Chemistry of Natural Compounds, Vol. 43, No. 5, 2007

BIOACTIVE ERGOSTEROL DERIVATIVES ISOLATED FROM THE FUNGUS *Lactarius hatsudake*

An-Ling Zhang, La-Ping Liu, Min Wang, and Jin-Ming Gao*

UDC 547.918

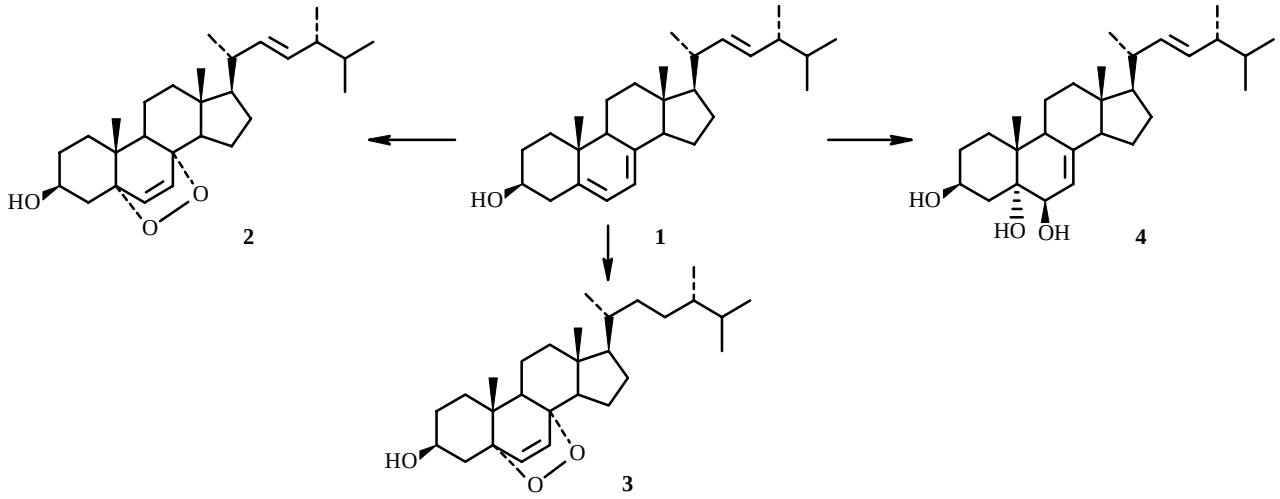
The family Russulaceae is one of the largest in the subdivision basidiomycotina in Whitthaker's Kingdom of Fungi and comprises hundreds of species [1]. While secondary metabolites occurring in the fruiting bodies of European *Lactarius* species have been well investigated [2], the genus mushrooms growing in China have received less attention, notwithstanding the larger number of existing species [3, 4].

The fruiting bodies of a basidiomycete fungus *Lactarius hatsudake* have long been used as antitumor and antiviral agents in Chinese folk medicine; the chloroform extract of the fruiting bodies of both milk mushrooms was found to show significant anti-HIV activity. In the course of our continuing search for fungi-derived biologically active metabolites from the Chinese medicinal fungi, bioactivity-directed fractionation of the extract of this species has resulted in the isolation of four ergosterol-type congeners **1–4**. Among them, compounds **2–3** were active against HIV replication in C8166 cells *in vitro*. The present paper describes for the first time the isolation and structural elucidation of these sterols **1–4** and their inhibition effects against HIV *in vitro*.

Compound **1,** yield 0.015%, colorless crystal, mp 152.6–154°C, ergosterol [5].

Compound **2**, yield 0.012%, colorless crystal, mp183°C, $[\alpha]_D$ –35° (*c* 0.8, $CHCl_3$), ergosterol peroxide [5–8].

Compound **3**, yield 0.0056%, white amorphous powder, mp 142.6–144°C, identified as 5α,8α-*epi*-dioxy-(24S)-ergosta-6-en-3β-ol by comparison of physicochemical data and spectral data with those in the literature [3].



Compound **4**, yield 0.0081%, colorless crystal, mp 224–226°C, $[\alpha]_D$ –23° (*c* 0.22, MeOH), which was characterized as (22*E*,24*R*)-ergosta-7,22-dien-3β,5α,6β-triol by comparing physicochemical data and spectral data with literature values [3, 5, 7]. These four biogenetically related ergostane-type sterols **1–4** isolated from *Lactarius hatsudake* were tested for *in vitro* inhibitory effects against HIV replication in C8166 cells [9]. Both sterol peroxides **2** and **3** showed *in vitro* weak to moderate anti-HIV activity, with an IC_{50} value of 1.26 and 0.05 μg/mL, and CC_{50} value of 0.63 and 0.58 μg/mL, respectively, with respect to that of AZT as positive control drug. The therapeutic index (TI) values were 1.90 for **2** and 12.30 for **3**. In general,

Natural Products Research Centre, College of Sciences, Northwest A & F University, Yangling, Shaanxi 712100, PR China, e-mail: jinminggaocn@yahoo.com.cn. Published in Khimiya Prirodnykh Soedinenii, No. 5, pp. 525-526, September-October, 2007. Original article submitted July 26, 2006.

0009-3130/07/4305-0637

a TI>5.0 is considered remarkable. 23,24-Dihydroergosterol peroxides **3** therefore displayed moderate anti-HIV activity. It was concluded from the present study that the peroxide group and the saturation of the side chain in the molecule is essential for anti-HIV activity. This is the first report of the occurrence of these sterols in this fungus and the anti-HIV activity of this class of ergosterol analogues with the peroxide moiety.

General Experimental Procedures. Melting points were obtained on an XRC-1 apparatus and uncorrected. Optical rotations were measured on a Horiba SEPA-300 polarimeter. NMR spectra were recorded on Bruker AM-400 and Bruker DRX-500 instruments with TMS as an internal standard. HREIMS and FAB-MS were recorded on a VG Auto Spec-3000 mass spectrometer. IR spectra were obtained in KBr pellets with a Bio-Rad FTS-135 infrared spectrophotometer.

Column chromatography was performed over silica gel (200–300 mesh). TLC was carried out on plates precoated with silica gel F_{254} (Qingdao Marine Chemical Ltd., People's Republic of China).

Fungal Materials. The fresh fruiting bodies of *Lactarius hatsudake* were collected from Yunnan Province in August 2002 and identified by Ms. X. H. Wang, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, People's Republic of China. A voucher specimen is deposited in the Herbarium of the Kunming Institute of Botany.

Extraction and Isolation. The dried fruiting bodies (568g) of *L. hatsudake* were extracted successively three times with $CHCl_3$/MeOH (1:1) at room temperature. The combined extracts were concentrated under reduced pressure to give a brown extract, which was partitioned between H_2O and $CHCl_3$. The resultant $CHCl_3$ extract (36g) after evaporation showed noticeable anti-HIV activity. The biologically active $CHCl_3$ soluble fraction of *L. hatsudake* was subjected to column chromatography on silica gel eluting with a solvent mixture of petroleum ether/acetone (50:1–1:1) to give 12 fractions. Fraction 2 after crystallization from *n*-hexane furnished **1** (86 mg). Fraction 4 after crystallization from *n*-hexane furnished **2** (68 mg)**.** Fraction 6 subjected to silica gel column chromatography (petroleum ether/EtOAc 7:3) afforded **3** (32 mg). Recrystallization of fr. 9 from petroleum ether/acetone provided **4** (46 mg).

ACKNOWLEDGMENT

This work was supported from the National Natural Science Foundation of China (No. 30370019, 30670221, 30770237) and the Program for New Century Excellent Talents in University (NCET-05-0852).

REFERENCES

1. R. H. Witthaker, *Science*, **163**, 150 (1969).
2. G. Vidari, Z. L. Che, and L. Garlaschell, *Tetrahedron Lett*., **39**, 6073 (1998).
3. J. M. Gao, Z. J. Dong, and J. K. Liu, *Lipids*, **36**, 175 (2001).
4. J. M. Gao, *Curr. Org. Chem.*, **10**, 849 (2006).
5. J.-M. Gao, J. Shen, A.-L. Zhang, W. Zhu, X. Zhang, and J.-K. Liu, *Chin. J. Org. Chem.*, **23**, 853 (2003).
6. D.-H. Kim, S.-J. Jung, I.-S. Chung, Y.-H. Lee, D.-K. Kim, S.-H. Kim, B.-M. Kwon, T.-S. Jeong, M.-H. Park, N.-S. Seoung, and N.-I. Baek, *Arch. Pharm. Res.*, **28**, 541 (2005).
7. J.-M. Gao, L. Hu, Z.-J. Dong, and J.-K. Liu, *Steroid*, **66**, 771 (2001).
8. H. Kawagishi, R. Katsumi, T. Sazawa, T. Mizuno, T. Hagiwara, and T. Nakamura, *Phytochemistry*, **27**, 2777 (1988).
9. Y. T. Zheng, K. L. Ben, and S. W. Jin, *Acta Pharm. Sin.*, **20**, 239 (1999).