文章编号:1001-6880(2014)10-1597-05

苦竹内生真菌 *Fusarium* sp. S13 的化学成分研究

王 勇[1],况 燚[1,2],杨胜祥[1,2*],刘 力[1*]

[1]浙江省林业生物质化学利用重点实验室;[2] 亚热带森林培育国家重点实验室,浙江临安 311300

摘 要:从苦竹内生真菌 *Fusarium* sp. S13 的发酵液提取物中分离得到 8 个化合物,通过波谱技术分别鉴定为:cerevisterol (**1**),(22*E*,24*S*)-24-methyl-5α-cholesta-7,22-diene-3β,5α,6β,9α- tetraol (**2**),ergosterol peroxide (**3**),3β,5α,9α-trihydroxy-6β-methoxyergosta-7,22-dien (**4**),ergosta-7,22-dien-6β-methoxy-3β,5α-diol (**5**),ergosta-4,6,8 (**14**),22-tetraen-3-one (**6**),25-hydroxy-ergosta-4,6,8 (**14**),22-tetraen-3-one (**7**),ergosta-7,22-dien-3β,6β-diol (**8**)。海虾致死实验结果显示化合物 **1-8** 均显示出不同程度的毒性。

关键词:苦竹;内生真菌;化学成分;细胞毒

中图分类号:R284.2 **文献标识码**:A

Chemical Constituents Produced by *Fusarium* sp. S13 Isolated from *Pleioblastus amarus*

WANG Yong[1],KUANG Yi[1,2],YANG Sheng-xiang[1,2*],LIU Li[1*]

[1]*Zhejiang Provincial Key Laboratory of Chemical Utilization of Forestry Biomass*;[2]*The Nurturing Station for the State Key Laboratory of Subtropical Silviculture,Zhejiang A & F University,Lin'an,Zhejiang Province 311300,China*

Abstract:8 compounds were isolateded from the fermented broth extract of the endophytic fungus *Fusarium* sp. S13 inhibited in the *Pleioblastus amarus*. Their structures were identified as cerevisterol (**1**),(22*E*,24*S*)-24-methyl-5α-cholesta-7,22-diene-3β,5α,6β,9α-tetraol (**2**),ergosterol peroxide (**3**),3β,5α,9α-trihydroxy-6β-methoxyergosta-7,22-dien (**4**),ergosta-7,22-dien-6β-methoxy-3β,5α-diol (**5**),ergosta-4,6,8 (**14**),22-tetraen-3-one (**6**),25-hydroxy-ergosta-4,6,8 (**14**),22-tetraen-3-one (**7**),and ergosta-7,22-dien-3β,6β-diol (**8**). The 8 steroids were found to exert some toxic effects on brine shrimp larvae.

Key words:*Pleioblastus amarus*;endophytic fungus;chemical constituents;brine shrimp;cytotoxicity

Endophytic fungi are microorganisms that live in the inter- and intracellular spaces of the tissues of apparently healthy host plants and do so in a variety of relationships, ranging from symbiotic to pathogenic. Recently statistical analysis showed that 51% of the biologically active metabolites obtained from endophytes are previously unknown, compared with only 38% of novel substances from soil microflora. Endophytes have been recognized as important sources of a variety of structurally novel active secondary metabolites with anticancer, antimicrobial and other biological activities[1]. As a result, the study of fungal endophytes is currently considered a reasonable approach to the discovery of novel, bioactive natural products [2].

Pleioblastus amarus Keng f. (Gramineae) occurs widely in south China. The shoots of the plant are used in Chinese herbal medicine as antipyretic and diuretic agent. Previous chemical work on the plant resulted in the isolation of several flavonoids and other compounds from the plant [3,4]. However, little is known about secondary metabolites of endophytes harbored inside the healthy tissues of *P. amarus*. In the course of our ongoing program devoted to the search for biologically active metabolites from microorganisms inhabiting the plants, we investigated the chemical constituents produced by an endophytic fungus *Fusarium* sp. S13 found inside *P. amarus*.

Received:October 8,2013 Accepted:February 17,2014

Funding Item:National Natural Science Foundation of China (Grant No. 31200262 and 31270619);Open Fund of Zhejiang Provincial Top Key Discipline of Forestry (KF201302);Talent Foundation of Zhejiang A&F University 2012FR041)

* Corresponding author E-mail: shengxiangyang2000@163.com; liuli582003@126.com

Materials and Methods

Instruments

IR spectra: Perkin - Elmer 1600 Series FT-IR; Perkin - Elmer 297 Infrared Spectrophotometer; Beckman DU-640; Shimadzu FT-IR (KBr tablets and film). UV/Vis spectra: Perkin - Elmer-Lambda 15 UV/Vis spectrometer. Optical rotation: Polarimeter (Perkin-Elmer, model 241). ^{1}H NMR spectra: Varian Inova 500 (499.8 MHz). ^{13}C NMR spectra: Varian Inova 500 (125.7 MHz). Chemical shifts were measured relatively to tetramethylsilane (TMS) as internal standard. 2D NMR spectra: H, H COSY spectra (^{1}H, ^{1}H Correlated Spectroscopy), HMBC spectra (Heteronuclear Multiple Bond Connectivity), HSQC spectra (Heteronuclear Multiple Quantum Coherence) and NOESY spectra (Nuclear Overhauser Effect Spectroscopy). Mass spectra: EIMS at 70 eV with Varian MAT 731, Varian 311A, AMD-402, high resolution with perflurokerosene as standard. ESIMS with Quattro Triple Quadruple mass spectrometer Finnigan Mat-Incos 50, ESIMS LCQ (Finnigan, Germany).

Silica gel (Merck) 60-120 mesh for columnchromatography and pre-coated TLC sheets (layer thickness 0.2 mm) and preparative TLC plate (layer thickness 1.25 mm) of silica gel 60 GF_{254} were used. Spots were detected on TLC under UV light or by heating after spraying with 5% H_2SO_4 in methanol.

Fungal material

The endophytic fungal strain was isolated from the fresh leaves of the tree *P. amarus* growing in the campus of Zhejiang A&F University, Linan, Zhejiang province, China. The isolate was identified as *Fusarium* sp. S13 by morphological analysis and was deposited at the Research Centre for Natural Medicinal Chemistry, Zhejiang A&F University.

Cultivation

After growing on PDA medium at 28 °C for 5 days, the fungus was inoculated in liquid medium containing: $CaCl_2$ 0.5 g, KH_2PO_4 0.1 g, KCl 0.05 g, $MgSO_4 \cdot 7H_2O$ 0.1 g, glucose 20.0 g, peptone 15.0 g, 1000 mL H_2O. The pH was adjusted to 6.0 before autoclaving. Fermentation was carried out in 1000 mL flasks each containing 200 mL medium on a rotary shaker at 150 rpm at 28°C for 5 days.

Extraction and Isolation

The culture broth (20 L) of *Fusarium sp.* S13 was filtered to give the mycelium and water phase. The culture filtrate was absorbed onto polymeric resin Amberlite XAD-16 (Rohm & Hass, Paris, France). Salt and high molecular materials were washed out with water, and other absorbed organic materials were eluted with MeOH to yield 8.3 g of dried extract after removing the solvent in vacuum. The dried extract was extracted with petroleum and EtOAC. The EtOAC extract (4.2 g) was fractionated on a silica gel column, followed by separation on Sephadex LH-20 (CH_2Cl_2/MeOH = 6:4 and MeOH), normal and reverse phase column chromatography and preparative TLC to afford compounds **1** (10.1 mg), **2** (6.2 mg), **3** (7.5 mg), **4** (6.9 mg), **5** (8.7 mg), **6** (7.7 mg), **7** (8.0 mg), and **8** (6.7 mg).

Brine shrimp bioassay

The brine shrimp toxicity was assayed by small modified microtiter-plate method using brine shrimp *Artemia salina* as a test organism [5,6]. Briefly, approximately 30 nuclei larvae hatched from eggs of A. salina in 0.2 mL of artificial sea water were incubated with a sample (5 ml in DMSO solution) in a deep-well microtiter plate at room temperature. After 24 h, the dead larvae were determined by counting the number of the dead animals in each well under microscope. To each test row, blind sample was accompanied by adding DMSO. The mortality rate was calculated using the formula: $M = [(A-B-N)/(G-N)] \times 100$.

M = percent of the dead larvae after 24 h; A = number of the dead larvae after 24 h; B = average number of the dead larvae in the blind samples after 24 h; N = number of the dead larvae before starting the test; G = number of selected larvae for test.

Results and Discussion

Identification

Compound 1 Colorless needle crystal. ^{1}H NMR ($CDCl_3$, 500MHz) δ: 4.07 (m, 1H, H-3), 3.62 (d, J = 4.8 Hz, 1H, H-6), 5.35 (dd, J = 4.8, 2.4Hz, 1H, H-7), 0.59 (s, 3H, H-18), 1.09 (s, 3H, H-19), 1.03

(d, J = 6.7 Hz, 3H, H-21), 5.16 (dd, J = 15.4, 8.0 Hz, 1H, H-22), 5.23 (dd, J = 15.4, 8.0 Hz, 1H, H-23), 0.82 (d, J = 6.2 Hz, 3H, H-26), 0.84 (d, J = 6.9 Hz, 3H, H-27), 0.92 (d, J = 6.6 Hz, 3H, H-28); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 32.8 (C-1), 30.4 (C-2), 67.2 (C-3), 39.3 (C-4), 75.9 (C-5), 73.1 (C-6), 117.3 (C-7), 143.2 (C-8), 43.2 (C-9), 37.0 (C-10), 22.0 (C-11), 38.9 (C-12), 43.6 (C-13), 54.7 (C-14), 22.9 (C-15), 28.0 (C-16), 55.9 (C-17), 12.3 (C-18), 18.4 (C-19), 40.4 (C-20), 19.6 (C-21), 131.9 (C-22), 135.3 (C-23), 42.8 (C-24), 33.1 (C-25), 19.9 (C-26), 21.1 (C-27), 17.6 (C-28). Comparing the NMR data with references[7], compound 1 was determined to be cerevisterol.

Compound 2 Colorless needle crystal. ^{1}H NMR (pyridine-d_5, 500 MHz) δ: 0.54 (s, 3H, H_3-18), 0.79 (d, J = 6.6 Hz, 3H, H-26), 0.80 (d, 3H, J = 6.6 Hz, H-27), 0.88 (d, 3H, J = 6.9 Hz, H-28), 0.95 (s, 3H, H-19), 0.97 (d, 3H, J = 6.6 Hz, H-21), 3.56 (m, 1H, H-6), 3.76 (m, 1H, H-3), 5.12 (dd, J = 5.7, 2.2 Hz, 1H, H-7), 5.17 (dd, J = 15.4, 7.7 Hz, 1H, H-22), 5.23 (dd, J = 15.4, 7.7 Hz, 1H, H-7), 6.80 (t, J = 7.1 Hz, 1H, H-23); ^{13}C NMR (pyridine-d_5, 125 MHz): δ 29.1 (C-1), 32.5 (C-2), 67.4 (C-3), 42.0 (C-4), 75.1 (C-5), 73.8 (C-6), 121.4 (C-7), 142.8 (C-8), 78.7 (C-9), 41.3 (C-10), 29.1 (C-11), 36.0 (C-12), 44.1 (C-13), 51.4 (C-14), 23.6 (C-15), 28.3 (C-16), 56.1 (C-17), 12.1 (C-18), 22.5 (C-19), 40.8 (C-20), 21.4 (C-21), 136.4 (C-22), 132.2 (C-23), 43.3 (C-24), 35.5 (C-25), 19.9 (C-26), 20.4 (C-27), 18.3 (C-28). Compound 2 was identified as (22E, 24S)-24-methyl-5α-cholesta-7, 22-diene-3β, 5α, 6β, 9α-tetraol by comparing the NMR data with reference [8].

Compound 3 Colorless needle crystal. ^{1}H NMR ($CDCl_3$, 500MHz) δ: 6.50 (d, J = 8.5 Hz, 1H, H-7), 6.24 (d, J = 8.5 Hz, 1H, H-6), 5.22 (dd, J = 7.6, 15.3 Hz, 1H, H-23), 5.14 (dd, J = 8.3, 15.5 Hz, 1H, H-22), 3.92 (m, 1H, H-3), 1.25 (s, 3H, H-19), 1.00 (d, J = 6.7, 3H, H-21), 0.91 (d, J = 6.9 Hz, 3H, H-28), 0.88 (s, 3H, H-18), 0.83 (d, J = 6.8 Hz, 3H, H-26), 0.82 (d, J = 6.8 Hz, 3H, H-27); ^{13}C NMR ($CDCl_3$, 125MHz) δ: 34.7 (C-1), 30.1 (C-2), 66.4 (C-3), 36.9 (C-4), 82.2 (C-5), 135.4 (C-6), 130.7 (C-7), 79.4 (C-8), 51.1 (C-9), 36.9 (C-10), 23.4 (C-11), 39.4 (C-12), 44.6 (C-13), 51.8 (C-14), 20.6 (C-15), 28.6 (C-16), 56.2 (C-17), 12.9 (C-18), 18.2 (C-19), 39.4 (C-20), 20.9 (C-21), 135.2 (C-22), 132.3 (C-23), 42.8 (C-24), 33.1 (C-25), 20.0 (C-26), 19.6 (C-27), 17.6 (C-28). The above spectral data were agree with the literature valuas of ergosterol peroxide[9].

Compound 4 Colorless solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.40 (t, J = 2.6 Hz, 1H, H-7), 5.28 (dd, J = 15.3, 7.3 Hz, 1H, H-23), 5.18 (dd, J = 15.3, 8.0 Hz, 1H, H-22), 4.06 (m, 1H, H-3), 3.17 (d, J = 5.1 Hz, 1H, H-6), 1.03 (d, J = 6.5 Hz, 3H, H-21), 1.00 (s, 3H, H-19), 0.92 (s, 3H, H-28), 0.84 (d, J = 7.5 Hz, 3H, H-27), 0.83 (d, J = 7.5 Hz, 3H, H-26), 0.60 (s, 3H, H-18), 3.39 (s, 3H, OMe-7); ^{13}C NMR ($CDCl_3$, 125MHz) δ: 32.9 (C-1), 31.1 (C-2), 68.0 (C-3), 39.5 (C-4), 76.5 (C-5), 82.6 (C-6), 115.2 (C-7), 143.8 (C-8), 44.0 (C-9), 37.4 (C-10), 22.3 (C-11), 39.8 (C-12), 44.1 (C-13), 55.1 (C-14), 23.0 (C-15), 28.1 (C-16), 56.2 (C-17), 12.5 (C-18), 18.5 (C-19), 40.5 (C-20), 19.8 (C-21), 132.3 (C-22), 135.6 (C-23), 43.0 (C-24), 33.2 (C-25), 20.1 (C-26), 21.3 (C-27), 17.6 (C-28), 58.4 (-OMe). The NMR data of compound 4 were identical to the 3β, 5α, 9α-trihydroxy-6β-methoxyergosta-7, 22-dien[7].

Compound 5 Colorless needle crystal. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 4.05 (m, 1H, H-3), 3.17 (d, J = 5.1 Hz, 1H, H-6), 5.40 (m, 1H, H-7), 0.95 (s, 3H, H-18), 1.00 (s, 3H, H-19), 1.02 (d, J = 6.6 Hz, 3H, H-21), 5.17 (dd, J = 15.3 Hz, 7.3, 1H, H-22), 5.21 (dd, J = 15.3 Hz, 8.0, 1H, H-23), 0.82 (d, J = 7.1 Hz, 3H, H-26), 0.83 (d, J = 7.4 Hz, 3H, H-27), 0.91 (d, J = 6.8 Hz, 3H, H-28), 3.39 (s, 3H, OMe-6); ^{13}C NMR ($CDCl_3$, 125MHz) δ: 31.0 (C-1), 32.7 (C-2), 67.9 (C-3), 39.8 (C-4), 76.4 (C-5), 82.5 (C-6), 115.1 (C-7), 143.7 (C-8), 43.9 (C-9), 37.3 (C-10), 23.0 (C-11), 39.5 (C-12), 44.0 (C-13), 55.0 (C-14), 22.3 (C-15), 28.0 (C-16), 56.1 (C-17), 12.3 (C-18), 18.3 (C-19), 40.4

(C-20), 21.2 (C-21), 135.5 (C-22), 132.1 (C-23), 43.0 (C-24), 33.2 (C-25), 19.6 (C-26), 19.9 (C-27), 17.8 (C-28), 58.3 (6-O-Me). Compound **5** was identified as ergosta-7, 22-dien-6β-methoxy-3β, 5α-diol by comparison of physicochemical data and spectral data with those in the literature [7].

Compound 6 Colorless needle crystal. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.72 (s, 1H, H-4), 6.01 (d, J = 9.4 Hz, 1H, H-6), 6.60 (d, J = 9.4 Hz, 1H, H-7), 0.95 (s, 3H, H-18), 0.98 (s, 3H, H-19), 1.05 (d, J = 6.6 Hz, 3H, H-21), 5.21 (m, 1H, H-22), 5.22 (m, 1H, H-23), 0.82 (d, J = 7.0 Hz, 3H, H-26), 0.84 (d, J = 6.8 Hz, 3H, H-27), 0.92 (d, J = 6.8 Hz, 3H, H-28); ^{13}C NMR ($CDCl_3$, 125MHz) δ: 34.1 (C-1), 34.3 (C-2), 199.2 (C-3), 123.1 (C-4), 164.2 (C-5), 124.5 (C-6), 133.9 (C-7), 124.6 (C-8), 44.5 (C-9), 36.8 (C-10), 19.0 (C-11), 35.6 (C-12), 44.0 (C-13), 155.9 (C-14), 25.3 (C-15), 27.7 (C-16), 55.8 (C-17), 19.0 (C-18), 16.8 (C-19), 39.2 (C-20), 21.2 (C-21), 135.0 (C-22), 132.5 (C-23), 43.0 (C-24), 33.2 (C-25), 20.0 (C-26), 19.8 (C-27), 17.7 (C-28). These data were identical to those recorded for an authentic specimen of ergosta-4, 6, 8(14), 22-tetraen-3-one [10].

Compound 7 Colorless needle crystal. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.74 (s, 1H, H-4), 6.04 (d, J = 9.7 Hz, 1H, H-6), 6.60 (d, J = 9.7 Hz, 1H, H-7), 0.97 (s, 3H, H-18), 1.00 (s, 3H, H-19), 1.08 (d, J = 6.6 Hz, 3H, H-21), 5.37 (m, 1H, H-22), 5.37 (m, 1H, H-23), 1.15 (s, 3H, H-26), 1.18 (s, 3H, H-27), 1.01 (d, J = 6.8 Hz, 3H, H-28); ^{13}C NMR ($CDCl_3$, 125MHz) δ: 34.1 (C-1), 34.2 (C-2), 199.5 (C-3), 123.5 (C-4), 164.2 (C-5), 124.5 (C-6), 133.9 (C-7), 124.5 (C-8), 44.5 (C-9), 36.8 (C-10), 19.0 (C-11), 35.6 (C-12), 44.0 (C-13), 155.9 (C-14), 25.4 (C-15), 27.7 (C-16), 56.1 (C-17), 19.0 (C-18), 16.3 (C-19), 39.26 (C-20), 21.2 (C-21), 138.2 (C-22), 129.9 (C-23), 49.0 (C-24), 72.5 (C-25), 27.0 (C-26), 26.3 (C-27), 15.5 (C-28). The NMR data of compound 7 were identical to the 25-hydroxy-ergosta-4, 6, 8(14), 22-tetraen-3-one [11].

Compound 8 Colorless powder. ^{1}H NMR ($CDCl_3$, 500MHz) δ: 3.60 (m, 1H, H-3), 5.18 (m, 1H, H-7), 0.81 (s, 3H, H-18), 0.84 (s, 3H, H-19), 0.92 (d, J = 6.8 Hz, 3H, H-21), 5.18 (m, 1H, H-22), 5.20 (dd, J = 15.2, 7.4 Hz, 1H, H-23), 0.82 (d, J = 6.9 Hz, 3H, H-26), 0.83 (d, J = 6.9 Hz, 3H, H-27), 0.95 (d, J = 6.8 Hz, 3H, H-28); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 28.1 (C-1), 31.2 (C-2), 70.9 (C-3), 37.3 (C-4), 70.2 (C-6), 121.9 (C-7), 141.7 (C-8), 43.6 (C-9), 40.4 (C-10), 28.2 (C-11), 35.2 (C-12), 43.4 (C-13), 54.9 (C-14), 22.8 (C-15), 28.0 (C-16), 56.0 (C-17), 12.2 (C-18), 21.1 (C-19), 40.4 (C-20), 21.5 (C-21), 135.6 (C-22), 132.0 (C-23), 42.9 (C-24), 33.8 (C-25), 19.6 (C-26), 19.9 (C-27), 17.7 (C-28). The above spectral data were in accord with the literature valuas of ergosta-7, 22-dien-3β, 6β-diol [12].

Result of brine shrimp bioassay

The growth inhibitory activity of compounds 1-8 was evaluated against brine shrimp (*Artemia salina*). Chaetomugilin A was used as a positive control, a fungal metabolite isolated from an endophyte *Chaetomium globosum* [6]. After incubation for 24 h, compounds **1**, **2**, **4**, and **8** were found to display significant toxicity toward brine shrimp larvae at a concentration of 10 (g/mL, with mortality rates (%) of 69.1%, 78.1%, 73.4%, and 70.4%. metabolite 5 exhibited moderate toxicity, with mortality rate of 61.5%, compounds 3, 6, and 7 showed weak growth inhibitory (43.3%, 52.1%, 55.7%, and 50.1%), while chaetomugilin A showed mortality rate of 78.3% at the same concentration. Compound **2** showed equal toxic activity against brine shrimp with the control chaetomugilin A.

Acknowledgements

This work was supported by the program for National Natural Science Foundation of China (Grant No. 31200262 and 31270619), Open Fund of Zhejiang Provincial Top Key Discipline of Forestry (KF201302), and Talent Foundation of Zhejiang A&F University (2012FR041).

References

1 Strobel GA, Daisy B, Castillo U, *et al*. Natural products from

endophytic microorganisms. *J Nat Prod*, 2004, 67: 257-268.

2 Gunatilaka AAL. Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. *J Nat Prod*, 2006, 69: 509-526.

3 Wang HB, Yao H, Gu WF, *et al*. Chemical constituents from the leaves of *Pleioblastus amarus*. *Chin Tradit Herbal Drugs*, 2004, 35: 739-740.

4 Cheng KJ, Chen J, Liang GL, *et al*. Taxiphyllin: A cyanogenic glucoside with tyrosinase inhibitory activity from the shoots of Pleioblastus amarus. *Nat Prod Res Dev*(天然产物研究与开发), 2005, 17: 733-735.

5 Yang SX, Gao JM, Zhang Q, *et al*. Toxic polyketides produced by Fusarium sp., an endophytic fungus isolated from *Melia azedarach*. *Bioorg Med Chem Lett*, 2011, 21: 1887-1889.

6 Qin JC, Zhang YM, Gao JM, *et al*. Bioactive metabolites produced by *Chaetomium globosum*, an endophytic fungus isolated from *Ginkgo biloba*. *Bioorg Med Chem Lett*, 2009, 19: 1572-1574.

7 Kawagishi H, Katsumi R, Sazawa T, *et al*. Cytotoxic steroids from the mushroom *Agaricus blazei*. *Phytochem*, 1988, 27: 2777-2779.

8 Migliuolo A, Notaro G, Piccialli V, *et al*. New tetrahydroxylated sterols from the marine sponge *Spongia officinalis*. *J Nat Prod*, 1990, 53: 1414-1424.

9 Gao JM, Dong ZJ, Liu JK. The constituents of the Basidiomycetes *Russula cyanoxantha*. *Acta Botanica Yunnanica*, 2000, 22: 85-89.

10 Lee IS, Kim JP, Na MK, *et al*. Cytotoxicity of ergosterol derivatives from the fruiting bodies of *Hygrophorus russula*. *Nat Prod Sci*, 2011, 17: 85-89.

11 Deng ZP, Sun LR, Ji M, *et al*. Steroids from *Bovistella radicata* (Mont.) Pat. *Biochem Syst Ecol*, 2007, 35: 700-703.

12 Madaio A, Piccialli V, Sica D. New polyhydroxysterols from the dictyoceratid sponges Hi*ppospongia communis*, *Spongia officinalis*, *Ircinia variabilis*, and *Spongionella gracilis*. *J Nat Prod*, 1989, 52: 952-961.

(上接第 1714 页)

2 Liao LM(廖立敏), Liu Y(刘月), Li JF(李建凤), *et al*. Study on microwave extraction of total flavonoids of lemon peel using response surface methodology. *J Huazhong Norm Univ*, *Nat Sci*(华中师范大学学报, 自科版), 2012, 46: 569-572.

3 Kumar M, Upreti RK. *In vitro* effect of azadirachin on aerobic bacteria of rat intestine. *Bull Environ Contam Toxicol*, 2003, 70: 1205-1212.

4 Poulose SM, Harris ED, Patil BS. Antiproliferative effects of citrus limonoids against human neuroblastoma and colonic adenocarcinoma cells. *Nutr Cancer*, 2006, 56: 103-112.

5 Luo SZ(罗水中), Pan LH(潘利华), He JJ(何建军), *et al*. Extraction and antibiotics of limonin in citrus seeds. *Acade Period Farm Prod Proc* (农产品加工·学刊), 2006, 10: 105-107.

6 Sawabe A, Moritab M, Kiso T, *et al*. Isolation and characterization of new limonoid glycosides from *Citrus unshiu* peels. *Carbohyd Res*, 1999, 315: 142-147.

7 Ishii T, Ohta H, Nogata Y. Limonoids in seeds of iyo tangor. *Food Sci Technol Res*, 2003, 9: 62-164.

8 Zeng XY(曾祥燕), Zhao LZ(赵良忠). Application of response surface methodology to optimize extraction process for nomilin from orange peel with supercritical carbon dioxide. *Food Sci*(食品科学), 2011, 32(22): 65-69.

9 Li JL(黎继烈), Zhang H(张慧), Zeng CZ(曾超珍), *et al*. Study on ultrasonic extraction technology of limonin from the kumquat. *J Chin Inst Food Sci Technol* (中国食品学报), 2009, 9: 96-101.

10 Wang H(王辉), Xu HX(徐环昕), Kou ZF(寇正福), *et al*. Separation and purification of limonin by adsorption resin from pomelo seed. *Ion Exchange Adsorp*(离子交换与吸附), 2010, 26: 256-263.