Pergamon

PII: S0031-9422(96)00808-4

Phytochemistry, Vol. 45, No. 3, pp. 559–562, 1997

0031-9422/97 $17.00+0.00

DITERPENOIDS FROM *ISODON GLUTINOSUS*

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(*Received in revised form* 16 *October* 1996)

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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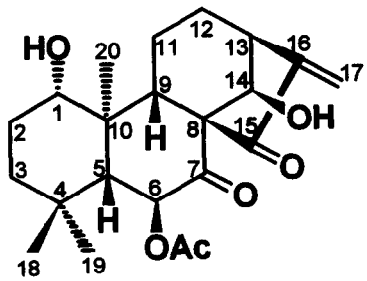

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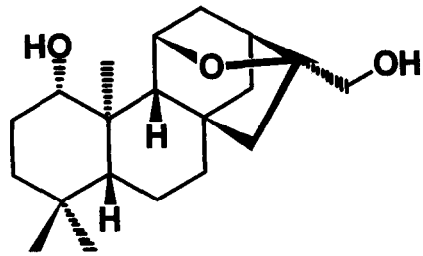

2

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 ^1H–^1H 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 ^1H—^1H 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, 11-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 ^1H 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.

Acknowledgements—We are grateful to Prof. H. W. Li, Department of Taxonomy, Kunming Institute of Botany, Academia Sinica, and to Mr Z. W. Lin, Laboratory of Phytochemistry, KIB, for the taxonomic verification and the procurement of the plant material, respectively.

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