Hepatoprotective Aliphatic Glycosides from Three *Goodyera* Species

Xiao-Ming Du,[a] Ning-Yi Sun,[b] Yang Chen,[a] Nobuto Irino,[b] and Yukihiro Shoyama*,[a]

Graduate School of Pharmaceutical Sciences, Kyushu University,[a] 3–1–1 Maidashi, Higashi-ku, Fukuoka 812–8582, Japan and Seiwa Pharmaceuticals Ltd.,[b] 1–12–15 Shiba-Daimon, Minato-ku, Tokyo 105–8585, Japan.

Received January 7, 2000; accepted February 18, 2000

Hepatoprotective aliphatic glycosides 3-(*S*)-3-β-D-glucopyranosyloxybutanolide (1) and its congener, 3-(*S*)-3-β-D-glucopyranosyloxy-4-hydroxybutanoic acid (2) were isolated as major constituents from the whole plants of three *Goodyera* species, *G. schlechtendaliana* Reichb. fil., *G. matsumurana* Schltr. and *G. discolor* Ker-Gawl. The structures of 1 and 2 have been determined by NMR, MS spectroscopic and chemical means. Compound 1 was converted into its methyl ester form (5) during the purification step, when the lactone ring was cleaved by catalysis of silica gel with the $CHCl_3$–MeOH–H_2O as a solvent. On the other hand, 1 was obtained in a high yield by the same purification procedure without MeOH. Based on this fact, a simple and economic method for the purification of 1 was confirmed. Compounds 1 and 2 were found to have a hepatoprotective effect on liver injury induced by carbon tetrachloride in primary cultured rat hepatocytes.

Key words *Goodyera*; goodyeroside; hepatoprotective effect

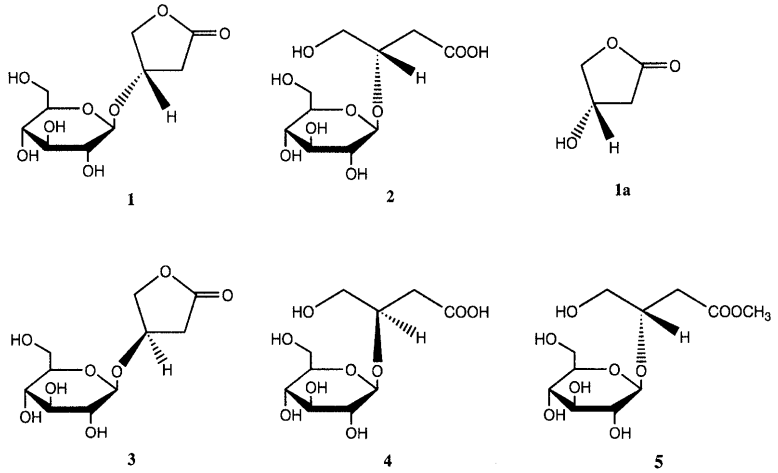
The genus *Goodyera* (Orchidaceae) comprises 55 species which are widely distributed in the world except for Africa.[1] Some species have been used since ancient times in Chinese folk medicine for fever, pain, snake-bite, and lung disease.[2,3] Several species of this genus were also used as a substitute for the precious crude drug, *Anoectochilus formosanus* Hayata.[4,5] Since no survey of the components of *Goodyera* species has been reported, we herein describe the isolation and characterization of aliphatic glycosides (**1** and **2**), named goodyerosides A and B, as major constituents from three *Goodyera* species, *G. schlechtendaliana* Reichb. fil., *G. matsumurana* Schltr. and *G. discolor* Ker-Gawl., natively grown in Japan and Southeast Asia.

RESULTS AND DISCUSSION

Compound **1** was isolated as colorless needles, mp 156—157 °C, $[\alpha]_D$ −71.2°. The ^{1}H- and ^{13}C-NMR spectra of **1** showed signals and coupling patterns similar to those of **3**, a colorless oil, $[\alpha]_D$ +48.4°, previously isolated from *Anoectochilus koshunensis* Hayata[6] and *A. formosanus* Hayata.[7] Positive ion FAB-MS of **1** gave a $(M+H)^+$ peak at *m/z* 265 and $(M+Na)^+$ peak at *m/z* 287, and the high resolution MS analysis of the quasimolecular ion peak $(M+Na)^+$ revealed the same molecular formula ($C_{10}H_{16}O_8$) as **3**,[6,7] suggesting that these compounds are epimers.

The enzymatic hydrolysis of **1** yielded D-glucose, which was confirmed by specific rotation measurement ($[\alpha]_D$ +50.8°), and an aglycon **1a**. Aglycon **1a** had the molecular formula $C_4H_6O_3$ based on electron-impact mass spectra (EI-MS). ^{1}H- and ^{13}C-NMR spectra showed all the same corresponding signals as those of **1** except for the D-glucose moiety. Compound **1a** was identified by comparison with an authentic sample of (3*S*)-(−)-3-hydroxybutanolide, synthesized from (*S*)-(−)-dimethylmalate.[8]

All the proton and carbon signals of **1** and **2** were assigned by ^{1}H–^{1}H correlated spectroscopy (COSY), nuclear Overhauser effect correlation spectroscopy (NOESY), ^{13}C and ^{13}C distortionless enhancement by polarization transfer (DEPT), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond correlation (HMBC) experiments (Tables 1 and 2). An anomeric proton signal at δ: 4.94 (1H, d, J=7.9 Hz) in the ^{1}H-NMR spectrum of **1** was assigned as a glucosyl anomeric proton, suggesting that the glycosidic bond had a β linkage.[6,7,9] In the NOESY spectrum, a cross peak between the anomeric proton and H-3 of the aglycon was observed. In the HMBC spectrum, long range correlation was observed from the anomeric proton to C-3 of the aglycon. Thus, **1** was determined to be 3-(*S*)-3-β-D-glucopyranosyloxybutanolide.



1 2 1a

3 4 5

* To whom correspondence should be addressed. e-mail: shoyama@shoyaku.phar.kyushu-u.ac.jp

Table 1. ^{1}H-NMR Spectral Data for **1**, **2**, **1a**, and **5**

	1[a]	**2**[b]	**1a**[a]	**5**[a]
2	2.88 (dd, 17.8, 5.2)	2.57 (dd, 15.5, 6.9)	2.92 (dd, 17.2, 5.6)	2.94 (dd, 15.8, 6.9)
	2.84 (dd, 17.8, 2.5)	2.48 (dd, 15.5, 5.6)	2.73 (dd, 17.2, 1.7)	3.02 (dd, 15.8, 5.5)
3	4.85 (m)	4.15 (m)	4.76 (ddd, 5.6, 3.6, 1.7)	4.73 (m)
4	4.69 (dd, 10.2, 1.5)	3.58 (dd, 12.3, 5.9)	4.47 (br d, 9.6)	4.17 (dd, 11.5, 5.4)
	4.41 (dd, 10.2, 4.7)	3.63 (dd, 12.3, 4.5)	4.42 (dd, 9.6, 3.6)	4.02 (dd, 11.5, 4.9)
G-1	4.94 (d, 7.9)	4.43 (d, 7.9)		5.06 (d, 7.9)
G-2	3.98 (dd, 7.9, 8.9)	3.18 (dd, 7.9, 8.9)		3.98 (dd, 7.9, 9.0)
G-3	4.22 (dd, 8.9, 8.9)	3.35 (dd, 8.9, 8.9)		4.24 (dd, 9.0, 9.0)
G-4	4.19 (dd, 8.9, 8.9)	3.22 (dd, 8.9, 8.9)		4.20 (dd, 9.0,9.0)
G-5	3.94 (ddd, 8.9, 5.3, 2.3)	3.34 (m)		3.95 (m)
G-6	4.54 (dd, 11.7, 2.3)	3.86 (dd, 11.9, 2.0)		4.51 (dd, 11.5, 2.6)
	4.35 (dd, 11.7, 5.3)	3.66 (dd, 11.9, 5.1)		4.35 (dd, 11.5, 5.6)
OMe				3.61 (s)

a) In C_5D_5N. *b*) In CD_3OD.

Table 2. ^{13}C-NMR Data for **1**, **2**, **1a**, and **5**

	1[a]	**2**[b]	**1a**[a]	**5**[a]
1	176.2	177.2	176.9	172.2
2	36.4	38.9	38.5	38.4
3	74.7	79.7	76.9	79.2
4	74.0	65.6	67.4	65.2
G-1	103.7	104.1		104.9
G-2	74.8	75.2		75.1
G-3	78.7	78.0		78.4
G-4	71.5	71.6		71.7
G-5	78.4	78.0		78.5
G-6	62.8	62.7		62.7
OCH_3				51.5

a) In pyridine-d_5. *b*) In CD_3OD.

Compound **2** showed a $(M+H)^+$ peak at *m/z* 283 in the positive ion FAB-MS, and a $(M-H)^-$ peak at *m/z* 281 in the negative ion FAB-MS. Its molecular formula $C_{10}H_{18}O_9$ was established by high resolution MS measurement of the quasi-molecular ion peak $(M-H)^-$. The monosaccharide obtained after acid hydrolysis was identified as D-glucose by specific rotation measurement ($[\alpha]_D$ +51.2°). When the ^{1}H- and ^{13}C-NMR spectra of **2** were compared with those of **4** [3-(*R*)-3-β-D-glucopyranosyloxy-4-hydroxybutanoic acid] previously isolated from *A. formosanus*,[7] similar signals and coupling patterns were observed. The ^{1}H-NMR spectrum showed two methylene groups and a methine signal. In the ^{13}C-NMR spectrum, four signals at δ: 177.2 (COOH), 38.9 (CH_2), 79.4 [CH(OR)] and an oxygenated carbon at δ: 65.6 (CH_2OH) were observed. These data indicated the existence of 3,4-dihydroxybutylic acid in **2**. The anomeric proton signal at δ: 4.43 (1H, d, J=7.9 Hz) in the ^{1}H-NMR spectrum was assigned as a glucosyl anomeric proton, suggesting that the glycosidic bond had a β linkage.[6,7,9] A cross peak between the anomeric proton and H-3 of the 3,4-dihydroxybutylic acid, and long range correlation from the anomeric proton to C-3 of the 3,4-dihydroxybutylic acid, were observed in the NOESY and HMBC spectra, respectively. Furthermore, **2** was converted to **1** by acid treatment in the same manner as **4** was to **3**, as reported previously.[7] Thus, **2** was characterized as 3-(*S*)-3-β-D-glucopyranosyloxy-4-hydroxybutanoic acid.

A fully acetylated derivative of **1** was recently isolated after the acetylation of **1** containing a mixture from the sprouts of *Crocus sativus*, but was not isolated in the free form of **1**.[10] Moreover, since we detected **5** in the purification step on a silica gel column chromatography elution with $CHCl_3$–MeOH–H_2O as a solvent in the preliminary experiment, it is easily suggested that the reason might depend on the occurrence of a methylation reaction in the purification step, because the authors used a silica gel column eluted by $CHCl_3$–MeOH–H_2O, resulting in difficulty of purifying **1**. On the other hand, we analyzed the concentration of **1** by HPLC to be more than 15% dr. wt. (data not shown). In order to confirm the artificial conversion of **1** to **5**, the combination of silica gel and an eluting solvent containing MeOH was investigated in detail. Purification of **1** using a longer column eluted with $CHCl_3$–MeOH–H_2O as a solvent enhanced the yield of **5**. Compound **1** completely changed into **5** by storage in $CHCl_3$–MeOH–H_2O solvent with silica gel at room temperature for one week. Based on the positive ion FAB-MS *m/z*: 297 $(M+H)^+$, **5** was considered to have the molecular formula $C_{11}H_{20}O_9$. The NMR spectra of **5** were closely related to those of **2** except for a methoxyl signal (δ_H: 3.61, 3H, s, δ_C: 51.5). Thus, **5** was the methyl ester of **2**. No conversion of **2** to **5** occurred under the same condition. Thus, the methylation reaction occurred when the γ-lactone ring was cleaved using silica gel as a catalyst with $CHCl_3$–MeOH–H_2O. As previously described, the conversion of **2** to **1** was easily enhanced by acid treatment. On the other hand, no change of **1** to **2** occurred under various other conditions (data not shown). Thus, the biogenetic conversion of **2** to **1** is suggested. Based on these results, a simple method can be used for the purification of **1** using a silica gel column eluted with a mixture of $CHCl_3$–EtOH solvent resulting in high yield, as described in the experimental section and previously registered in a patent.[11]

The antihepatotoxic activities of **1** and **2**, and those of the epimeric compounds **3** and **4** were studied on injury induced by CCl_4 in primary cultured rat hepatocytes, and were demonstrated by measuring the levels of lactate dehydrogenase (LDH), glutamic oxaloacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT). The results are summarized in Table 3. In the CCl_4-treated control group, there were marked increases in LDH, GOT, and GPT activities, compared with the normal group. In contrast, these levels were suppressed in the **1**, **2**, **3**, and **4** (0.1, 1 and 10 μg/ml) treated groups. Although the *S*-isomers (**1** and **2**) appeared to be more effective than those of the *R*-isomers (**3** and **4**), no

Table 3. Hepatoprotective Activity of **1**, **2**, **3** and **4** on CCl_4-Induced Cultured Hepatocyte Injury

Samples	Concentration (μg/ml)	LDH (units/ml)	GOT (units/ml)	GPT (units/ml)
Normal		180.28±6.62	58.20±0.48	8.55±0.48
Control		1987.58±44.36	519.40±57.38	97.92±4.81
1	0.1	1349.43±69.71**	229.50±22.28**	78.55±3.21
	1.0	1263.25±77.48**	203.33±21.20**	72.30±6.29
	10.0	1142.70±106.34**	180.30±28.22**	65.28±10.03*
2	0.1	1664.27±25.05	251.31±17.99**	84.01±1.64
	1.0	1460.48±104.73*	218.05±32.85**	78.30±5.06
	10.0	1328.28±21.87**	193.26±11.31**	69.68±1.71
3	0.1	1395.78±162.01**	223.57±32.25**	79.30±8.31
	1.0	1327.15±112.27**	215.55±23.26**	76.63±6.67
	10.0	1285.40±118.19**	195.18±31.54**	68.45±13.23*
4	0.1	1663.30±85.63	297.60±29.44**	88.18±4.98
	1.0	1228.38±153.39**	209.23±24.78**	72.43±6.78
	10.0	1175.45±110.25**	200.30±24.68**	70.40±6.82

* $p<0.05$, ** $p<0.01$ *vs.* control group.

significant differences were observed between them. Although glycyrrhizin, which has been widely used as a clinical antihepatitis drug,[12] was tested as a positive control, no effect was observed under 100 μg/ml (data not shown) as previously reported.[13] The result in the present study indicates that the whole plants of the three *Goodyera* species may be useful for the treatment of liver diseases, due to the hepatoprotective activity of **1** at a high concentration in these crude drugs.

MATERIALS AND METHODS

General Procedures Melting points were determined using a Yazawa BY-2 apparatus and are uncorrected. Optical rotations were measured on a Jasco P-1010 digital polarimeter. FAB-MS were measured on a JMS-700 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL 270 (^{1}H at 270 MHz, ^{13}C at 67.8 MHz) and JEOL GX-400 (^{1}H at 400 MHz, ^{13}C at 100 MHz) spectrometers, using tetramethylsilane (TMS) as an internal standard. Chiral HPLC was carried out on a Gasukuro Kogyo Model 576, with a Shodex RI SE-61 differential refractometer and a Cyclobond 2000 column (β-cyclodextrin bonded, 4.6 mm i.d×250 mm; eluate: 80% CH_3CN; flow rate: 1.0 ml/min). Kieselgel 60 F_{254} (Merck) was used for analytical TLC. Silica gel 60 (70—230 and 230—400 mesh, Merck) was used for column chromatography. Preparative TLC was carried out on Kieselgel 60 F_{254} plates (Merck).

Plant Material Whole plants of *G. schlechtendaliana*, *G. matsumurana* and *G. discolor* were collected in Okinawa, Taiwan and Vietnam. Voucher specimens have been deposited in the Laboratory of Natural Products of Seiwa Pharmaceuticals, Ltd., as well as the Herbarium of Medicinal Plant Garden, Graduate School of Pharmaceutical Sciences, Kyushu University.

Extraction and Isolation (1) *G. schlechtendaliana*: The dried plants of *G. schlechtendaliana* (20 g) were percolated with MeOH at room temperature to give 6.8 g of extract, which was suspended in H_2O. Removal of $CHCl_3$ soluble materials carried out by partition afforded an H_2O-soluble fraction. The H_2O-soluble fraction was applied to an ODS (Cosmosil 75C_{18}-OPN) column eluted with H_2O to obtain fr. 1 and 2. Fr. 1 was purified by preparative TLC ($CHCl_3$–MeOH–H_2O, 6 : 4 : 1) to yield **2** (51 mg). Fr. 2 was purified with silica gel column chromatography eluted with $CHCl_3$–EtOH (7 : 3) to yield **1** (3.2 g).

(2) *G. matsumurana*: The dried plants of *G. matsumurana* (3 g) were treated in the same manner as described above, to give **1** (453 mg) and **2** (9 mg).

(3) *G. discolor*: The dried plants of *G. discolor* (3.8 g) were also treated in the same manner as described above, to yield **1** (588 mg) and **2** (10 mg).

3-(*S*)-3-β-D-Glucopyranosyloxybutanolide (1) Colorless needles, mp 156—157 °C (crystallized from EtOH). $[\alpha]_D^{25}$ −71.2° (c=0.55, H_2O). Positive ion FAB-MS *m/z*: 265 $(M+H)^+$, 287 $(M+Na)^+$. HR-FAB-MS *m/z*: 287.0843 $(M+Na)^+$ (Calcd for $C_{10}H_{16}O_8Na$ 287.0845). ^{1}H-NMR (400 MHz, pyridine-d_5) δ_H: given in Table 1. ^{13}C-NMR (100 MHz, pyridine-d_5) δ_C: given in Table 2.

3-(*S*)-3-β-D-Glucopyranosyloxy-4-hydroxy Butanoic Acid (2) Colorless oil, $[\alpha]_D^{25}$ −19.3° (c=0.40, H_2O). Positive ion FAB-MS *m/z*: 283 $(M+H)^+$, 305 $(M+Na)^+$. Negative ion FAB-MS *m/z*: 281 $(M-H)^-$. HR-FAB-MS *m/z*: 281.0883 $(M-H)^-$ (Calcd for $C_{10}H_{17}O_9$ 281.0872). ^{1}H-NMR (270 MHz, CD_3OD) δ_H: given in Table 1. ^{13}C-NMR (100 MHz, CD_3OD) δ_C: given in Table 2.

Enzymatic Hydrolysis of 1 A solution of **1** (50 mg) and β-glucosidase (Sigma Chemical Co., 60 mg) in H_2O (5 ml) was shaken in a water bath at 37 °C for 5 h. The reaction mixture was extracted with EtOAc (2 ml×3), and the combined EtOAc layer was washed with H_2O, dried over Na_2SO_4, then concentrated to give an oil, followed by chromatography over silica gel (*n*-hexane–EtOAc, 1 : 1) to afford **1a** (13 mg). The H_2O layer was evaporated *in vacuo* to dryness, and the residue was applied to a Sephadex LH-20 column (MeOH) and then purified by silica gel column chromatography ($CHCl_3$–MeOH–H_2O, 6 : 4 : 1) to afford D-glucose (17 mg). Syrup, $[\alpha]_D^{25}$ +50.8° (c=1.5, H_2O).

(3*S*)-(−)-3-Hydroxybutanolide (1a) Oil, $[\alpha]_D^{25}$ −81.7° (c=1.2, EtOH). EI-MS *m/z*: 102 $(M)^+$. Identical with synthetic (3*S*)-(−)-3-hydroxybutanolide by comparison of the NMR spectra, optical rotation and retention time by chiral HPLC (t_R: 4.52 min).

Synthesis of (3*S*)-(−)-3-Hydroxybutanolide To a solu-

tion of dimethyl (*S*)-(−)-malate (1 g) in dry tetrahydrofuran (THF, 12 ml), borane-dimethyl sulfide (0.6 ml) was added dropwise and the mixture was stirred at 20 °C for 0.5 h. Then, $NaBH_4$ (10 mg) was added at 0—10 °C for 20 min, and the resulting mixture was stirred for an additional 0.5 h at 20 °C, followed by the addition of dry MeOH (4 ml), with stirring continued for 0.5 h. Solvent was removed and the residue was purified by silica gel chromatography (EtOAc) to give a colorless oil (480 mg), which was added to a solution of CH_2Cl_2 (3 ml) containing CF_3CO_2H (0.2 ml). The reaction mixture was stirred at room temperature for 15 h. The crude product was purified by silica gel chromatography (*n*-hexane–EtOAc, 1 : 1) to give (3*S*)-(−)-3-hydroxybutanolide (319 mg). Oil, $[\alpha]_D^{25}$ −80.9° (*c*=0.9, EtOH). EI-MS *m/z*: 102 $(M)^+$. Its ^1H- and ^{13}C-NMR spectra (270 and 67.8 MHz, respectively) were superimposable on those of **1a**.

Acid Hydrolysis of 2 Compound **2** (23 mg) was dissolved in 1 M H_2SO_4 (2 ml) and heated at 95 °C for 1 h. The reaction mixture was diluted with H_2O and extracted with Et_2O. The H_2O layer was neutralized by $Ba(OH)_2$ and the precipitates filtered off. The filtrate was desalted by chromatography over Sephadex LH-20 (MeOH), then purified by silica gel column ($CHCl_3$–MeOH–H_2O, 6 : 4 : 1) to give D-glucose (9 mg). Syrup, $[\alpha]_D^{25}$ +51.2° (*c*=0.9, H_2O).

Conversion of 2 to 1 by Acid Treatment A solution of **2** (18 mg) in H_2O (0.5 ml) was treated with 0.5 M H_2SO_4 (0.5 ml), and the reaction mixture was left to stand at room temperature overnight. The mixture was neutralized by $Ba(OH)_2$ and then filtered. The filtrate was evaporated under a stream of N_2 and the residue was chromatographed over silica gel ($CHCl_3$–EtOH, 7 : 3) to give **1** (12 mg). Needles, mp 156—157 °C, $[\alpha]_D^{25}$ −70.8°(*c*=0.82, H_2O). The ^1H- and ^{13}C-NMR data were superimposable on those of **1**.

Cleavage of the Lactone Ring in 1 by Silica Gel Compound **1** (25 mg) was chromatographed over silica gel (20 cm×1.5 cm i.d) and eluted with $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5), to give **5** (10 mg) and a mixture of **1** and **5** (12 mg). Compound **5**: colorless oil, positive ion FAB-MS *m/z*: 297 $(M+H)^+$, 319 $(M+Na)^+$. ^1H-NMR (270 MHz, pyridine-d_5) δ_H: given in Table 1. ^{13}C-NMR (100 MHz, pyridine-d_5) δ_C: given in Table 2.

Preparation of Primary Cultured Rat Hepatocytes Rat liver parenchymal cells were isolated using a collagenase perfusion method following the procedure developed by Seglen.[14] The portal vein of rat liver was exposed and a cannula was inserted. The liver was perfused with Ca^{2+}-free Hanks' balanced salt solution (HBSS) containing 0.5% bovine serum albumin (BSA) and 0.5 mM EGTA aerated with 95% O_2/5% CO_2 at 37 °C. The flow rate of the washing buffer was maintained at 30 ml/min. After the liver was perfused for 10 min, recirculation was started with collagenase solution containing Ca^{2+}-free HBSS, 0.05% collagenase, 4 mM $CaCl_2$, and 0.005% trypsin inhibitor, at a flow rate of 15 ml/min. Isolated hepatocytes (1.4×10^5 cells/cm^2) were cultured in William's E medium supplemented with 10% calf serum, 50 μg/ml gentamycin, 1 μM dexamethasone, and 10 nM insulin under 5% CO_2 in air at 37 °C in 24-well plastic plates.

CCl_4-Exposure of Primary Cultured Hepatocytes After a 24 h pre-culture, the hepatocytes were exposed to fresh medium containing 10 mM CCl_4 with or without the test samples. After CCl_4 exposure for 60 min, the medium was sampled for determination of enzyme activity (LDH, GOT and GPT).

Statistical Analysis Each value was expressed as a mean±S.E. (*n*=4). The significance of variation in data was calculated by Duncan's test, and the results were considered significant when $p<0.05$.

REFERENCES

1) Asahi Shinbunsha, "Asahi Encyclopedia the World of Plants," Vol. 9, Asahi Shinbun Press, Tokyo, 1997, pp. 243—244.
2) Jiangsu New Medical College, "Dictionary of the Traditional Chinese Medicines," Shanghai Scientific Technologic Publishing House, Shanghai, 1986, pp. 2282—2283.
3) Liang W.-L., Chen R.-C., Ching Y.-J., Su C.-H., Yang L.-L., Yen K.-Y., *Formosan Sci.*, **43**, 47—58 (1990).
4) Lin C.-C., Namba T., *Shoyakugaku Zasshi*, **35**, 262—271 (1981).
5) Lin C.-C., Namba T., *Shoyakugaku Zasshi*, **35**, 272—286 (1981).
6) Ito A., Kasai R., Yamasaki K., Sugimoto H., *Phytochemistry*, **33**, 1133—1137 (1993).
7) Du X.-M., Yoshizawa T., Shoyama Y., *Phytochemistry*, **49**, 1925—1928 (1998).
8) Du X.-M., Kohinata K., Kawasaki T., Guo Y.-T., Miyahara K., *Phytochemistry*, **48**, 843—850 (1998).
9) Saito S., Hasegawa T., Inaba M., Nishida R., Fujii T., Nomizu S., Moriwake T., *Chem. Lett.*, **1984**, 1389—1392.
10) Cao W., Li Y., Zhu D., *Planta Med.*, **65**, 425—427 (1999).
11) Du X.-M., Sun N.-Y., Yoshizawa T., Japan. Kokai Tokkyo Koho, 11-92492, (1997).
12) Safayhi H., Sailer E. R., *Planta Med.*, **63**, 487—493 (1997).
13) Arao T., Udayama M., Kinjo J., Nohara T., Funakoshi T., Kojima S., *Biol. Pharm. Bull.*, **20**, 988—991 (1997).
14) Seglen P. O., *Methods Cell Biol.*, **13**, 29—83 (1976).