Uncinoside A and B, Two New Antiviral Chromone Glycosides from *Selaginella uncinata*

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Five compounds have been isolated from the dried whole plants of *Selaginella uncinata*, two of them were new chromone glycosides, 5-hydroxy-2,6,8-trimethylchromone 7-*O*-β-D-glucopyranoside (uncinoside A) and 5-acetoxyl-2,6,8-trimethylchromone 7-*O*-β-D-glucopyranoside (uncinoside B). Their structures were elucidated by spectroscopic methods including one- and two-dimensional NMR techniques. The other three compounds were identified as 8-methyl eugenitol, amentoflavone and hinokiflavone. Uncinoside A and B showed potent antiviral activities against respiratory syncytial virus (RSV) with IC_{50} value of 6.9 and 1.3 μg/ml, moderate antiviral activities against parainfluenza type 3 virus (PIV 3) with IC_{50} value of 13.8 and 20.8 μg/ml, respectively.

Key words *Selaginella uncinata*; uncinoside A; uncinoside B, antiviral activity

Viruses are the leading cause of respiratory infections in children and adults and are a major cause of morbidity and mortality worldwide. Respiratory viral infections contribute to be leading cause of morbidity, mortality, and economic loss throughout the world.[1)] Respiratory syncytial virus (RSV) is a major cause of pneumonia and bronchiolitis in infants, in children, and even in adults.[2)] Ribavirin and immunoglobulin preparations with high titers of RSV-specific neutralizing antibodies are currently approved for use to treat and prevent RSV infections. However, neither of these is cost-effective and simple to administer.[3)] Parainfluenza type 3 virus (PIV 3) is another respiratory virus caused infections in children. No chemotherapy agents and vaccine have been used to treat PIV 3 infections.[4)] Therefore, the development of new antiviral agents with different mechanisms is most desirable. Traditional medicines utilizing natural products have been shown to contain antiviral compounds.[5—9)] The screening of plants as a possible source of antiviral has led to the discovery of potent inhibitors of *in vitro* viral growth and the use of the ethnopharmacological approach enhances the probability of identifying new bioactive plant compounds.[10)] Our interest has focused on the treatments for respiratory viral infections. In order to find more potent inhibitors of RSV and PIV 3, we have been looking for some inhibitory substances from natural sources.[11—17)]

Selaginella uncinata SPRING is a Chinese herb medicine widely distributed in south China, which has been used to treat infectious diseases and tumors. Previous phytochemical studies on the constituents of genus *Selaginella* led to the discovery of many compounds, including flavonoids and lignans.[18,19)] As part of our continuous searching for novel antivial agents from medicinal plants, ethanol extract of the whole plants of *S. uncinata* was found to show significant inhibitory activities against RSV. The active extract led to the isolation and characterization of two new compounds, named uncinoside A (**1**) and uncinoside B (**2**)[20)] together with three known compounds. Here we report the isolation and structure elucidation of the two new chromone glycosides and their antiviral activities against RSV, PIV 3, and influenza type A virus (Flu A).

Results and Discussion

An EtOH extract of the whole plant of *S. uncinata* was concentrated and partitioned with $CHCl_3$ and EtOAc. The $CHCl_3$ fraction was separated by silica gel column chromatography to yield two new chromone glycosides uncinoside A (**1**) and uncinoside B (**2**), and one known 5,7-dihydroxy-2,6-8-trimethylchromone (8-methyleugenitol) (**3**). The EtOAc fraction was separated by silica gel and polyamide column chromatography and prepared thin-layer chromatography (TLC) to afford two known biflavonoids: amentoflavone (**4**)[11,21)] and hinokiflavone (**5**)[21,22)] (Chart 1). The structure of the compound **3** was determined by the X-ray diffraction analysis and spectral data. The structures of the other two known biflavonoids were confirmed by comparison of the physical and spectral data with those reported.

Uncinoside A (**1**), yellow powder, obtained from the ethanol extract of this plant, mp 263—264 °C; positive result of Molisch's reaction; UV λ_{max} (MeOH) nm. 217, 263, 300; IR (KBr) cm^{-1} 3477 (OH), 2931 (CH), 1669 (C=O) and 1610 (C=C) indicated the presence of OH, C=O and C=C functionalities. Compound **1** exhibited a $[M+H]^+$ ion at *m/z* 383 in positive FAB-MS corresponding to a molecular formula of $C_{18}H_{22}O_9$. Complete acid hydrolysis of **1** afforded glucose, identified by comparison with authentic samples by TLC. The FAB-MS data 383 $[M+H]^+$, 221 $[M-Glc+H]^+$, confirmed above conclusion. The ^1H- and ^{13}C-NMR spectra (Table 1) of **1** clearly showed the presence of chromone skeleton, and there were three methyl and one hydroxyl substitutions on aglycon respectively, which was identified by comparison of it's NMR and IR data with those reported in the literatures.[23—26)] The sugar residue was clearly indicated by an anomeric carbon signal at δ_C 104.4, and the corresponding anomeric proton signal at δ_H 4.63 (1H, d, J=7.0 Hz). The D-configuration has been assumed for the glucoses in keeping with Massiot and Lavaud's assertion regarding the D-sugars commonly found in the plant kingdom: "The enantiomers of these sugars (glucose, galactose, *etc.*) are not found in plants, a fact used as a clue in the determination of these sugars."[27)] Evaluation of the spin–spin couplings and chemical shifts allowed the identification of β-D-glucose.

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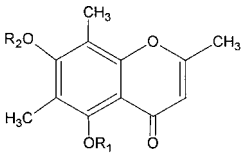
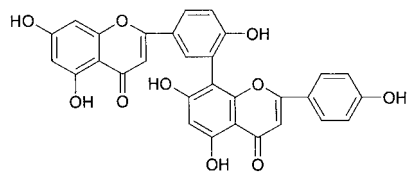


1. R_1 = H, R_2 = Glc

2. R_1 = Ac, R_2 = Glc

3. R_1 = H, R_2 = H



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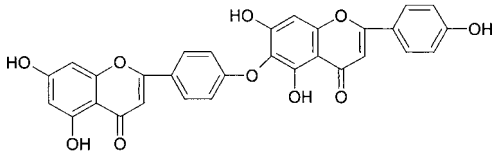

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Chart 1. Structures of Compounds **1**—**5**

The connectivity of the methyl, hydroxyl and glucose at the chromone skeleton of **1** was determined by an analysis of the heteronuclear multiple bond correlation spectroscopy (HMBC) (Fig. 1). In HMBC spectrum of **1**, correlation peaks were observed between H-1′ (δ_H 4.63) of glucose and C-7 (δ_C 158.75) of the aglycon, also between H-11 of 8-methyl (δ_H 2.29) and C-8 (δ_C 109.79), C-8a (δ_C 152.57), C-7 (δ_C 158.75). The correlation peaks between H-10 (δ_H 2.17) of 6-methyl and C-6 (δ_C 114.28), C-5 (δ_C 155.93) were also observed. It was concluded that the glucose was bonded to the hydroxyl at C-7 position of aglycon, and at the positions of C-6 and C-8, there were two methyl substitutions respectively. Moreover, the HMBC spectrum revealed the correlation peaks between H-9 (δ_H 2.42) of C-2 methyl and C-2 (δ_C 168.42), C-3 (δ_C 108.03), which conformed the substitution of methyl at C-2 position. The correlation peaks were observed between H-3 (δ_H 6.28) and C-4a (δ_C 106.52), C-2 (δ_C 168.42) and C-9 (δ_C 20.05) . The correlation peaks between 5-OH (δ_H 12.94) and C-5 (δ_C 155.93), C-6 (δ_C 114.28) and C4a (δ_C 106.52) were also observed. Thus, the structure of compound **1** was established to be 5-hydroxy-2,6,8-trimethylchromone7-*O*-*β*-D-glucopyranoside, named uncinaside A.

Uncinoside B (**2**), white powder, also obtained from the ethanol extract of this plant, mp 165—167 °C; positive result of Molisch's reaction; UV λ_{max} (MeOH) nm. 215, 260, 302; IR (KBr) cm^{-1} 3365 (OH), 2916 (CH), 1747 (C=O), 1657 (OC=O) and 1620 (C=C) indicated the presence of OH, C=O, OC=O and C=C functionalities. Compound **2** exhibited a $[M+H]^+$ ion at *m/z* 425 in positive FAB-MS corresponding to a molecular formula of $C_{20}H_{24}O_{10}$. Complete acid hydrolysis of **2** afforded glucose, identified by comparison with authentic samples by TLC. The ^{1}H- and ^{13}C-NMR spectra (Table 1) of **2** are very similar to **1**, except that there were two added carbon signals of acetyl in **2**. The chemical shifts were δ_C 172.51, δ_C 20.38 in ^{13}C-NMR and δ_H 1.85 (3H, s) in ^{1}H-NMR respectively. The FAB-MS data 425 $[M+H]^+$, 263 $[M-Glc+H]^+$, 220 $[M-Glc-Ac]^+$ confirmed above conclusion. The connectivity of the glucose, methyl and acetyl at the chromone skelten was determined by HMBC spectrum. The acetyl group is connected at 5-OH position of aglycone. This was also confirmed by the fact that the absence signal of 5-OH in ^{1}H-NMR spectrum, which was clearly observed in **1**. Thus, the structure of compound **2** was determined to be 5-acetyoxyl-2,6,8-trimethylchromone 7-*O*-*β*-D-glucopyranoside, named uncinaside B.

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of Compounds **1** and **2**

C	Compound **1**[a]		Compound **2**[a]	
	δ_C[b]	δ_H[c]	δ_C[b]	δ_H[c]
2	168.4		170.0	
3	108.0	6.28 (1H, s)	109.1	6.09 (1H, s)
4	182.7		185.0	
4a	106.5		108.2	
5	155.9	12.94 (1H, s, 5-OH)	157.8	
6	114.3		116.3	
7	158.8		160.2	
8	109.8		111.6	
8a	152.6		154.6	
9	20.1	2.42 (3H, s)	20.5	2.37 (3H, s)
10	9.0	2.17 (3H, s)	8.9	2.17 (3H, s)
11	9.1	2.29 (3H, s)	9.5	2.24 (3H, s)
5-OAc			172.5 (C=O)	
			20.4 (Me)	
1′	104.4	4.63 (1H, d, *J*=7.0)	105.7	1.85 (3H, s)
2′	74.1		75.3	4.65 (1H, d, *J*=7.5)
3′	76.3		75.6	
4′	69.9		71.7	
5′	77.0		77.7	
6′	61.0		64.3	

a) Measured in 500 MHz. *b*) Measured in 125 MHz. *c*) Coupling constants (*J* in Hz) are in parentheses.

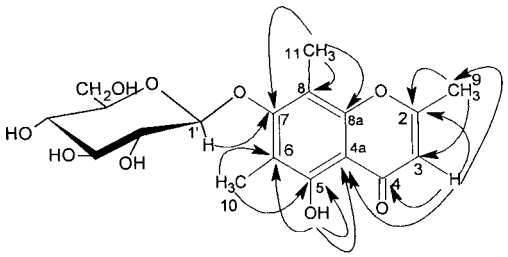

Fig. 1. Selected HMBC Correlation of Compound **1**

Compound **3** was obtained as yellow crystals, mp 282—284 °C. Its electron ionization-mass spectroscopy (EI-MS) gave the $[M]^+$ peak at *m/z* 220, corresponding to the molecular formula $C_{12}H_{12}O_4$. Compared its IR, ^{1}H- and ^{13}C-NMR spectral data and analyzed by acid hydrolysis experiment, **3** was the aglycone of **1**. An X-ray crystallographic study was performed to confirm the structure of **3**. A view of the solid

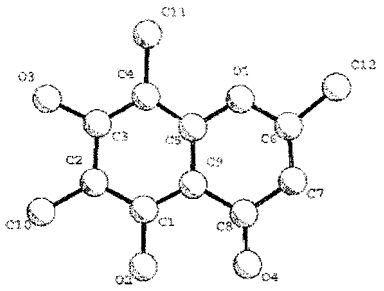

Fig. 2. Stereoview of **3** from X-Ray Diffraction Analysis

Table 2. Inhibitory Effects of Compounds **1** and **2** Isolated from *Selaginella uncinata* on RSV and PIV 3-Induced Cytopathogenicity in HEp 2 Cells

Compound	RSV			PIV 3		
	IC_{50} (μg/ml)[a]	TC_{50} (μg/ml)[b]	TI[c]	IC_{50} (μg/ml)[a]	TC_{50} (μg/ml)[b]	TI[c]
1	6.9	82.5	12.0	13.8	82.5	6.0
2	1.3	83.3	64.0	20.8	83.3	4.0
Ribavirin[d]	2.6	62.5	24.0	5.2	62.5	12.0

a) IC_{50} is the concentration of the sample required to inhibit virus-induced CPE 50%. *b*) TC_{50} is the concentration of the 50% cytotoxic effect. *c*) $TI=TC_{50}/IC_{50}$. *d*) Ribavirin, an approved drug for the treatment of RSV infections.

state conformation is provided in Fig. 2. The bond lengths were in accord with expectations. Thus, **3** was determined as 5,7-dihydroxyl-2,6,8-trimethylchromone (8-methyleugenitol).

The inhibitory activities of **1** and **2** against RSV, PIV 3 and Flu A (H_1N_1) were determined using the method described in the Experimental section. Uncinoside A (**1**) and uncinoside B (**2**) isolated from *S. uncinata* did not show any antiviral activities against Flu A (H_1N_1). The *in vitro* antiviral activities of the compounds **1** and **2** against RSV and PIV 3 are summarized in Table 2. Uncinoside B (**2**) showed potent anti-RSV activity with an IC_{50} value of 1.3 μg/ml and a TI value of 64.0, a large therapeutic index comparable to that of ribavirin with a TI value of 24.0, an approved drug for the treatment of RSV infections in human. **1** and **2** also showed moderate antiviral activities against PIV 3 with IC_{50} values of 13.8 and 20.8 μg/ml, TI values of 6.0 and 4.0, respectively.

Amentoflavone (**4**) had been recently reported to have potent antiviral activity against RSV with IC_{50} values of 5.5 μg/ml.[11] It also showed significant activity against Flu A and B viruses and exhibited moderate anti-herpes simplex virus (HSV)-1 and anti-HSV-2 activities with IC_{50} values of 17.9 μg/ml (HSV-1) and 48.0 μg/ml (HSV-2).[28] It also demonstrated moderate activity against human immunodeficiency virus (HIV)-1 reverse transcriptase (RT), with an IC_{50} value of 119 μg/ml.[29] Amentoflavone were also isolated from the whole herbs of *S. sinensis* and the leaves of *Celaenodendron mexicanum*, *Cupressocyparis leylandii*, *Ginkgo biloba*, *Rhus succedanea* and *Garcinia multiflora*.[11,30—33] Hinokiflavone (**5**) did not show any antiviral activities against RSV, PIV 3, and Flu A and B.[28]

Experimental

General Melting points were determined using Kofle instrument and are uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV spectra were recorded using a SHIMADZU UV-2100PC spectrophotometer. IR absorption spectra were obtained with Nicolet Impact 420 FT-IR instrument as a film on KBr disk. FAB-MS were recorded on VG Autospec 3000 system, and ESI-MS on Finnigan TSQ 7000. ^{1}H and ^{13}C spectra were obtained with INOVA 500 instrument operating at 500 MHz for ^{1}H, 125 MHz for ^{13}C, respectively. X-ray intensity of compound **3** was measured on a Bruker SMART 1000 CCD diffractometer. Chemical shifts are reported in parts per million on the δ scale with TMS as the internal standard, and coupling constants are in Hertz. Column chromatographies were performed with silica gel (Qingdao Haiyang Chemical Group Co. Ltd., China). TLC were performed on precoated Silica 60 F_{254} plates (0.2 mm thick, Merck) with $CHCl_3$–MeOH system and spots were detected by UV illumination and by spraying with 10% ethanolic H_2SO_4 reagent.

Plant Material Herb of *S. uncinata* was collected at Yunan Province, P. R. China, in September 1998. A voucher sample is preserved in the Museum for Materia Medica, National Institute for the Control of Pharmaceutical and Biological Products, Beijing.

Extraction and Isolation The dried whole herbs (6 kg) of *S. uncinata* were extracted with 90% EtOH (10 l×3). The ethanol solution was filtered and evaporated under reduced pressure to give a brownish ethanol extract (150 g). The EtOH extract was dissolved in water containing 0.5 N HCl and filtered. The acid solution was adjusted to pH 9—10 with NH_3H_2O and filtered. The basic solution was further extracted with $CHCl_3$ and ethyl acetate to give a chloroform extract (8.5 g), an ethyl acetate extract (5.0 g) and the residue. A part (8.5 g) of $CHCl_3$ extract was chromatographied on silica gel with $CHCl_3$–MeOH gradient system to give 6 fractions. The 6 fractions were combined into three fractions, Frs. 1—3, based on silica TLC ($CHCl_3$–MeOH, 90 : 10) results. 5,7-dihydroxyl-2,6,8-trimethylchromone (8-methyleugenitol) (**3**) (5 mg) was obtained from Fr. 1 by re-crystallization. Fraction 2 was purified by silica gel eluting with $CHCl_3$–MeOH to give uncinoside B (**2**) (16 mg). The Fr. 3 was purified by $CHCl_3$–MeOH to give uncinoside A (**1**) (40 mg). A part (5.0 g) of ethyl acetate extract was chromatographied on silica gel with $CHCl_3$–MeOH gradient system to give 4 fractions. Fraction 2 was purified by silica gel eluting with $CHCl_3$–MeOH (10 : 1) to give amentoflavone (**4**) (15 mg) and hinokiflavone (**5**) (12 mg).

Uncinoside A (**1**): Slight-yellow powder, mp 273—274 °C. UV λ_{max} (MeOH) nm: 207, 243, 256, 336. IR ν_{max} cm^{-1}: 3477, 1649, 1610—1500. FAB-MS *m/z*: 383 $[M+H]^+$. ^{1}H- and ^{13}C-NMR data of **1** are shown in Table 1.

Uncinoside B (**2**): White powder, mp 165—167 °C. UV λ_{max} (MeOH) nm: 203, 226, 243, 256. IR ν_{max} cm^{-1}: 3365, 1747, 1620—1500. FAB-MS *m/z*: 425 $[M+H]^+$. ^{1}H- and ^{13}C-NMR data of **2** are shown in Table 1.

5,7-Dihydroxy-2,6,8-trimethylchromone(8-methyleugenitol) (**3**): Molecular formula $C_{12}H_{12}O_4$, M.W. 220.22. Crystal size 0.20×0.20×0.60 mm, space group $P2_1$, unit cell parameters a=7.150(1), b=17.817(1), c=8.155(1) Å, β=95.16(4)°, V=1034.6(2) Å^3, Z=4. D_{calcd}=1.414 g·cm^{-3}. The diffraction data were collected on the MAC DIP-2030K Imaging Plate diffractometer with Mo$K\alpha$ radiation, $2\theta_{max}$ 50.0°. 1708 independent reflections, observed 1691 were used to the refinement. Structure was solved by direct methods and refinement by the full matric least square methods, the final Rf=0.056, Rw=0.054 ($w=1/\sigma^2|F|$).

Acid Hydrolysis of Compounds 1 and 2 A solution of **1** or **2** (3 mg) in 50% MeOH (5 ml) containing 2 N HCl (3 ml) was refluxed for 3 h, concentrated under reduced pressure, and diluted with H_2O (6 ml). It was extracted with $CHCl_3$ and the residue was recovered from the organic phase. The aqueous phase was concentrated and glucose was identified by the TLC with a standard using $CHCl_3$–MeOH (8 : 2).

Materials Dulbecco's modified Eagle's medium (DMEM) and phosphate buffered saline (PBS) were purchased from Sigma Co. (U.S.A.). Trypsin–EDTA (×10) and trypsin (1 : 250) were from Gibco Co. (U.S.A.). Fetal bovine serum (FBS) was from Biofluids Inc. (U.S.A.). Ribavirin (Sigma Chemical Co.)

Viruses and Cells RSV strain Long, PIV 3, MDCK cells and HEp 2 cells were obtained from American Type Culture Collection. Flu A (H_1N_1) strain was obtained from Guangzhou province, P. R. China.

Cytotoxicity Assay The cytotoxicity of test sample on virus was measured by the cytopathic effect assay (CPE).[12,13] Cell toxicity was monitored by determining the effect of the natural products on cell morphology and cell viability. Serial twofold dilutions of the natural products were added to confluent cell monolayers and the cells were cultivated at 37 °C for 2—5 d. The morphology of the cells was inspected daily and observed for microscopically detectable alterations, including the loss of monolayer, rounding, shrinking of the cells, granulation, and vacuolisation in the cytoplasm. The cytopathic effect was scored (scores: 0=0% CPE, 1=0—25% CPE, 2=25—

50% CPE, 3=50—75% CPE, 4=75—100% CPE). The 50% toxic concentration (TC_{50}), the concentration required to cause visible changes in 50% of intact cells, was estimated from graphic plots. The maximal non-cytotoxic concentration (MNCC) was determined as the maximal concentration of the natural products that did not exert toxic effect detected by microscopic monitoring.

Cytopathic Effect Reduction Assay The antiviral activity of test samples against viruses was measured by the CPE inhibition assay.[12,13,16,34] Two-fold serial dilutions of natural products were seeded into cells monolayers cultivated in 96-well culture plates, using the MNCC as the higher concentration. An infection control was made in the absence of natural products. An equal volume of virus suspension (100 $TCID_{50}$/ml) was added to the cells monolayers. The plates were incubated at 37 °C in a humidified CO_2 atmosphere (5% CO_2) for 2—5 d. After that, CPE was observed. The virus induced CPE was scored as described above in cytotoxicity assay. The reduction of virus multiplication was calculated as % of virus control (%virus control=$CPE_{exp}/CPE_{virus\ control}\times100$). The concentration reducing CPE by 50% with respect to virus control was estimated from graphic plots and was defined as 50% inhibited concentration (IC_{50}) expressed in (μg/ml). The therapeutic index (TI) was calculated from the ratio TC_{50}/IC_{50}.

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References

1) Kaiser L., Couch R. B., Galasso G. J., Glezen W. P., Webster R. G., Wright P. F., Hayden F. G., *Antiviral Res.*, **42**, 149—176 (1999).
2) Treanor J., Falsey A., *Antiviral Res.*, **44**, 79—102 (1999).
3) Kneyber M. C. J., Mou H. A., Groot R. D., *Eur. J. Pediatr.*, **159**, 339—411 (2000).
4) Galasso G. J., Whitley R. J., Merigan T. C., "Antiviral Agents and Human Viral Diseases," 4th ed., Lippincott-Raven Publishers, Philadelphia, 1997, pp. 369—413.
5) Kurokawa M., Ochiai H., Nagasaka K., Neki M., Xu H., Kadota S., Sutardjo S., Matsumoto T., Namba T., Shiraki K., *Antiviral Res.*, **22**, 175—188 (1993).
6) Hayashi K., Nishino H., Niwayama S., Shiraki K., Hiramatsu A., *Antiviral Res.*, **17**, 323—333 (1992).
7) Syddislis R. J., Owen D. G., Lophr J. L., Rosler K. H. A., Blomster R. N., *Antimicrob. Agents Chemother.*, **35**, 2363—2466 (1991).
8) Tang J., Colacino J. M., Larsen S. H., Spitzer W., *Antiviral Res.*, **13**, 313—326 (1990).
9) Yao X. J., Wainberg M. A., Parniak M. A., *Virology*, **187**, 56—62 (1992).
10) Baker T., Borris R., Carte B., Cragg G., Gupta M., Iwu M., Madulid D., Tyler V., *J. Nat. Prod.*, **58**, 1325—1357 (1995).
11) Ma S. C., But P. P. H., He Y. H., Lee S. H. S., Lee S. F., Lin R. C., *Biol. Pharm. Bull.*, **24**, 311—312 (2001).
12) Ma S. C., He Z. D., But P. P. H., Ooi V. E. C., Xu H. X., Lee S. H. S., Lee S. F., *Chem. Pharm. Bull.*, **49**, 1471—1473 (2001).
13) Ma S. C., Du J., But P. P. H., Deng X. L., Zhang Y. W., Ooi V. E. C., Lee S. H. S., Lee S. F., *J. Ethnopharmacol.*, **79**, 203—211 (2002).
14) Jiang R. W., Ma S. C., But P. P. H., Ye W. C., Chan S. P., Mak T. C. W., *Tetrahedron Lett.*, **43**, 2415—2418 (2002).
15) Jiang R. W., Ma S. C., But P. P. H., Mak T. C. W., *J. Nat. Prod.*, **64**, 1266—1272 (2001).
16) Li Y. L., Ma S. C., Yang Y. T., But P. P. H., *J. Ethnopharmacol.*, **79**, 365—368 (2002).
17) Jiang R. W., Ma S. C., He Z. D., Huang X. S., But P. P. H., Wang H., Chan S. P., Ooi V. E. C., Xu H. X., Mak T. C. W., *Bioorg. Med. Chem.*, **10**, 2161—2170 (2002).
18) Lin R. C., Alexios L. S., Elisabeth S., Francois T., Michel K., *Planta Med.*, **60**, 168—170 (1994).
19) Lin R. C., Elisabeth S., Francois T., Michel K., *J. Nat. Prod.*, **50**, 422—426 (1987).
20) Ma L. Y., Wei F., Ma S. C., Lin R. C., *Chinese Chemical Letters*, **13**, 748—751 (2002).
21) Kenneth R. M., Carolyn S., Hans G., *Phytochemistry*, **26**, 3335—3337 (1987).
22) Taichi O., Ohgi Y., *Chem. Pharm. Bull.*, **31**, 919—924 (1983).
23) Yu D. Q., Yang J. S., "Fenxi Huaxue Shouce," Chemical Industry Press, Beijing, 1999, p. 840.
24) Wu Q. L., Liao Y. H., Wang S. P., Wang L. W., Feng Y. X., Yang J. S., Xiao P. G., Hong S. L., *Chin. Chem. Lett.*, **8**, 41—42 (1997).
25) Carlos J., Manuel M., Mary C. V., Ricardo R., Luis C., France S., *Phytochemistry*, **28**, 1992—1993 (1989).
26) Bohlmann F., Misra L. N., Jakupovic J., *Planta Med.*, **50**, 174—176 (1984).
27) Massiot G., Lavaud C., "Studies in Natural Products Chemistry," Vol. 15, Elsevier Sciences, Amsterdam, 1995, pp. 187—224.
28) Lin Y. M., Flavin M. T., Schure R., Chen F. C., Sidwell R., Barnaral D. L., Huffman J. H., Kern E. R., *Planta Med.*, **65**, 120—125 (1999).
29) Lin Y. M., Anderson H., Flavin M. T., Pai Y. H., Mata-Greenwood E., Pengsuparp T., Peezzuto J. M., Schinazi R. F., Hughes S. H., Chen F. C., *J. Nat. Prod.*, **60**, 884—888 (1997).
30) Camacho M. R., Mata R., Castaneda P., Kirby G. C., Warhurst D. C., Croft S. L., Philipson J. D., *Planta Med.*, **66**, 463—468 (2000).
31) Krauze-Baranowska M., Cisowski W., Wiwart M., Madziar B., *Planta Med.*, **66**, 572—573 (1999).
32) Lin Y. M., Anderson H., Flavin M. T., Pai Y. H., Mata-Greenwood E., Pengsuparp T., Pezzuto J. M., Schinazi R. F., Hughes S. H., Chen F. C., *J. Nat. Prod.*, **60**, 884—888 (1997).
33) Lobstein-Guth A., Briancon-Scheid F., Victoire C., Haag-Berruier M., Anton R., *Planta Med.*, **54**, 555—556 (1988).
34) Ubillas R., Jolad S. D., Bruening R. C., Kernan M. R., King S. R., Sesin D. F., Barrett M., Stoddart C. A., Flaster T., Kuo J., Ayala F., Meza E., Castanel M., McMeekin D., Rozhon E., Tempesta M. S., Barnard D., Huffman J., Smee D., Sidwell R., Soike K., Brazier A., Safrin S., Orlando R., Kenny P. T., Berova N., Nakanishi K., *Phytomedicine*, **1**, 77—106 (1994).