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NOTE

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A New Cadinane Sesquiterpene with Significant Anti-HIV-1 Activity from the Cultures of the Basidiomycete *Tyromyces chioneus*

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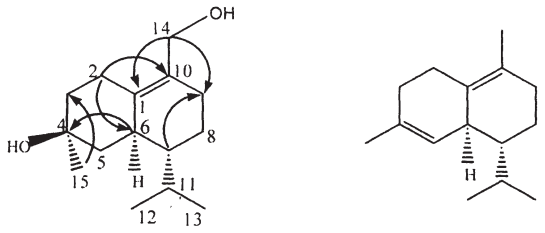
Abstract A new cadinane sesquiterpene (4β,14-dihydroxy-6α,7βH-1(10)-cadinene, **1**) was isolated from the cultures of the basidiomycete, *Tyromyces chioneus*. Its structure was established on the basis of spectral measurements (MS, IR, 1D and 2D NMR experiments). **1** showed significant anti-HIV-1 activity with EC_{50}=3.0 μg/ml (SI=25.4).

Keywords *Tyromyces chioneus*, culture broth, cadinane sesquiterpene, anti-HIV-1

Introduction

Yunnan Province, southwest of China, is one of the areas with the richest and most diverse bioresources in the world. Among these bioresources, fungi produce a broad variety of secondary metabolites. *Tyromyces chioneus* belonging to order Polyporales of the family Polyporaceae is an inedible fungi and mainly occurs on wood as a substrate. So far, little work has been done on the chemical constituents of *T. chioneus*. As one part of our search for naturally occurring bioactive metabolites from the higher fungi in Yunnan province [1~6], a new cadinane sesquiterpene (**1**) was isolated from the cultures of the basidiomycete *T. chioneus*. In this paper, we describe the isolation and structure elucidation of **1** as well as its anti-HIV-1 activity.



4,3,14-dihydroxy-6α,7,βH-1(10)-cadinene(**1**) 1(10),4-cadinadiene(**2**)

Fig. 1 Structures of **1** and **2**, and key HMBC correlations of **1**.

Materials and Methods

General

Optical rotation was measured on an Horiba SEPA-300 polarimeter. IR spectra were obtained on a Bruker Tensor 27 instrument as KBr pellets. NMR spectra were recorded on Bruker AV-400 and Bruker DRX-500 spectrometers in CD_3OD and $CDCl_3$ solvents with TMS as an internal standard. EI-MS was recorded with a VG Autospec-3000 spectrometer. 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.

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Fungal Material

The basidiomycete *T. chioneus* was collected at Ailao Mountain of Yunnan Province, China, in July 2003 and identified by Prof. Mu Zang, Kunming Institute of Botany, Chinese Academy of Sciences (CAS). The voucher specimen was deposited at the Herbarium of Kunming Institute of Botany, CAS.

Fermentation and Isolation

The culture medium consisted of potato (peeled) 200 g, glucose 20 g, KH_2PO_4 3 g, $MgSO_4 \cdot 7H_2O$ 1.5 g, citric acid 0.1 g and thiamine hydrochloride 10 mg in 1 liter of deionized water. Reagent bottles were used as shaker flasks (size: 500 ml; volume of media: 300 ml). The pH was adjusted to 6.5 before autoclaving. Fermentation was carried out on a shaker at 22°C and 150 rpm for 10 days.

The whole culture broth of *T. chioneus* (12 liters) was filtered and then extracted twice with EtOAc. The organic layer was concentrated *in vacuo* to give an oily residue (1.3 g) that was applied to a silica gel column, which was eluted stepwise with a $CHCl_3$ - MeOH solvent system. Fr. III (19 mg), eluted with $CHCl_3$ - MeOH (100 : 1, v/v), was further purified by repeated chromatography on a Sephadex LH-20 column eluted with $CHCl_3$ - MeOH (1 : 1, v/v) to yield **1** (8.5 mg).

Physico-chemical Properties

4β,14-dihydroxy-6α,7β*H*-1(10)-cadinene (**1**). Colorless Oil; $[\alpha]_D^{29.8}$ −20.3 (*c* 0.8, MeOH); IR ν_{max} (KBr) cm^{-1}: 3440, 2959, 2927, 2870, 1630, 1063; HRESI-MS (+), *m/z* 261.1834 $[M+Na]^+$ (calcd. for $C_{15}H_{26}O_2Na$, 261.1830); EI-MS (70 eV) *m/z* (rel. int.): 220 (12) $[M-H_2O]^+$, 202 (13), 189 (13), 177 (100), 159 (45), 147 (27), 133 (25), 119 (40); ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (CD_3OD and $CDCl_3$): see Table 1.

Reagents and Cell Culture

AZT (=3′-azido-3′-deoxythymidine) and MTT (=3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were purchased from *Sigma*. Cells were donated by the Medical Research Council (MRC), AIDS Reagent Project, UK, and grown in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (Gibro), 2 mM L-glutamine, 10 mM HEPES (=4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid), 50 μM 2-mercaptoethanol, 100,000 IU/ml penicillin, and 100 μg/ml streptomycin sulfate. All cells and virus were stored and resuscitated by common methods.

Cytotoxicity of Compound 1 on C8166 Cells

C8166 was one of the host cells for HIV-1. On a microtier plate, 100 μl of 4×10^5/ml cells were seeded. Then 100 μl of various concentrations of **1** was added and the cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 for 72 hours. The cellular toxicity was assessed by an MTT colorimetric assay, the plates were read with a *Bio-Tek ELx* 800 ELISA reader at 595/630 nm, and the 50% inhibitory concentration (IC_{50}) was calculated.

Cytopathic-Effect (CPE) Inhibition Assay of 1

In the presence of 100 μl of various concentrations of **1**, C8166 cells (4×10^5/ml) were infected with HIV-1_{IIIB} at a multiplicity of infection (M.O.I) of 0.06. The final volume was 200 μl. The plates were incubated in a humidified incubator at 37°C and 5% CO_2. AZT (Sigma Chemical Co.) was used for drug control. After 3 days of culture, the cytopathic effect was measured by counting the number of syncytia (multinucleated giant cells) in each well under an inverted microscope, and the 50% effective concentration (EC_{50}) was calculated.

Results and Discussion

1 was obtained as an oil. Based on positive ion HRESI-MS ($[M+Na]^+$ *m/z* 261.1834, calcd. for 261.1830) and NMR spectra data, the molecular formula was determined to be $C_{15}H_{26}O_2$, corresponding to 3 degrees of unsaturation. The IR spectrum indicated the presence of hydroxyl group at 3440 cm^{-1} and C=C double bond at 1630 cm^{-1}. The ^{1}H-NMR spectrum (Table 1) exhibited one methylene proton bearing a hydroxyl group [δ_H 3.98 (1H, d, J=11.6 Hz), 4.11 (1H, d, J=11.6 Hz) and three methyl signals due to one tertiary methyl group [δ_H 1.31 (3H, s)] and two secondary methyl groups [δ_H 0.97 (3H, d, J=6.8 Hz), 0.81 (3H, d, J=6.8 Hz)].

The ^{13}C-NMR and DEPT spectra (Table 1, in CD_3OD) displayed 15 carbon resonances, including three methine carbons (δ_C 47.7, 39.7, 28.7), six methylene carbons (δ_C 62.4, 48.3, 41.6, 28.7, 27.4, 22.4), two olefinic carbons (δ_C 137.0, 130.3), one quaternary carbon (δ_C 71.3) and three methyl carbons (δ_C 25.6, 22.1, 17.1). These observations, in combination with the molecular formula, indicated the presence of two OH groups and two rings. The similarity of ^{13}C-NMR data of partial structure for **1** [C-1 (δ_C 129.5), C-2 (δ_C 26.6), C-6 (δ_C 38.5), C-7 (δ_C 46.0), C-8 (δ_C 21.7), C-12 (δ_C 16.7), and C-13 (δ_C 21.3)] with those of 1(10),4-cadinadiene (**2**) [7] [C-1 (δ_C 129.9), C-2 (δ_C 26.7), C-6 (δ_C 39.5), C-7 (δ_C 45.4), C-8 (δ_C 21.2), C-12 (δ_C 15.6), and C-13 (δ_C 21.7)] suggested that **1** might be a cadinane-type sesquiterpene. This hypothesis was confirmed by careful analysis of ^{1}H-^{1}H

Table 1 NMR spectral data for **1** and **2**. (δ in ppm, J in Hz)

Position	**1** (CD_3OD)		**1** ($CDCl_3$)	**2** ($CDCl_3$)
	δ (H)	δ (C)	δ (C)	δ (C)
1	—	130.3 (s)	129.5	129.9
2	2.78 (1H, ddd, 14.0, 3.2, 2.4) 1.78 (1H, m)	27.4 (t)	26.6	26.7
3	1.41 (1H, m), 1.71 (1H, m)	41.6 (t)	41.0	31.9
4	—	71.3 (s)	70.9	133.9
5	1.94 (1H, m), 1.12 (1H, m)	48.3 (t)	47.5	124.6
6	1.94 (1H, m)	39.7 (d)	38.5	39.5
7	1.03 (1H, m)	47.7 (d)	46.0	45.4
8	1.65 (1H, m), 1.19 (1H, m)	22.4 (t)	21.7	21.2
9	2.03 (1H, m), 2.19 (1H, br d, 16.9)	28.7 (t)	27.9	32.3
10	—	137.0 (s)	136.3	124.1
11	1.84 (1H, m)	28.7 (d)	27.5	26.7
12	0.81 (3H, d, 6.8)	17.1 (q)	16.7	15.6
13	0.97 (3H, d, 6.8)	22.1 (q)	21.3	21.7
14	3.98 (1H, d, 11.6), 4.11 (1H, d, 11.6)	62.4 (t)	62.7	18.4
15	1.31 (3H, s)	25.6 (q)	25.6	23.5

COSY, HSQC, and HMBC spectrum.

Analysis of ^{1}H-^{1}H COSY and HSQC spectrum led to the identification of the partial structures $CH_2(2)$–$CH_2(3)$, $CH_2(8)$–$CH_2(9)$ and $CH_3(12)$–$CH(11)$–$CH_3(13)$. The HMBC correlations of Me-15 (δ_H 1.31) with C-3, C-4 and C-5, and H-14 with C-1, C-9 and C-10 indicated that C-3, C-5, Me-15 and C-1, C-9, C-14 were connected to the quaternary carbons C-4 and C-10, respectively. Additional HMBC experiments showed the critical correlations from H-2 to C-1, C-3, C-6 and C-10, from H-7 to C-6 and C-9, from H-6 to C-4, and from both Me-12 (δ_H 0.81) and Me-13 (δ_H 0.97) to C-7 and C-11. The above analysis determined the planar structure of **1**. The relative configurations of **1** were deduced from analysis of the ROESY spectrum, which shows cross-peaks of H-6 with Me-12 and Me-15, H-8α (δ_H 1.65) with Me-13, and H-7 with H-9β (δ_H 2.19) indicating that H-6 and Me-15 were α-oriented, while H-7 was β-oriented. In the light of the evidence mentioned above, the structure of **1** was determined as 4β,14-dihydroxy-6α,7β*H*-1(10)-cadinene.

1 showed cytotoxicity against C8166 cells (IC_{50}=76.9 μg/ml) and significant anti-HIV-1 activity with EC_{50}=3.0 μg/ml and SI (selectivity index) 25.4.

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