School of Traditional Chinese Materia Medica[1]; Shenyang Pharmaceutical University, College of Chemical Engineering[2]; Qingdao University of Science & Technology, Qingdao, Department of instrumental analysis[3], Shenyang Pharmaceutical University, Shenyang P.R. China

## A new cyclotetrapeptide from marine fungus *Trichoderma reesei*

YI SUN[1], LI TIAN[2], YONG-FU HUANG[1], YI SHA[3], YUE-HU PEI[1]

*Received March 25, 2006, accepted April 10, 2006*

*Prof. Yue-Hu Pei, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, P.R. China*
*peiyueh@vip.163.com*

*Pharmazie 61: 809–810 (2006)*

A new cyclotetrapeptide, trichoderide A, was isolated from the marine fungus *Trichoderma reesei*. The structure was identified by spectral methods, and the stereochemical assignments were made by chiral HPLC of the hydrolyzed compound. Trichoderide A showed moderate cytotoxity against human A375-S2 melanoma cell line.

Marine microorganisms have become an especially productive source of structurally unique cancer cell growth inhibitors and *in vivo* active anticancer drugs (Schwartsmann et al. 2003; Mayer et al. 2003). Marine-derived fungi also represent great potential for the discovery of new pharmacologically cytotoxic metablites (Bugni et al. 2004). As a result of our previous research directed at discovering new cytotoxic compounds, trichoderide A (**1**) was isolated for structural elucidation and characterization of biological activities.

Compound **1**, white solid, had the molecular formula $C_{22}H_{35}N_5O_7$ deriving from its HR-FAB MS at m/z 482.2519 ($[M + H]^+$, calcd for 482.2536). General analyses of $^1H$, $^{13}C$ and HMQC signals showed characteristic peptide resonances including six carbonyls (δ 174.26, 172.67, 170.08, 168.61, 166.55, 166.45), four NH groups (overlapped from δ 9.25 to 9.32), four α-CH group (δ 59.01, 57.74, 53.23, 50.73), three $CH_3$ groups (δ 19.67, 15.26, 15.26) and a number of $CH_2$ groups. In the $^1H$ NMR spectrum of **1**, four proton signals δ 4.26 (1 H, m, H-1), 4.14 (1 H, br t, J = 8.2 Hz, H-7), 4.20 (1 H, dd, J = 8.6, 8.3 Hz, H-9) and 4.22 (1 H, dt, J = 11.0, 2.5 Hz, H-14) were attributable to the α-protons of four amino acid units. The structure of **1** including the sequence of the amino acids could be established on the basis of connectivity observed in the HMBC and NOESY experiments (Fig.). In the HMBC, the amide carbonyl carbons δ 166.45 (C-3), 170.08 (C-8), 166.55 (C-13) and 168.61 (C-22) each showed two-bond correlations with the Hα protons (δ 4.26, 4.14, 4.20 and 4.22), and the three-bond correlations with the Hβ protons δ 1.46 (3 H, d, J = 7.2 Hz, H-2), 2.21and 2.18 (2 H, m, H-6), 2.14 (1 H, m, H-10) and 2.01 (1 H, m, H-15a) respectively, which indicated four amino acid units: Ala, Pro, Val and Orn. In the NOESY, the sequential information was partially deduced to include Ala-Orn between the Hα protons at δ 4.26 and 4.24. To confirm

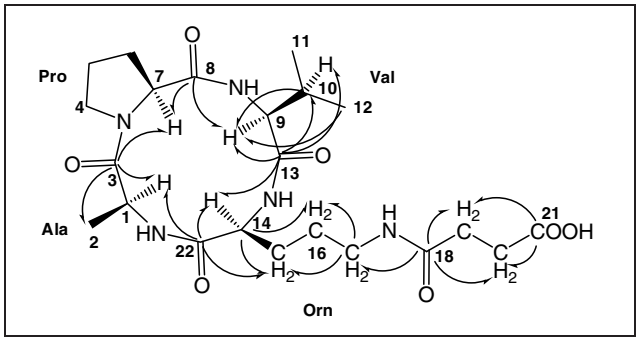


Fig.: The HMBC correlations of trichoderide A

the whole sequence of the four amino acids, the parameters of JCH couplings were changed from 4, 8 to 12 Hz for HMBC experiment. Thus, it could be observed from different JCH couplings that each amide carbonyl carbon was coupled with the Hα proton of the neighboring amino acid, which the sequence was verified as Ala-Pro-Val-Orn. In addition, the HMBC correlations from δ 172.67 (C-18) to δ 2.79 (2 H, t, J = 6.3 Hz, H-19), 2.87 (2 H, t, J = 6.3 Hz, H-20) and 3.58 (1 H, m, H-17b), as well as δ 174.26 (C-21) to δ 2.79 and 2.87 revealed that the dehydroxyl succinoyl unit was associated with ornithine residue. Therefore, the structure of **1** was determined as shown in the Fig. By acid hydrolysis and the chiral HPLC analysis of **1**, all four amino acid units were shown to have L-configuration.

The cytotoxicity of trichoderide A was evaluated against A375-S2 melanoma cell line using MTT assay, which showed moderate effect with a value of 18.5 μg/ml.

## Experimental

### *1. Apparatus*

The NMR spectra were recorded on a Bruker-ARX-600 spectrometer ($^1H$ at 600 MHz and $^{13}C$ at 150 MHz). HR-FAB MS spectra were taken with a Q-trap LC-MS-MS system using Turbo ionspray source. The optical rotation was measured on Perkin-Elmer 241 polarimeter.

### *2. Fungal material*

The fungus *Trichoderma reesei* which was obtained from sea mud in the tideland in Lianyungang, Jiangsu Province, China, in July 2003, was identified by Prof. Li Tian. A voucher specimen (YZ48–08) is deposited in the key laboratory of Marine Biology in the State Oceanic Administration, China.

### *3. Cultivation and extraction*

The culture medium of the strain contained starch liquor 200 ml, peptone 2 g, yeast 1 g, glucose 15 g, NaCl 25 g, $MgCl_2 \cdot 6H_2O$ 1.5 g, KCl 0.2 g, $FePO_4$ 0.01 g, $H_2O$ 1000 ml. It was cultured at 15 °C on a rotary shaker for 10 days. On the tenth day, the fermentation broth, including cells, was harvested and then was centrifugated to separate mycelial mass from aqueous layer. The mycelial mass was exhaustively extracted with acetone and the aqueous layer was extracted with EtOAc. By TLC method, both fractions were mixed to get the crude extract (15 g).

### *4. Isolation and characterization of 1*

The extract was chromatograghed on a silica gel column using a gradient of acetone in chloroform, the sixth fraction (10 : 1) was further subjected by preparative HPLC (YMC C18, 22 × 250 mm, 10 μm; 100% $H_2O$) to afford **1** (5.1 mg). Compound **1**: $[\alpha]_D^{20}$ − 0.65 (c 0.02, MeOH); $^1H$ NMR (600 MHz, $C_5D_5N$)/$^{13}C$ NMR (150 MHz, $C_5D_5N$): 4.26 (1 H, m, H-1)/53.23, 1.46 (3 H, d, J = 7.2 Hz, H-2)/19.67, 166.45 (C-3), 3.54 (1 H, dt, J = 10.5, 8.5 Hz, H-4a)/44.87, 3.44 (1 H, dt, J = 10.5, 8.1 Hz, H-4b), 1.66 (1 H, m, H-5a)/22.38, 1.64 (1 H, m, H-5b), 2.21 (1 H, m, H-6a)/27.92, 2.18 (1 H, m, H-6b), 4.14 (1 H, br t, J = 8.2 Hz, H-7)/59.01, 170.08 (C-8), 4.20 (1 H, dd, J = 8.6, 8.3 Hz, H-9)/50.73, 2.14 (1 H, m, H-10)/28.85, 1.60 (6 H, d, J = 7.2 Hz, H-11,12)/15.26, 166.55 (C-13), 4.22 (1 H, dt, J = 11.0, 2.5 Hz, H-14)/57.74, 2.32 (1 H, m, H-15a)/34.82, 2.01 (1 H, m, H-15b), 1.62 (2 H, m, H-16)/21.69, 3.64 (1 H, m, H-17a)/45.02, 3.58 (1 H,

m, H-17b), 172.67 (C-18), 2.79 (2 H, t, J = 6.3 Hz, H-19)/28.97, 2.87 (2 H, t, J = 6.3 Hz, H-20)/29.17, 174.26 (C-21), 168.61 (C-22); for selected HMBC correlations, see Fig.

### *5. Chiral amino acid analysis*

Trichoderide A (**1**, 2.0 mg) was hydrolyzed in 1 mL of 6 N HCl at 110 °C for 20 h, and then dried under vacuum. The hydrolysate was eluted from a C18 column (Dikma) using MeOH/$H_2O$ (10 : 90). The elute was dried under vacuum and reconstituted with 100 μL of $H_2O$ prior to analysis [CHIRAL PAK CR(+), 4.61 × 50 mm; detection: UV 200 nm; injected amount: 5 nmol; mobile phase: pH 1.5 $HClO_4$ in $H_2O$, flow rate 0.4 ml/min]. The hydrolysate was chromatographed alone and co-injected with standards to confirm assignments. Retention times (min) of the four amino acids of **1** were as follows: L-Ala (4.68), L-Val (7.06), L-Pro (3.82), and L-Orn (5.25), which were identical with the authentic amino acids. The standard retention times (min) of the corresponding D-amino acids were D-Ala (3.54), D-Val (5.85), D-Pro (3.08), and D-Orn (4.30).

Acknowledgements: This work was supported by 863 Hi-Tech Research and Development Program of China (Grant No. 2001AA624020) and the Fund for the 973-project from Ministry of Science and Technology (Grant No. G1998051100), China.

**References**

Bugni TS, Ireland CM (2004) Marine-derived fungi: a chemically and biologically diverse group of microorganisms Nat Prod Rep 21: 143–163.
Mayer AMS, Gustafson KR (2003) Marine pharmacology in 2000: Antitumor and cytotoxic compounds. Int J Cancer 105: 291–299.
Schwartsmann G, da Rocha AB, Mattei J, Lopes R (2003) Marine-derived anticancer agents in clinical trials Expert opin. Investig Drugs 12: 1367–1383.

Research Department of Natural Medicine[1], China-Japan Research Institute of Medical and Pharmaceutical Sciences[2], Shenyang Pharmaceutical University, Shenyang, P.R. China

## Two new triterpenoids from the carpophore of *Xanthoceras sorbifolia* Bunge

Wei Li[1], Xian Li[1], Jia Yang[2], Lin-Hao Li[2], Ning Li[1], Da-Li Meng[1]

*Received March 17, 2006, accepted April 27, 2006*

*Prof. Xian Li, Research Department of Natural Medicine, Shenyang Pharmaceutical University, Box 49, Shenyang, 110016, P.R. China*
*proflixian@163.com*

*Pharmazie 61: 810–811 (2006)*

Two new triterpenoids 3-*O*-β-d-glucopyranosyl (1→6)-β-d-glucopyranosyl, 28-*O*-β-d-glucopyranosyl (1→6) [α-l-rhamnopyranosyl (1→2)]-β-d-glucopyranosyl, 16-deoxybarringtogenol C (**1**) and 22-*O*-acetyl-21-*O*-(4′-*O*-angeloyl)-β-d-fucopyranosyl theasapogenol B (**2**), were isolated from the dried carpophore of *Xanthoceras sorbifolia* Bunge (Sapindaceae). **1** and **2** were found to have activity of inhibiting the proliferation of two human tumor cell lines.

*Xanthoceras sorbifolia* Bunge (Sapindaceae) is a shrub mainly growing in Inner Mongolia, China. Its bark and fruits are used to treat rheumatism and enuresis of children as a folk medicine. Previous phytochemical studies on this plant revealed the presence of saponins (Chen et al. 1985a, 1985b), flavonoids (Ma et al. 2000). We investigated the chemical constituents of the carpophore of *X. sorbifolia* Bunge and report here the isolation and identification of two new triterpenoids (Fig.), as well as their inhibiting activities against human tumor cell lines.

Compound **1**, white powder from MeOH, was deduced to have the molecular formula $C_{60}H_{100}O_{28}$, on the basis of its ESIMS (m/z 1291 $[M + Na]^+$) and NMR data and was considered to be a triterpen glycoside due to a positive Liebermann-Burchard and Molish reactions. Furthermore, the fragmentation patterns indicated the loss of sugar moieties (m/z 1291 $[M + Na]^+$, 1145 $[M + Na\text{-rha}]^+$, 1129 $[M + Na\text{-}162]^+$, 983 $[M + Na\text{-}146\text{-}162]^+$, 967 $[M + Na\text{-}146\text{-}2 \times 162]^+$, 821 $[M + Na\text{-}146\text{-}2 \times 162]^+$, 659 $[M + Na\text{-}146\text{-}3 \times 162]^+$). On hydrolysis, two kinds of monosaccharide units were obtained and identified as glucose and

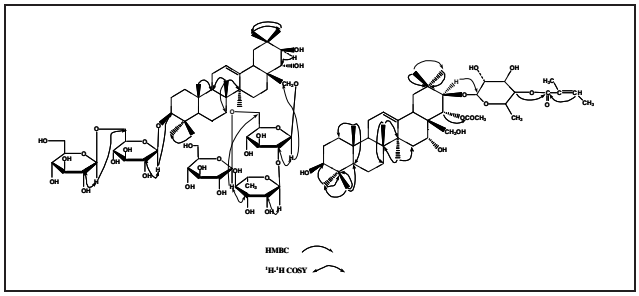


Fig.: Important correlations of compound **1** and **2** = 1