J. Antibiot. 58(12): 828–831, 2005

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THE JOURNAL OF ANTIBIOTICS

A New Cytotoxic Lanostane Triterpenoid from the Basidiomycete *Hebeloma versipelle*

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Received: July 28, 2005 / Accepted: December 5, 2005

Abstract A new cytotoxic lanostane triterpenoid, 24(*E*)-3β-hydroxylanosta-8,24-dien-26-al-21-oic acid (**1**), was isolated from the fruiting bodies of the mushroom *Hebeloma versipelle*. The structure was elucidated on the basis of spectroscopic studies including 2D NMR experiments. Compound **1** moderately inhibited the growth of several tumor cell lines (IC_{50} 10～25.0 μg/ml).

Keywords lanostane triterpenoid, *Hebeloma versipelle*, basidiomycete, cytotoxicity

Introduction

The fungi of the genus *Hebeloma* belonging to the family Cortinariaceae (Basidiomycetes) comprise many inedible or toxic species, but a few species were investigated with respect to their secondary metabolites. From *H. crustulinifirm* and *H. sinapizans*, a cytotoxic triterpene has been isolated [1], and several new lanostane-type triterpene esters have also been found in *H. spoliatum* and *H. senescens* [2～4], and fourteen neurotoxic cucurbitane-type glycosides have been obtained from the poisonous mushroom *H. vinosophyilurn* [5～7]. Besides triterpenoids a few new caryophyllenes, 6,7-seco-caryophyllenes and related sesquiterpenoids were isolated from the liquid cultures of *H. longicaudum* [8～10]. As a part of our studies on biologically active substances from higher fungi in Yunnan Province, China [11～16], the fruiting bodies of the mushroom *H. versipelle* were investigated. In this report, we described the isolation, structural elucidation and cytotoxic activity of a new lanostanoid, namely, 24(*E*)-3β-hydroxylanosta-8,24-dien-26-al-21-oic acid (Fig. 1).

Materials and Methods

General

Melting point was obtained on an XRC-1 micro-melting point apparatus and uncorrected. Optical rotations were measured on a Horiba SEPA-300 polarimeter. IR spectra were obtained on a Tensor 27 with KBr pellets. UV spectra were recorded on a Shimadzu UV-2401PC spectrophotometer. NMR spectra were recorded on Bruker DRX-500 spectrometer in C_5D_5N solvent. EI-MS was recorded with a VG Autospec-3000 spectrometer and HRESI-MS was recorded with an API QSTAR Pulsar 1 spectrometer.

Silica gel (200～300 mesh, Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol.

Mushroom Material

The fresh fruiting bodies of *H. versipelle* were collected at Ninglang County of Yunnan Province, China, in July 2004. The voucher specimen was deposited in the Herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences.

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Extraction and Isolation

The air-dried and powdered fruiting bodies of *H. versipelle* (1 kg) were extracted with $CHCl_3$/MeOH (1 : 1, v/v) at room temperature to afford a deep brown gum (178.5 g). 119 g of this gum was diluted with H_2O, and partitioned with petroleum ether, EtOAc and *n*-BuOH, successively. The EtOAc extract (4 g) was subjected to column chromatography eluting with petroleum ether/acetone gradient. The fraction (0.8 g) from petroleum ether/acetone (4 : 1, v/v) was further purified by repeated Sephadex LH-20 ($CHCl_3$/MeOH 1 : 1) and Pre-TLC ($CHCl_3$/MeOH 12 : 1, Rf 0.35) to give the pure compound **1** (11 mg).

Physico-chemical Properties

24(*E*)-3β-Hydroxylanosta-8,24-dien-26-al-21-oic acid (**1**). White amorphous powder, m.p. 222°C crystal ($CHCl_3$/CH_3COCH_3 2 : 1, v/v), $[\alpha]_D^{23.5}$ +31 (*c* 0.07, MeOH). UV λ_{max}^{MeOH} nm (log ε) 230 (3.43). IR (KBr) 3432, 2942, 2874, 2836, 2711, 1723, 1691, 1644, 1456, 1375, 1183, 1029 cm^{-1}. ^1H and ^{13}C NMR spectra: see Table 1. HRESI-MS *m/z* 493.3294 (M+Na, Calc. for $C_{30}H_{46}O_4Na$ 493.3293). EI-MS *m/z* 470 (M, 25), 455 (M–Me, 21), 437 (M–Me–H_2O, 100), 419 (7), 409 (8), 391 (12), 341 (9), 297 (17), 281 (23), 255 (16), 241 (20), 213 (24), 187 (70), 159 (81), 119 (81).

Cell Lines and Culture

All cell lines were grown in RPMI-1640 medium (GIBCO) supplemented with 10% heat-inactivated bovine serum, 2 nM *l*-glutamine, 10^5 IU/liter penicillin, 100 mg/liter streptomycin and 10 mM HEPES, pH 7.4. Cells were kept at 37°C in a humidified 5% CO_2 incubator.

Cell Growth Inhibition Assay

Growth inhibition of compound **1** on tumor cells was measured by the microculture tetrazolium (MTT) assay [19, 20] with minor modification [21]. Briefly, adherent tumor cells were seeded into 96-well microculture plates and allowed to adhere for 24 hours before drug addition, while suspended cells were seeded just before drug addition. Each tumor cell line was exposed to compound at 0.01, 0.1, 1, 10 and 100 mg/liter concentrations for different periods (adherent cells for 72 hours, suspended cells for 48 hours) and each concentration was tested in triplicate. At the end of exposure, 20 μl of 5 g/liter MTT [3-(4,5-dimethylthiazol-2yl)-2,5-di-phenyl-tetrazolium bromide, Sigma Chemical Co.] was added to each well and the plates were incubated for 4 hours at 37°C, then "triplex solution (10% SDS - 5% isobutanol - 0.012 M HCl)" was added and the plates were incubated for 12～20 hours at 37°C. The optical density (OD) was read on a plate reader at a wavelength of 570 nm. Media and DMSO control wells, in which compound **1** was absent, were included in all the experiments in order to eliminated the influence of DMSO. The inhibitory rate of cell proliferation was calculated by the following formula:

$$\text{Growth inhibition (\%)} = [OD_{control} - OD_{treated}/OD_{control}] \times 100\%.$$

The cytotoxicity of compound **1** on tumor cells was expressed as IC_{50} values (the drug concentration reducing by 50% the absorbance in treated cells, with respect to untreated cells) and was calculated by LOGIT method.

Results and Discussion

Compound **1** was obtained as white amorphous powder. The HRESI-MS of **1** gave an $[M+Na]^+$ peak at *m/z* 493.3294 (calc. for $C_{30}H_{46}O_4Na$ 493.3293) and corresponded to a molecular formula of $C_{30}H_{46}O_4$, requiring eight degrees of unsaturation. Its IR and UV spectra showed the presence of an α,β-unsaturated carbonyl [ν_{max} 2711 and 1690 cm^{-1} (aldehyde), 1643 cm^{-1} (C=C); λ_{max} 230 nm], a hydroxyl (ν_{max} 3432 cm^{-1}) and a carbonyl (ν_{max} 1723 cm^{-1}) as functional groups. The ^1H NMR spectrum (Table 1) exhibited a total of six methyl signals, *i.e.*, five tertiary methyls at δ_H 1.01 (s, H-19), 1.01 (s, H-30), 1.05 (s, H-29), 1.07 (s, H-18), 1.24 (s, H-28), and an olefinic methyl at δ_H 1.77 (br s, H-27). A trisubstituted olefinic proton was revealed at δ_H 6.56 (br t, 7.1, H-24), together with a CHO group at δ_H 9.53 (s, H-26). The 30 carbon signals were sorted into six methyls, ten methylenes and six methines, one of which had an oxygen substituent at δ_C 78.1 (d, C-3), an olefinic methine at δ_C 154.4 (d, C-24) and an aldehyde methine at δ_C 195.2 (d, C-26), and eight quaternary carbons, one of which contained a carbonyl carbon at δ_C 178.5 (s, C-21), and three olefinic quaternary carbons at δ_C 134.3 (s, C-9), 135.2 (s, C-8) and 139.8 (s, C-25) by DEPT experiments. These data suggested that **1** is a lanostane triterpenoid, which was also concluded by comparison of those data with the similar known compound, trametenolic acid B [17].

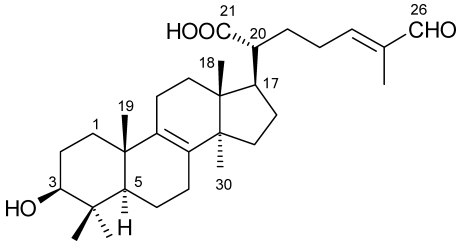

Fig. 1 Structure of **1**.

Table 1 ^{1}H and ^{13}C NMR Data of **1** in C_5D_5N

Position	δ_C	δ_H
1	36.2	1.18 (1H, br s); 1.61 (1H, br d, 12.9)
2	28.7	1.81 (1H, m); 1.88 (1H, m)
3	78.1	3.44 (1H, dd, 8.4, 7.5)
4	39.6	
5	50.9	1.17 (1H, br d, 11.2)
6	18.8	1.54 (1H, m); 1.74 (1H, m)
7	26.9	2.09 (2H, m)
8	135.2	
9	134.3	
10	37.4	
11	21.3	1.99 (2H, m)
12	29.4	1.98 (2H, m)
13	45.0	
14	49.9	
15	30.9	1.30 (1H, dd, 10.0, 11.5); 1.73 (1H, m)
16	27.5	1.47 (1H, m); 2.08 (1H, m)
17	47.7	2.42 (1H, m)
18	16.4	1.07 (3H, s)
19	19.5	1.01 (3H, s)
20	49.1	2.63 (1H, ddd, 3.2, 11.1, 11.1)
21	178.5	
22	31.6	1.85 (1H, m); 1.97 (1H, m)
23	27.5	2.53 (2H, m)
24	154.4	6.56 (1H, br t, 7.1)
25	139.8	
26	195.2	9.53 (1H, s)
27	9.2	1.77 (3H, br s)
28	28.7	1.24 (3H, s)
29	16.4	1.05 (3H, s)
30	24.6	1.01 (3H, s)

The structure of **1** was deduced from detailed analysis of ^{1}H and ^{13}C NMR data aided by 2D NMR including ^{1}H, ^{1}H-COSY, HMQC, HMBC and ROESY experiments (Fig. 2). The COSY spectrum revealed connectivity of C-1 to C-2, C-2 to C-3, C-5 to C-6, C-6 to C-7, C-11 to C-12, C-15 to C-16, C-16 to 17, C-17 to C-20 and C-22, C-22 to C-23, C-23 to C-24. The HMBC correlations of Me-18 to C-12 and C-17, Me-19 to C-1 and C-9, Me-27 to C-24 and C-26, Me-28 and Me-29 to C-3 and C-5, Me-30 to C-8, C-13 and C-15, H-3 [δ_H 3.44 (dd, 8.4, 7.5)] to C-29, H-24 to C-22, C-26 and C-27, and H-26 to C-24 and C-27 revealed one double bond at C-8, one trisubstituted olefinic bond at C-24, which, together with an aldehyde group attached at C-25, one hydroxy group attached at C-3, and one carbonyl group attached at C-20.

The stereochemistry of **1** was determined by a ROESY experiment (Fig. 2) and comparison of the ^{13}C-NMR data with those of trametenolic acid B [17]. The obvious correlations between H-3 and H-5 indicated that the stereochemistry at C-3 was proved to be β-OH. The clear correlation between H-24 and H-26 confirmed that the configuration of the double bond (Δ^{24}) is *E* [18]. On the basis of evidences mentioned above, the structure of **1** was determined as 24(*E*)-3β-hydroxylanosta-8,24-dien-26-al-21-oic acid.

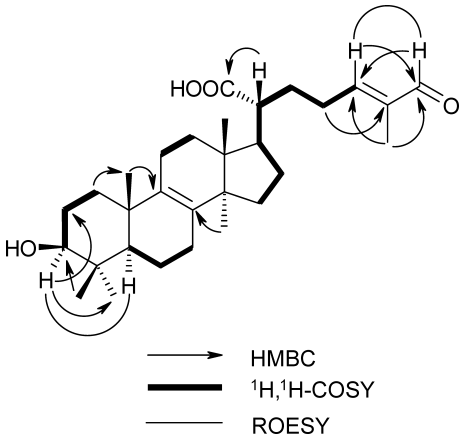

Fig. 2 Key HMBC, ^{1}H, ^{1}H-COSY and ROESY correlations of **1**.

Compound **1** exhibited to possess cytotoxic activities against tumor cell lines, HL60, A549, SGC-7901 and Bel-7402, with IC_{50} values, 11.2, 20.9, 22.6 and 25.0 μg/ml, respectively. It is noted that compound **1** possessing α,β-unsaturated aldehyde group at the side chain of lanostanoids from higher fungi is rather rare.

Acknowledgements This project was supported by the National Natural Science Foundation of China (30470027 and 30225048) and Natural Science Foundation of Yunnan Province (2000C0001P).

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