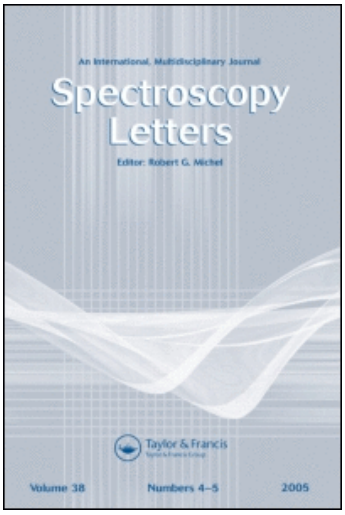

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H. Huang[a]; Q. -R. Chao[b]; J. Hu[b]; R. X. Tan[a]
[a] Institute of Biotechnology, Department of Biological Science & Technology, Nanjing University, Nanjing, P. R. China [b] State Key Laboratory of Coordination Chemistry, Nanjing University, Nanjing, P. R. China

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STRUCTURE OF ORESBIUSIDE FROM *ISODON ORESBIUS*

Key Word: *Isodon oresbius*, Labiatae, *ent*-kaurene glycoside, oresbiuside, NMR

[a] H. Huang, [b] Q. -R. Chao, [b] J. Hu and [a] R. X. Tan*

a) Institute of Biotechnology, Department of Biological Science & Technology, Nanjing University, Nanjing 210093, P. R. China
b) State Key Laboratory of Coordination Chemistry, Nanjing University, Nanjing 210093, P. R. China

ABSTRACT

Repeated fractionation of the *n*-BuOH extract of the aerial parts of *Isodon oresbius* gave a new diterpene glycoside, named oresbiuside (**1**), whose structure was established as 18-(β-D-glucopyransyl)-7α,12α,14β,15β-tetrahydroxy-*ent*-kaur-16-ene (**1**) mainly by a combination of 1D and 2D NMR techniques (^{1}H and ^{13}C NMR, DEPT, COSY, NOESY, HMBC and HMQC).

* Address correspondence to Prof. R. X. Tan.

INTRODUCTION

Isodon species (Labiatae) are known to contain cytotoxic and antibacterial *ent*-kaurane diterpenoids. However, only a few diterpene glycosides were isolated from this genus. The diterpene glycosides reported so far from *Isodon* species are limited to rabdoside, rabdoside[1], parvifoliside[2], shikokiaside A and B[3]. *I. oresbius* (W. W. Smith) Kudo is widely distributed in the southwestern region of China. It has been used as folk medicine for the treatment of blood clots in internal organs such as liver and kidneys[4]. The aerial parts of *I. oresbius* collected at Lijiang County, Yunnan Province, P. R. China, have been shown to contain flavonoids[4] and other phenolic compounds[5]. In continuation of our investigation of the structurally novel and/or biologically active principles from *Isodon* plants, we have studied the EtOH extract of *I. oresbius* growing at Diqing County in the same province to afford a new diterpene glycoside, oresbiuside (**1**). We hereby wish to report the structure determination of the new compound through one- and two-dimensional NMR techniques including ^{1}H and ^{13}C NMR, DEPT, COSY, NOESY, HMQC and HMBC spectra.

EXPERIMENTAL

Melting points were measured on a YANACO MP-S_2 apparatus and are uncorrected. IR spectrum was taken on a Perkin-Elmer 577 instrument and recorded in KBr discs. Mass spectra were obtained from a ZAB-HS mass spectrometer. All NMR spectra were recorded on a Bruker DRX-400 NMR spectrometer with CD_3OD used as solvent, and TMS int. standard. Column chromatography was performed on Si gel (100-200 or 200-300 mesh), and TLC analyses was carried out using precoated Si gel plates.

The aerial parts of *I. oresbius*, collected in October 1996 at Diqing County, Yunnan Province, P. R. China, were authenticated by Prof. H. W. Li, Kunming Institute of Botany, Academia Sinica, Kunming 650204, P. R. China, where a

voucher specimen was deposited. The air-dried plant material (3.5 kg) were powdered and then extracted with 80% EtOH to give an EtOH extract (620 g) that was dissolved in EtOH-H_2O (1:9), and then partitioned successively with petrol, EtOAc and *n*-BuOH to give fractions P (90 g), E (120 g) and N (140 g), respectively. Fr.N was repeatedly chromatographed over Si gel, eluting with $CHCl_3$ containing gradually increased amounts of CH_3OH, followed by recrystallization to give finally oresbiuside (**1**, 60 mg) as white powder; mp: 190-192°; IR ν_{max}^{KBr} cm^{-1}: 3500-3200, 1700, 1620, 1440, 1050; HRFABMS *m/z* (negative mode): 527.2494 $[M-H]^-$ (calcd. for $C_{26}H_{39}O_{11}$: 527.2492); ^1H and ^{13}C NMR data, unequivocally assigned by COSY, HMQC, HMBC and NOESY techniques, were given in Table 1.

RESULTS AND DISCUSSION

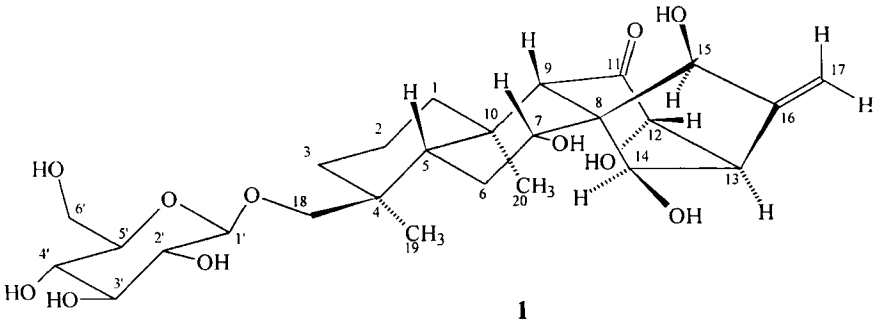
The molecular formula of compound **1** was shown to be $C_{26}H_{40}O_{11}$ by the HRFAB mass spectrum exhibiting a quasi-molecular ion at *m/z* 527.2494 $[M-H]^-$ ($C_{26}H_{39}O_{11}$ requires: 527.2492). This molecular formula was in agreement with its ^{13}C NMR spectrum which gave a total of 26 resonance lines consisting of 2 methyl, 7 methylene (two oxygenated C at δ 79.2 and 62.9), 12 methine (nine oxygen-bearing C between δ 70 and 105), and 5 quaternary carbon signals. The presence of a β-D-glucopyranosyl group was demonstrated by the anomeric proton signal at δ 4.45 (1H, d, J=7.8 Hz) and the carbon resonances at δ 105.0 d, 75.1 d, 78.2 d, 71.8 d, 78.0 d and 62.9 t, ascribable to the glucosyl moiety. Subtracting the elemental composition ($C_6H_{11}O_5$) of glucosyl residue from the molecular formula equaled $C_{20}H_{29}O_6$. This observation, along with two methyl singlets (δ 1.49 and 1.01), an *exo*-methylene signal (δ 5.56 and 5.44) and an oxygenated methylene resonance (δ 3.62 and 3.51) in the ^1H NMR spectrum, indicated that it was most probably an *ent*-kaurene diterpene glucoside[6]. This hypothesis was supported by its ^{13}C NMR data and DEPT experiments. All proton and carbon signals were

TABLE 1. ^{1}H & ^{13}C NMR, HMQC and HMBC Spectral Data of Oresbiuside (**1**)

		HMQC		HMBC
C	δ_C	DEPT	δ_H (J in Hz)	
1	40.9	CH_2	α: 1.87 m; β: 1.45 m	H-5, H-9, H-20
2	18.9	CH_2	α: 1.63 m; β: 1.75 m	*
3	36.5	CH_2	α: 1.77 m; β: 1.51 m	H-18a/b, H-19
4	38.6	C		H-5, H-18a/b, H-19
5	46.2	CH	1.73 br d (12.1)	H-9, H-18a/b, H-19, H-20
6	30.2	CH_2	α: 1.95 ddd (12.1,11.7, 11.3)	H-5, H-7
			β: 2.11 br dd (11.3, 3.7)	
7	75.0	CH	4.04 dd (11.7, 3.7)	H-9, H-14, H-15
8	53.2	C		H-6α/β, H-7, H-9, H-13
9	63.9	CH	2.54 br s	H-5, H-12, H-14, H-15
10	41.0	C		H-5, H-9, H-20
11	211.3	C		H-9, H-12, H-13
12	80.3	CH	3.81 br d (3.5)	H-9, H-13, H-14, H-17a/b
13	55.8	CH	3.01 br d (3.5)	H-12, H-14, H-15, H-17a/b
14	73.1	CH	5.44 br s	H-9, H-12, H-15
15	75.0	CH	5.33 br s	H-9, H-13, H-17a/b
16	151.7	C		H-13, H-14, H-15, H-17a/b
17	114.3	CH_2	**a**: 5.56 br s; **b**: 5.44 br s	H-13, H-15
18	79.2	CH_2	**a**: 3.62 d (9.8); **b**: 3.51 d (9.8)	H-5, H-19, H-1'
19	18.7	CH_3	1.01 s	H-5, H-18a/b
20	19.4	CH_3	1.49 s	H-5, H-9
1'	105.0	CH	4.45 d (7.8)	H-2', H-3'
2'	75.1	CH	3.42 dd (9.0, 7.8)	*
3'	78.3	CH	3.57 dd (9.0, 9.0)	*
4'	71.8	CH	3.54 m^{+}	*
5'	77.9	CH	3.54 m^{+}	*
6'	62.9	CH_2	**a**: 4.05 br d (11.5)	H-4', H-5'
			b: 3.85 dd (11.5, 4.8)	

* Not well resolved; $^{+}$ Not the first order.

assigned unambiguously by a combination of 2D NMR spectra (COSY, HMQC, HMBC and NOESY). The chemical shift (δ 79.2) of C-18 suggested the linkage of 18-glucosyloxy group. This assumption was further confirmed by the ^{3}J heteronuclear coupling of C-18 with the anomeric proton (δ 4.45, d, J=7.8 Hz) in the HMBC spectrum. The double doublets (J=11.7, 4.1 Hz) of H-7 at δ 4.04, along with the splitting patterns of H-6α and H-6β (Table 1) required the presence of 7α-hydroxy group. Furthermore, H-9 signal appeared as a broadened singlet suggesting that C-11 was quaternary. Further scrutiny of the ^{13}C NMR data led to the proposal of 11-ketone function as the C-9 resonance was shifted downfield by 7.6 ppm from that of *16,17-dihydroxykauran-19-oic acid β-D-glucopyranosyl ester* due to the β-effect of the ketone group[7]. As shown by the COSY spectrum of compound **1**, the doublet (J=3.5 Hz) of H-12 at δ 3.81, broadened by its long range coupling to H-9 through a "W-conformation"[8], coupled to the broadened doublet (J=3.5 Hz) of H-13 at δ 3.01 demonstrating the presence of 12α-hydroxy



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group. In addition, a pair of oxygenated methine singlets at δ 5.44 and 5.33 showed allylic couplings to the *exo*-methylene signals of H-17 at δ 5.44 and 5.56. This observation could only be explained by the proposal of a 14,15-dihydroxy functionality. This hypothesis was further reinforced by the NOESY spectrum of compound **1**, which ascertained as well the formulated configurations of C-4, C-5, C-7, C-8, C-10, C-12, C-13, C-14 and C-15 by the discerned NOE correlations

(H-20 with H-19 and H-14α, H-9 with H-5 and H-7, H-12 with H-17b, but no correlation between H-9 and H-15). Accordingly, the structure of oresbiuside (**1**) was elucidated as 18-(β-D-glucopyransyl)-7α,12α,14β,15β-tetrahydroxy-*ent*-kaur-16-ene.

The antifungal bioassay showed that this new diterpene glycoside was not active even at 200 μg/ml against the human and plant pathogenic fungi (*Candida albicans* and *Cladosporium cucumerinum*) following the procedure described previously[9].

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