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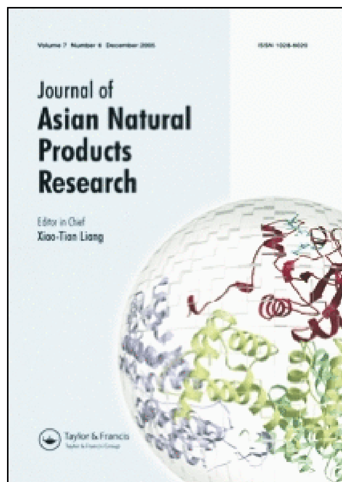

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New furostanol glycosides from the rhizomes of *Dioscorea futschauensis* R. Kunth

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NEW FUROSTANOL GLYCOSIDES FROM THE RHIZOMES OF *DIOSCOREA FUTSCHAUENSIS* R. KUNTH

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Two new furostanol glycosides, 26-*O*-β-D-glucopyranosyl-3β,26-dihydroxy-23(*S*)-methoxyl-25(*R*)-furosta-5,20(22)-dien-3-*O*-α-L-rhamnopyranosyl(1 → 2)-[β-D-glucopyranosyl(1 → 3)]-β-D-glucopyranoside (dioscoreside E, **1**) and 26-*O*-β-D-glucopyranosyl-3β,26-dihydroxy-25(*R*)-furosta-5,20(22)-dien-3-*O*-α-L-rhamnopyranosyl(1 → 2)-[β-D-glucopyranosyl (1 → 3)]-β-D-glucopyranoside (prtotogracillin, **2**), together with 11 known furostanol glycosides were isolated from the rhizomes of *Dioscorea futshauensis* R. Kunth. Their structures were elucidated on the basis of spectroscopic analysis (NMR and FABMS). Their anti-fungal activity against the plant pathogenic fungus *Pyricularia oryzae* and cytotoxic activity on K562 cancer cell line were evaluated *in vitro*.

Keywords: *Dioscorea futschauensis*; Furostanol glycosides; *Pyricularia oryzae*; Anti-fungal; Cytotoxic

INTRODUCTION

Steroidal glycosides are the predominant metabolites of the plants of genus *Dioscorea* traditionally used as a source of material for production of steroidal hormones in the pharmaceutical industry. Recently, more and more structurally novel steroidal glycosides have been isolated from the plants of genus *Dioscorea* [1–4]. They have shown a wide range of bioactivities: cytotoxic, hemolytic, antibacterial, and antifungal [5,6]. In our search for anti-neoplastic bioactive agents from the traditional Chinese medicines, we have investigated the ethanol extracts of *Dioscorea collettii* var. *hypoglauca* to afford 14 steroidal glycosides [7–10] that showed good cytotoxic activity on K562 cell line and anti-fungal activity against *Pyricularia oryzae in vitro*. Some of the glycosides were involved in the preliminary anti-cancer screening panel of NCI, and further recommended for *in vivo* testing in the hope that they will become a new class of anti-cancer drugs [11–13]. This work stimulated our interest in the study of steroidal glycosides. The ethanol extracts of *Dioscorea*

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futschauensis R. Kunth (Dioscoreaceae) also showed good activity against the growth of *P. oryzae*, a bioassay for detecting anti-cancer agents, in our preliminary screening. Rhizomes of *D. futschauensis* are used as the traditional Chinese medicine "Mian Bi Xie" for the treatment of rheumatism and urinary tract disease. It is widely distributed in Southeast China and collected in the Pharmacopoeia of People's Republic of China (2000). By TLC analysis, several different glycosides spots were detected in the ethanol extract of *D. futschauensis* on comparison with that of *D. collettii.* We have reported several anti-fungal spirostanol glycosides and furostanol glycosides from this plant [14,15]. Herein, we describe the isolation and characterization of 13 furostanol glycosides, including two new furostanol glycosides (**1**, **2**; Fig. 1), as well as their anti-fungal activity against *Pyricylaria oryzae* and cytotoxic activity on the K562 cell line.

RESULTS AND DISCUSSION

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Furostanol glycosides (**1**–**13**) were isolated and purified by a combination of chromatographic methods from the rhizomes of *D. futschauensis*. Compounds **3**–**13** were identified as 26-*O*-β-D-glucopyranosyl-3β,26-dihydroxy-25(*R*)-furosta-5,20(22)-dien-3-*O*-α-L-rhamnopyranosyl(1 → 2)-β-D-glucopyranoside (**3**), pseudoprotodioscin (**4**), dioscoreside C (**5**), protodioscin (**6**), protoneodioscin (**7**), protogracillin (**8**), protoneogracillin (**9**), 22-methoxyl protodioscin (**10**), 22-methoxyl-protoneodioscin (**11**), 22-methoxyl-protogracillin (**12**) and 22-methoxyl-protoneogracillin (**13**) by detailed analysis of NMR data and comparison with previously published results [2,4,8].

Compound **1** was obtained as a white amorphous powder, and gave positive results for the Liebermann–Burchard reaction and Molish reagents. The negative HR-FAB-MS spectrum revealed the composition ($C_{52}H_{83}O_{23}$) by the quasi-molecular ion peak at *m/z* 1075.5347 $[M - H]^-$. The IR spectrum showed absorption bands at 3420, 2950, 1380, 1040 cm^{-1}. The ^1H NMR spectrum of **1** showed the presence of five methyl groups at δ 0.69 (s, Me-18), 1.06 (s, Me-19), 1.09 (d, 3H, $J = 6.0$ Hz, Me-27), 1.77 (s, Me-21), and 1.75 (br.s, Rha Me-6″), a methoxyl group at δ 3.34 (s, methoxy-23), an olefinic proton at δ 5.31 (br.s, H-6), and four anomeric protons at δ 6.38 (br.s, Rha-1″), 4.92 (d, 1H, $J = 6.8$ Hz, Glc-1′), 5.10 (d, 1H, $J = 7.5$ Hz, Glc-1‴) and 4.84 (d, 1H, $J = 7.2$ Hz, Glc-1⁗) which suggested the presence of three glucoses and one rhamnose units. The ^{13}C NMR spectrum confirmed the presence of a methoxyl group, two pair of olefinic carbons, and four anomeric carbons of sugars. Full assignments of the proton and carbon signals of the aglycon part of **1** were achieved on the basis of DEPT, ^1H–^1H COSY, HMQC and HMBC spectral analysis (Table I). The relative stereochemistry of the aglycon of **1** was resolved by comparison with dioscoreside C (**5**). The proton and carbon signals of the aglycon of **1** were quite consistent with those of dioscoreside C, indicating the same skeleton and stereochemistry as dioscoreside C [2,4]. All the analysis tends to establish the aglycon of compound **1** as 25(*R*)-furosta-5,20(22)-diene-23(*S*)-methoxyl-3β,26-diol.

On acid hydrolysis, the sugar moiety was detected as D-glucose and L-rhamnose by silica gel TLC upon comparison with authentic samples. The negative FAB-MS of **1** also confirmed the presence of sugar moiety, which gave a quasi-molecular ion peak $[M - H]^-$ at *m/z* 1075, and four prominent fragments $[M - H - Glc]^-$ at *m/z* 913, $[M - H - Rha - Glc]^-$ at *m/z* 767, $[M - H - Glc \times 2 - Rha]^-$ at *m/z* 605, and $[M - H - Glc \times 3 - Rha]^-$ at *m/z* 443. By analyzing the DEPT, ^1H–^1H COSY, HMQC spectra, the ^1H and ^{13}C NMR signals (Table II) of the sugar moiety could be assigned. The linkage sites of the sugar moiety on the aglycon and inter-linkages among sugar moiety were determined by HMBC spectral analysis. In the HMBC spectrum, the anomeric proton signals at δ 6.38 (Rha-1″),

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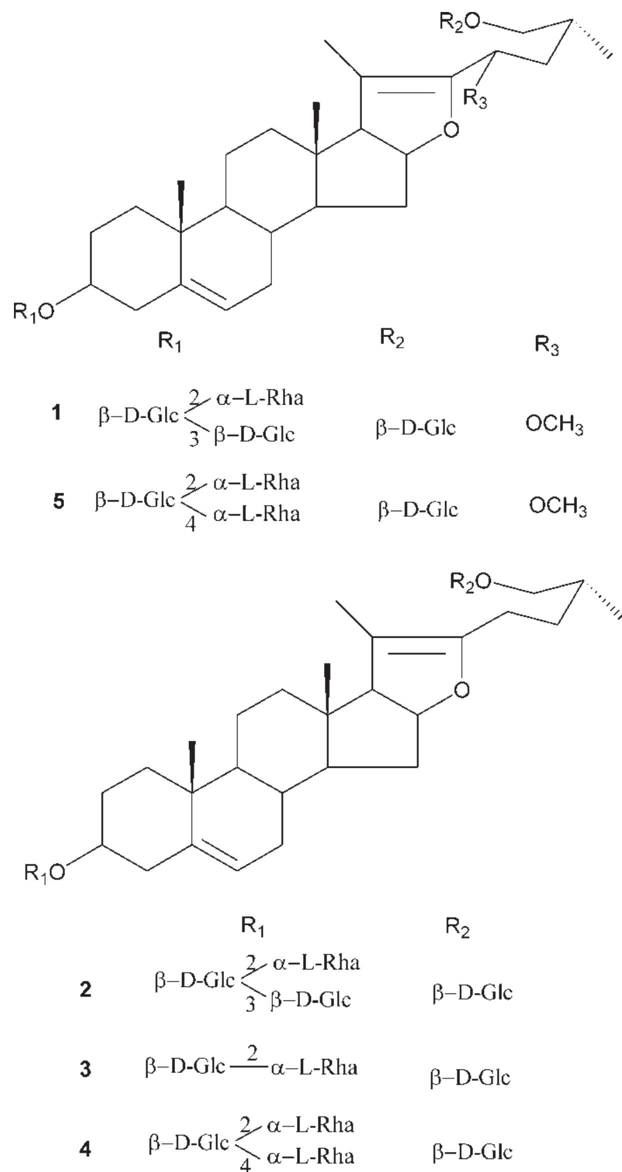

FIGURE 1 Structures of compounds **1**–**5**.

5.10 (Glc-1‴), 4.92 (Glc-1′) and 4.84 (Glc-1⁗) showed cross-peaks with the carbon signals at δ 77.6 (Glc-2′), 89.5 (Glc-3′), 78.2 (C-3) and 75.3 (C-26), respectively. A β-configuration at the anomeric position may be inferred from the values of the coupling constants for three glucopyranosyl units (6.0, 7.5 and 7.2 Hz). The α-configuration of the anomeric carbon of the rhamnose was assured by comparison of the chemical shift values of carbons 3 and 5 with those of the corresponding carbons of methyl α- and β-rhamnopyranoside [16]. Consequently, the structure of compound **1** is proposed as 26-*O*-β-D-glucopyranosyl-3β,26-dihydroxy-23(*S*)-methoxyl-25(*R*)-furosta-5,20(22)-dien-3-*O*-α-L-rhamnopyranosyl (1 → 2)-[β-D-glucopyranosyl(1 → 3)]-β-D-glucopyranoside, and named as dioscoreside E.

Compound **2** was isolated as a white powder, and gave a positive result for the Liebermann–Burchard reaction and Molish reagents. The molecular formula, $C_{51}H_{81}O_{22}$,

TABLE I NMR data for the aglycons of **1** and **2** in pyridine-d_5

	1[a]		***2***[a]	
Position	δ_H *(J in Hz)*	δ_C	δ_H *(J in Hz)*	δ_C
1	0.96, 1.73 (o)	37.5	0.94, 1.74 (o)	37.5
2	1.88, 2.07 (o)	30.2	1.84, 2.10 (o)	30.1
3	3.86 (m)	78.2	3.84 (m)	78.5
4	2.70, 2.78 (m)	38.7	2.72, 2.79 (m)	39.0
5		140.8		141.2
6	5.31 (br. s)	121.9	5.32 (br.s)	122.3
7	1.46, 1.86 (o)	32.4	1.46, 1.88 (o)	32.5
8	1.54 (o)	31.7	1.56 (o)	31.5
9	0.88 (o)	50.3	0.86 (o)	50.3
10		37.2		37.2
11	1.45 (o)	21.2	1.46, 1.71	21.3
12	1.14, 1.70 (o)	40.8	1.12, 1.76	39.7
13		43.5		43.5
14	0.88 (o)	55.0	1.10 (o)	55.0
15	1.48, 2.10 (o)	34.5	1.46, 2.10 (o)	34.5
16	4.86 (o)	84.6	4.83 (o)	84.5
17	2.49 (d, 10 Hz)	64.8	2.44 (d, 10.0 Hz)	64.6
18	0.69 (s)	14.3	0.75 (s)	14.1
19	1.06 (s)	19.4	1.06 (s)	19.4
20		108.6		103.6
21	1.77 (s)	11.4	1.64 (s)	11.8
22		152.2		152.6
23	4.20 (o)	73.4	2.23 (o)	23.7
24	1.68, 2.24 (o)	37.7	1.48, 1.85 (m)	31.5
25	2.28 (m)	30.7	1.94 (m)	33.6
26	3.72, 4.00 (o)	75.3	3.62, 3.96 (o)	75.2
27	1.09 (d, 6.0 Hz)	17.6	1.02 (d, 6.0 Hz)	17.4
23-OCH_3	3.34 (s)	56.1		

[a] ^{1}H NMR, 500 MHz in pyridine; ^{13}C NMR, 125 MHz, all signals were assigned by ^{1}H–^{1}H COSY, HMQC and HMBC spectra. Overlapped signals are indicated by "(o)".

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was determined from the quasi-molecular ion peak at *m/z* 1045.4327 $[M - H]^-$. Detailed examination of 1D- and 2D-NMR spectra of **2** and comparison with those of pseudoprotodioscin (**4**) revealed their considerable structural similarity [2,4], and allowed the assignment of proton and carbon signals from the aglycon of **2** (Table I). The only difference between them was in the signals of the sugar chain attached at C-3 of the sapogenol moiety. Acid hydrolysis followed by TLC analysis showed the presence of D-glucose and L-rhamnose. The assignment of the sugar moiety was resolved by a combination of ^{1}H–^{1}H COSY and HMQC data (Table II), which indicated the presence of two terminal β-D-glucopyranosyl units, one terminal α-L-rhamnopyranosyl unit, and a 2,3-disubstituted-β-D-glucopyranosyl unit. Direct evidence for the sugar sequence and the linkage sites was determined by HMBC analysis as described for **1**. The C-25 configuration was deduced to be *R* by comparing the NMR data with that of pseudoprotodioscin. Based on the above results, the structure of **2** was elucidated as 26-*O*-β-D-glucopyranosyl-3β,26-dihydroxy-25(*R*)-furosta-5,20(22)-dien-3-*O*-α-L-rhamnopyranosyl(1 → 2)-[β-D-glucopyranosyl(1 → 3)]-β-D-glucopyranoside, and named as pseudoprotogracillin.

Compounds **1–5** exhibited moderate activity against the plant pathogenic fungus *Pyricularia oryzae* with MMDC (minimal morphological deformation concentration) values of 100, 150, 140, 150, and 100 μM. Compounds **2** and **4** also showed cytotoxic activity on the K562 cancer cell line with IC_{50} values of 24 and 96 μM, respectively. The anti-fungal and cytotoxic activities of compounds **6–13** have been reported in earlier [8].

TABLE II NMR data for sugar moiety of **1** and **2** in pyridine-d_5

	1[a]		***2***[a]	
Position	δ_H *(J in Hz)*	δ_C	δ_H *(J in Hz)*	δ_C
3-O-Glc				
1′	4.92 (d, 6.0 Hz)	100.0	4.94 (d, 6.9 Hz)	100.0
	4.18 (o)	77.6	4.18 (o)	77.1
2′	4.19 (o)	89.5	4.16 (o)	89.5
	4.03 (o)	69.6	4.05 (o)	69.6
3′	3.82 (m)	77.9	3.82 (m)	77.9
	4.24, 4.42 (o)	62.4	4.26, 4.42 (o)	62.5
4′	6.38 (br. s)	102.2	6.38 (br.s)	102.2
	4.86 (o)	72.5	4.86 (m)	72.5
5′	4.58 (o)	72.8	4.55 (o)	72.8
	4.25 (m)	74.1	4.30 (o)	74.2
6′	4.90 (o)	69.6	4.93 (o)	69.6
	1.75 (br.s)	18.7	1.74 (d, 6.0 Hz)	18.7
Rha(1 → 2)				
1″	5.10 (d, 7.5 Hz)	104.5	5.09 (d, 7.5 Hz)	104.6
	4.02 (o)	75.0	4.02 (o)	75.0
2″	4.19 (o)	78.5	4.19 (o)	78.5
	4.10 (o)	71.7	4.10 (o)	71.8
3″	4.00 (o)	77.9	4.00 (o)	77.9
	4.28, 4.53 (o)	62.4	4.28, 4.53 (o)	62.5
4″	4.84 (d, 7.2 Hz)	104.9	4.83 (d, 7.5 Hz)	104.9
	4.02 (m)	75.2	4.03 (o)	75.2
5″	4.18 (o)	78.5	4.18 (o)	78.5
	4.19 (o)	71.5	4.19 (o)	71.6
6″	3.83 (o)	78.6	3.94 (o)	78.6
	4.36, 4.50 (o)	62.8	4.36, 4.57	62.9
Glc(1 → 3)				
1‴				
2‴				
3‴				
4‴				
5‴				
6‴				
26-O-Glc				
1⁗				
2⁗				
3⁗				
4⁗				
5⁗				
6⁗				

[a] ^{1}H NMR, 500 MHz in pyridine; ^{13}C NMR, 125 MHz, All signals were assigned by ^{1}H-^{1}H COSY, HMQC and HMBC spectra. Overlapped signals are indicated by "(o)".

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EXPERIMENTAL

General Experimental Procedures

Melting points were recorded on a Yanaco MP-S3 micro-melting point apparatus, and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 13°. IR: Jasco A-102 (KBr). ^{1}H and ^{13}C NMR: JEOL JNM-GX400 (^{1}H 300 MHz, ^{13}C 75 MHz) spectrometer. FAB-MS: JEOL JMS-DX302. HPLC: Liquid Chromatography: LC-10 (RI detector, Japan Analytical Industry Co., Ltd.) using an ODS column (Waters, 20 × 250 mm, 5 μm) with mobile-phase flow rate of 4.0 mL min^{-1}. C. C. silica gel H (10–40 μm, Qingdao Haiyang Chemical Factory). TLC: silica gel G (10–40 μm, Qingdao Haiyang Chemical Factory). C. C. ODS (Senshu Scientific Co., Ltd.). Spots were visualized by spraying with 10% H_2SO_4 followed by heating.

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Plant Material

Rhizomes of *Dioscorea futschauensis* R. Kunth (Dioscoreaceae) were collected in 1999 from Fujian Province (China), and were identified by Professor Qishi Sun (Division of Pharmacognosy, Shenyang Pharmaceutical University). A voucher specimen (99049) has been deposited at the herbarium of Shenyang Pharmaceutical University, Liaoning Province.

Extraction and Isolation

Air-dried powdered rhizomes (3 kg) of *D. futschauensis* were refluxed with 75% EtOH (8 L × 2). The ethanol extract was evaporated under reduced pressure to give a residue (280 g) that was suspended in 3000 ml of water and extracted with an equal volume of n-BuOH (2 ×) to afford an n-butanol soluble fraction DB (100 g). Fr. DB (50 g) was subjected to column chromatography on silica gel H (500 g) and eluted step-wise by $CHCl_3$–MeOH (100:1, 100:2, 97:3, 95:5, 90:10, 85:15, 80:20, 70:30 and 60:40, each 5000 ml) to give 27 fractions according to TLC analysis. Frs. DB-15-DB-25 were active against the growth of *P. oryzae*. Fr. DB-22 (100 mg) was further separated by preparative HPLC (75% MeOH–H_2O) to give compound **3** (20 mg). Fr. DB-23 (2.2 g) was subjected to column chromatograph on ODS (45–85% MeOH–H_2O) to afford four fractions, DB-23-1-DB-23-4. Fr. DB-23-2 (0.6 g) was further purified by preparative HPLC (70% MeOH–H_2O) to afford compounds **1** (8 mg), **2** (200 mg), **4** (100 mg), and **5** (9 mg). Fr. DB-24 (1.5 g) was also separated by chromatograph on ODS (50–75% MeOH–H_2O) before being purified by HPLC. Five sub-fractions, DB-24-1-DB-24-5, were obtained. Compounds **6** (10 mg), **7** (4 mg), **8** (8 mg), and **9** (5 mg) were isolated from Fr. DB-24-2 (0.1 g) by preparative HPLC (55% MeOH–H_2O). Compounds **10** (20 mg), **11** (10 mg), **12** (30 mg), and **13** (12 mg) were purified from Fr. DB-24–4 (0.3 g) by preparative HPLC (65% MeOH–H_2O). It has been reported that compounds **6**–**9** can be obtained from **10**–**13**, respectively, by refluxing in a 50% acetone-water solution for 36 h.

Compound **1**: amorphous powder, mp 256–258°C (dec.); $[\alpha]_D^{24}$: −50.3 (pyridine; *c* 0.05). IR(KBr) ν_{max} (cm^{-1}): 3420(OH), 2950, 1640, 1380, 1040 (glycosyl C–O); ^1H NMR and ^{13}C NMR: Tables I and II; FAB-MS (negative) *m/z* 1075 $[M-H]^-$, 913 $[M-H-Glc]^-$, 767 $[M-H-Rha-Glc]^-$, 605 $[M-H-Glc\times 2-Rha]^-$, and 443 $[M-H-Glc\times 3-Rha]^-$; HR-FAB-MS *m/z* 1075.5347 $[M-H]^-$ (calcd for $C_{52}H_{83}O_{23}$, 1075.5354).

Compound **2**: amorphous powder, mp 238–240°C (dec.); $[\alpha]_D^{24}$ −45.6 (pyridine; *c* 0.1). IR(KBr) ν_{max} (cm^{-1}): 3385 (OH), 2930, 1645, 1456, 1370, 1046 (glycosyl C—O); ^1H and ^{13}C NMR: Tables I and II; FAB-MS (negative) *m/z*: 1045 $[M-H]^-$, 883 $[M-H-Glc]^-$, 737 $[M-H-Rha-Glc]^-$, 575 $[M-H-Glc\times 2-Rha]^-$, and 413 $[M-H-Glc\times 3-Rha]^-$; HR-FAB-MS *m/z* 1045.4327 $[M-H]^-$ (calcd for $C_{51}H_{81}O_{22}$, 1045.4339).

Acid Hydrolysis

Each compound (2 mg) was heated with 2 M HCl (2 ml) in a sealed tube at 100°C for 4 h. The reaction mixture was extracted with ethyl acetate. After evaporating off the organic layer, the aqueous phase was neutralized with $NaHCO_3$ and lyophilized. The lyophilized residue was dissolved in pyridine (0.2 mL), and co-eluted (TLC) with an authentic sample using the solvent system EtOAc-n-BuOH-H_2O (20:70:10, v/v). The plates were sprayed with naphthoresorcinol reagent by heating at 100°C.

Bioassay

Antifungal assays against plant pathogenic fungus *Pyricularia oryzae* and the cytotoxicity assays on cultured K562 cell line were carried out as previously reported [7,14].

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References

[1] Shen, P., Wang, S.L., Liu, X.K., Yang, C.R., Cai, B. and Yao, X.S. (2002), *J. Asian Nat. Prod. Res.* **4**, 211–215.
[2] Dong, M., Feng, X.Z., Wu, L.J., Wang, B.X. and Ikejima, T. (2001), *Planta Med.* **67**, 853–857.
[3] Dong, M., Feng, X.Z., Wang, B.X., Wu, L.J. and Ikejima, T. (2001), *Tetrahedron* **57**, 501–506.
[4] Dong, M., Wu, L.J., Chen, Q. and Wang, B.X. (2001), *Acta Pharmacol. Sin.* **36**, 42–45.
[5] Furuya, S., Takayama, F., Mimaki, Y., Sashida, Y., Satoh, K. and Sakagami, H. (2000), *Anticancer Res.* **20**, 4189–4194.
[6] Lee, S.C., Tsai, C.C., Chen, J.C., Lin, J.G., Lin, C.C., Hu, M.L. and Lu, S. (2002), *Acta Pharmacol. Sin.* **23**, 503–508.
[7] Hu, K., Dong, A.J., Yao, X.S., Kobayashi, H. and Iwasaki, S. (1996), *Planta Med.* **62**, 573–575.
[8] Hu, K., Dong, A.J., Yao, X.S., Kobayashi, H. and Iwasaki, S. (1997), *Planta Med.* **63**, 161–165.
[9] Hu, K., Dong, A.J., Yao, X.S., Kobayshi, H. and Iwasaki, S. (1997), *Phytochemistry* **44**, 1339–1342.
[10] Hu, K., Dong, A.J., Kobayshi, H., Jing, Y.K., Iwasaki, S. and Yao, X.S. (1999), *J. Nat. Prod.* **62**, 299–301.
[11] Hu, K. and Yao, X.S. (2001), *Anticancer Drugs* **12**, 541–547.
[12] Hu, K. and Yao, X.S. (2002), *Anticancer Res.* **22**, 1001–1005.
[13] Hu, K. and Yao, X.S. (2002), *Planta Med.* **68**, 297–301.
[14] Liu, H.W., Kobayshi, H., Qu, G.X. and Yao, X.S. (2001), *Chin. Chem. Lett.* **12**, 613–616.
[15] Liu, H.W., Kobayshi, H., Qu, G.X. and Yao, X.S. (2002), *Chin. Chem. Lett.* **13**, 241–244.
[16] Agrawal, P.K., Jain, D.C., Gupta, P.K. and Thakur, R.S. (1985), *Phytochemistry* **24**, 2479–2485.

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