5β	H-5β
C-20	H-9β,5β	H-9β
OAc(C=O)	H-Ac,6α	

doglutinosin A (**1**, 1.0 g) and isodoglutinosin B (**2**, 100 mg).

Isodoglutinosin A (**1**). Crystals; mp 144–146°; $C_{22}H_{30}O_6$, UV λ_{max}^{EtOH} nm (log ε): 231 (4.15); IR ν_{max}^{KBr} cm^{-1}: 3550, 3460, 1745, 1725, 1720, 1641, 1370, 1283, 1260, and 1087; ^1H NMR data—see Table 1; ^{13}C NMR data—see Table 2; EIMS *m/z*: 390 $[M]^+$, 373, 348, 330, 312, 297, 284, 259 and 231.

Isodoglutinosin B (**2**). Crystals; mp 144–146°; IR ν_{max}^{KBr} cm^{-1}: 3585, 3300, 1440, 1385, 1120 and 1020; ^1H NMR data—see Table 1; ^{13}C NMR data—see Table 2; EIMS *m/z*: 320 $[M]^+$, 302, 287, 261, 243, 220, 203, 185 and 171.

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REFERENCES

1. Fujita, E. and Node, M., *Progress in Chemistry of Organic Natural Product*, 1983, **46**, 144.
2. Huang, H., Study on chemical constituents of three Labiatae *Isodon* plants. Ph.D. Thesis, Part I, China Pharmaceutical University, Nanjing, 1995.
3. Fujita, T., Takeda, Y., Sun, H. D., Minami, Y., Marunaka, T., Takeda, S., Yamada, Y. and Togo, T., *Planta Medica*, 1988, **5**, 377.
4. Sun, H. D., Lin, Z. W. and Shen, P. Q., *Acta Botanica Yunnanica* 1987, **9**, 247.
5. Lin, Z. W., He, X. D., Shen, X. Y. and Sun, H. D., *Acta Botanica Yunnanica*, 1992, **14**, 327.
6. Hanson, J. R., Siverns, M., Piozzi, F. and Savona, G., *Journal of the Chemical Society, Perkin I*, 1976, 114.