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SESQUITERPENE HYDROXYLACTONE FROM *LACTARIUS SUBVELLEREUS*

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Key Word Index—*Lactarius subvellereus*; Basidiomycetes; Russulaceae; fruiting body; 13-oxo-lactara-γ-lactone; lactarane sesquiterpenes; subvellerolactone A.

Abstract—A new 13-hydroxy-lactara-6,8-dien 5-oic acid γ-lactone has been isolated from the fruiting bodies of *Lactarius subvellereus*. Its structure and stereochemistry were determined on the basis of spectral analysis, including 2D NMR spectroscopy, especially HMBC correlation. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The fungus *Lactarius subvellereus* Peck. (Russulaceae) is used in Chinese folk medicine. Its EtOAc extract has attracted our interest at a consequence of its cytotoxic and antitumour activities. We recently reported on the isolation of five sesquiterpenes and other compounds from this fungus and cytotoxic effects against some cancer cells were shown [1]. Further investigation on the relatively non-polar fraction of *L. subvellereus* has led to the isolation of one new sesquiterpene (**1**) of uncertain configuration on C-13. It has the unusual and characteristic lactarane skeleton which appears unique to the *Lactarius sesquiterpenes*.

RESULTS AND DISCUSSION

The ether fraction of the methanol extract of the fruiting bodies of *L. subvellereus* was subjected to repeated chromatography to afford subvellerolactone A (**1**). Its IR spectrum revealed the absorptions of an ester carbonyl at 1736 cm^{-1} and hydroxyls at 3348 cm^{-1}. The maximum absorption in the UV spectrum at 290 nm indicated the presence of a conjugated system. The high resolution mas spectrum led to the formula $C_{15}H_{20}O_4$ and the mass spectrum exhibited peaks due to the loss of water and a methyl group at *m/z* 246 $[M-H_2O]^+$ and 231 $[M-H_2O-Me]^+$ (base peak). The presence of a γ-hydroxy-α,β-unsaturated butenolide system with the double bond located at the junction of two rings was confirmed on the basis of the NMR spectral data: one ester carbonyl group (δ_c 173.4/173.3), two unsaturated quaternary carbons (δ_c 152.5/152.3 and 126.2/125.6) and one methine linked to two oxygens (δ_C 98.2/97.9, δ_H 6.00/5.87 *d*). In addition, the ^{13}C NMR spectrum showed signals attributed to three methyls (δ 17.3, 26.1/26.0 and 28.5), two methylenes (δ 46.1 and 50.4/50.3), one oxygenated methine (δ 72.0/71.6), two methines (δ 44.7/44.6 and 37.9/37.6), one quaternary carbon (δ 36.4/36.3), one tertiary (δ 111.6/111.4) and quaternary (δ 168.2/167.4) unsaturated carbons. These data suggested that subvellerolactone A was a sesquiterpene lactone with a lactarane skeleton (Table 1).

The position of the second double bond was unambiguous because it was conjugated with the one in the lactone ring. The ^1H NMR spectrum showed only one vinyl proton signal (δ 5.99/5.94, *s*), and, the ^{13}C NMR specturm indicated one low-field unsaturated quaternary carbon (δ 168.2/167.4, C-9). Thus, the second double bond had to be located between C-8 and C-9. The ^1H NMR spectrum showed signals of three methyl groups typical of the lactarane skeleton, one of them being a doublet ($J = 6.7$ Hz, 12-Me). The inter couplings were confirmed by measuring the ^1H, ^1H COSY spectrum (Table 1). According to the formula, the compound had two hydroxy groups, which should be placed in positions 4 and 13. On the basis of the homoallylic coupling between them [2] and the long range correlation between C-6 and H-8 in the HMBC experiment, we deduced that the carbonyl group should be located on C-5. The HMBC spectrum was extremely valuable in the assignment of all carbons and hydrogens (Table 1).

The large value of $^3J(3,4)$ (9.3 or 8.4 Hz) suggested that the OH-4 was quasi-equatorial, anti to H-2 in the

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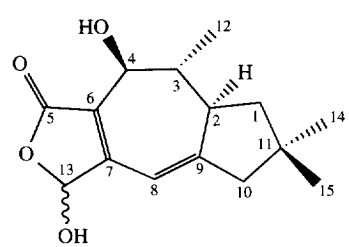
Table 1. ^1H and ^{13}C NMR spectral data of subvellerolactone A (500 MHz, $CDCl_3$, TMS)

H	δ	J(Hz)	^1H-^1HCOSY correlation with	C	δ(DEPT)	^1H-^{13}CCOSY correlation with	Significant HMBC correlation with
1α	1.52/1.53 *t*	12.1/12.1	H-1β/H-2	1	46.1(CH_2)	H-1α,β	H-10α,14,15
1β	1.80 *m*		H-1α/H-2				
2	2.62 *m*		II-1αβ/H-3	2	44.7/44.6(CH)	H-2	H-1α,8,10α,12
3	1.80 *m*		H-2/H-4/H-12	3	37.9/37.6(CH)	H-3	H-1α,4,12
4	4.31/4.28 *d*	9.3 or 8.4	H-3	4	72.0/71.6(CH)	H-4	H-3,12
5				5	173.4/173.3(C)		
6				6	126.2/125.6(C)		H-4,8,13
7				7	152.5/152.3(C)		II-4
8	5.99/5.94 *br.s*		H-10β(w)	8	111.6/111.4(CH)	H-8	H-10α
9				9	168.2/167.4(C)		H-1β or 3, 10α
10α	2.30/2.31 *d*	17.7	H-10β	10	50.4/50.3(CH_2)	H-10α,β	H-1β,8,14,15
10β	2.41 *dd*	17.7/2.6	H-10α/H-8(w)				
11				11	36.4/36.3(C)		H-1α,10α,10β,14,15
12	1.13 *d*	6.7	H-3	12	17.3(CH_3)	H-12	H-4
13	6.00/5.87 *d*	4.7/1.6		13	98.2/97.9(CH)	H-13	
14	0.91/0.90 *s*			14	26.1/26.0(CH_3)	H-14	H-1α,10α,10β,15
15	1.11/1.13 *s*			15	28.5(CH_3)	H-15	H-1α,14

compound (indicating a *trans* quasi-axial orientation of H-3 and H-4 [3]. Usually the configuration of Me-3 is syn to the ring junction protons (H-2 and H-9) when C-12 is geminal to a hydrogen and anti when it is geminal to an hydroxy group [4]. The proposed biosynthesis of the lactarane skeleton indicated that the protons at C-2 and C-9 both possessed α configurations [5], so we concluded that Me-3 possessed α-configuration and OH-4 possessed β-configuration. When H-3 occupied the α-position, its signal was shifted to lower field (H-3α δ 2.15) [6]. Twelve pairs of peaks with similar chemical shift values (the ratio of each pair height was about 1:1), respectively, in the ^{13}C NMR specturm (Table 1) indicated that subvellerolactone A was an epimeric mixture which gave only a single spot on silica gel TLC. Thus, we deduced that the many pairs of peaks is due to a C-13 epimeric mixture. The obvious differences in the ^1H signals of H-13 and H-8 (Table 1) support this. In fact, it is a cyclic acetal of a 1,2-aldehyde-carboxylic acid, which may be the oxidation products of the corresponding furan. The fast equilibrium between both epimers makes the separation of the mixture impossible. Considering the above data, the structure for the compound was established (**1**)

The ^{13}C-^1H COSY, ^1H-^1H COSY and HMBC spectra were all consistent with the structure of subvellerolactone A (Table 1).



1 Subvellerolactone A

EXPERIMENTAL

General. IR: thin film; 1D-NMR and 2D-NMR: 500 MHz(^1H) and 125 MHz(^{13}C), Bruker AM-500; MS: MAT 711.

Fungus material. Lactarius subvellereus was collected from Guangxi, People's Republic of China, in 1994, and identified by Prof. Weikung Xu, etc., of Guangxi Institute of Botany.

Extraction and isolation. 5 kg of dry fruiting bodies were extracted with MeOH. The extract was evapd *in vacuo* to remove the MeOH. The crude residue was extracted with EtOAc and then with Et_2O. The Et_2O soln was extracted with 0.5% NaOH to remove the fatty acid components. The collected organic layers (pH 7–8) were washed with H_2O and then evaporated to dryness *in vacuo* to give 48.1 g of a crude residue. The residue (44.5 g) was chromatographed on VLC with a mixt. of $CHCl_3$–MeOH. Sesquiterpenes in the frs were visualized as differently coloured spots by spraying the silica gel TLC plates with an Aubepine–H_2SO_4–EtOH soln. The frn containing subvellerolactone A was rechromatographed on CC (petrol–Et_2O) and then on PTLC [C_6H_{12}–Me_2CO 7:3].

Subvellerolactone A. 7 mg, yellowish oil. UV λ_{max} (MeOH) nm: 290; IR ν_{max}(thin film) cm^{-1}: 3348 (OH), 2917, 1736 (C=O), 1634 (C=C); ^1H, ^{13}C NMR, ^{13}C ^1H COSY, ^1H ^1H COSY and HMBC (500 MHz, $CDCl_3$, TMS): Table 1. HRMS: $[M]^+$ 264.1592 (calcd for $C_{15}H_{20}O_4$ $[M]^+$ 264.1632); MS *m/z* (rel. int.): 264 $[M]^+$ (17), 246 $[M-H_2O]^+$ (77), 231 $[M-H_2-Me]^+$ (100), 217 (97), 203 (26), 190 (62), 172 (44), 157 (43), 134 (27), 105 (51), 91 (89), 69 (47), 55 (70), 41 (89).

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