Fitoterapia 89 (2013) 183–187

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Three new secoiridoids, swermacrolactones A–C and anti-hepatitis B virus activity from *Swertia macrosperma*

Hong-Ling Wang [a,b], Chang-An Geng [a], Yun-Bao Ma [a], Xue-Mei Zhang [a], Ji-Jun Chen [a,*]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, PR China
[b] University of Chinese Academy of Sciences, Beijing 100039, PR China

ARTICLE INFO

Article history:
Received 5 February 2013
Accepted in revised form 26 May 2013
Available online 10 June 2013

Keywords:
Secoiridoids
Swermacrolactones A–C
Anti-HBV activity
Swertia macrosperma
Gentianaceae

ABSTRACT

Three new secoiridoids, swermacrolactones A–C (**1**–**3**), together with fourteen known compounds were isolated from *Swertia macrosperma*. Their structures were elucidated based on extensive spectroscopic analyses (IR, UV, MS, 1D and 2D NMR). By anti-HBV assay on the Hep G 2.2.15 cell line *in vitro*, the most active compound, luteolin (**9**), inhibited the secretion of hepatitis B virus surface antigen (HBsAg) and hepatitis B virus e antigen (HBeAg) with IC_{50} values of 0.02 and 0.02 mM, respectively.

1. Introduction

Plants of the genus *Swertia* (Gentianaceae), annual or perennial herbs, are mainly distributed in Asia, Africa and North America. There are about 170 *Swertia* species all over the world, of which 79 species and 10 variants are present in China [1,2]. Reported in the literature, xanthones, secoiridoids and triterpenoids are the main constituents of the *Swertia* species. Although the extracts and monomer compounds possess many pharmacological activities, for example, oleanolic acid, gentiopicroside, pseudonolin and methylbellidiforlin have a hepatoprotective property, its anti-hepatitis B virus (HBV) constituents remain unclear [3–5]. Our previous investigation on *Swertia* species had led to the isolation of several xanthones, secoiridoids and lactonic enamino ketones with their anti-HBV activity [6–15]. *Swertia macrosperma*, belonging to the family Gentianaceae, was widely distributed in Tibet, Guizhou, and Yunnan provinces of China for the treatment of hepatitis. Our bioassay suggested that the water extract exhibited inhibitory activities on the secretion of HBsAg and HBeAg with IC_{50} values of 0.22 and $<0.14\ mg\cdot mL^{-1}$ [12]. In the course of our continuous search for active anti-HBV compounds from natural products, compounds **1**–**17** were isolated from *S. macrosperma* and assayed on Hep G 2.2.15 cell line *in vitro*. This paper described their isolation, structural elucidation and anti-HBV activities *in vitro*.

2. Experimental

2.1. General experimental procedures

Optical rotations were determined on a Jasco model 1020 polarimeter (Horiba, Tokyo, Japan). UV spectra were measured on a Shimadzu UV2401PC spectrophotometer (Shimadzu, Kyoto, Japan). IR (KBr) spectra were recorded on a Bio-Rad FTS-135 spectrometer (Bio-Rad, Hercules, California, USA). 1D and 2D NMR were recorded on Bruker AM-400, Bruker DRX-500 or AVANCE III-600 spectrometers (Bruker, Bremerhaven, Germany). Mass spectra were ran on a VG Spec-3000 spectrometer (VG, Manchester, UK) and Waters AutoSpec Premier P776 (Waters, USA). Silica gel (200–300 mesh) for column chromatography and TLC plates (GF_{254}) were obtained from Qingdao Makall Chemical

* Corresponding author. Tel.: +86 871 65223265; fax: +86 871 65227197.
E-mail address: chenjj@mail.kib.ac.cn (J.-J. Chen).

0367-326X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.fitote.2013.06.002

Company (Makall, Qingdao, China). Semi-preparative HPLC was carried out on Waters Alliance 2695 liquid chromatography with an Eclipse XDB-C_{18} (9.4 × 250 mm) column (Waters, Milford, MA, USA). Fractions were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

2.2. Plant material

The whole plants of *S. macrosperma* (C. B. Clarke) C. B. Clarke were collected in Yuxi, Yunnan Province, P. R. China, in November 2008, which was identified by Dr. Professor Li-Gong Lei (Kunming Institute of Botany, Chinese Academy of Sciences). A voucher specimen (No. 20081102) was deposited in the Laboratory of Antivirus and Natural Medicine Chemistry, Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The air-dried and powered plant material (5.0 kg) was extracted with water (12 L) under reflux for three times, 1 h for each time. The extract was concentrated under *vacuum* to give a residue (585 g), which was suspended in H_2O and partitioned between petroleum ether, EtOAc and *n*-BuOH for three times (2 L × 3), successively. The EtOAc fraction (80 g) was subjected to silica column chromatography (Si CC, 800 g, 8 × 50 cm) and eluted with $CHCl_3$–MeOH–H_2O (100:0:0, 95:5:0, 80:20:2, 70:30:3, 60:40:4, 0:100:0, *v/v*, each 10 L) gradient to afford five fractions (A–E). Fr. B (25 g) was further separated to obtain five subfractions (B1–B5) by Si CC with $CHCl_3$–Me_2CO (80:20, 70:30, 60:40, 50:50, each 1.5 L) as the eluent. Fr. B1 (4.5 g) was performed on Si CC (4 × 22 cm, 112 g) and eluted with petroleum ether-Me_2CO (80:20, 70:30, each 800 mL) to yield compounds **1** (5 mg) and **15** (12 mg). Fr. B2 (0.8 g) was applied to Si CC (2.0 × 25 cm, 30 g) with an eluent of petroleum ether-EtOAc (70:30, 60:40, each 500 mL) to produce compounds **2** (5 mg) and **3** (7 mg). Fr. B3 (8.2 g) was treated with $CHCl_3$–MeOH (95:5, 90:10, 80:20, 70:30, 60:40, each 2.0 L) to obtain 4 subfractions (Frs. B3-1–B3-4). Compounds **4** (10 mg), **5** (8 mg), **6** (5 mg) and **7** (6 mg) were isolated from Fr. B3-2 (1.2 g) by Si CC (2.0 × 30 cm, 38 g) with $CHCl_3$–MeOH–H_2O (95:5: 0, 90:10:1, each 500 mL). Fr. B3-3 (0.6 g) was treated with the same procedure as Fr. B1 to get four fractions (Frs. B3-3-1–B3-3-4). Fr. B3-3-1 (120 mg) was purified by column chromatography over silica gel (1.5 × 23 cm, 10 g, $CHCl_3$–Me_2CO, 50:50, each 300 mL), and followed by semi-preparative HPLC (MeOH–H_2O, 30:70) to provide compounds **12** (4 mg, t_R = 15.72 min) and **13** (6 mg, t_R = 16.85 min). Fr. B3-3-1 (108 mg), Fr. B3-3-2 (208 mg) and Fr. B3-3-3 (86 mg) were further separated by Si CC (3 × 25 cm, 65 g) with $CHCl_3$–MeOH–H_2O (90:10:1), respectively, to furnish compounds **8** (8 mg), **9** (25 mg), **10** (32 mg), **11** (7 mg), **14** (11 mg), **16** (6 mg) and **17** (5 mg). All obtained compounds had a degree of purity > 90%, based on the TLC method in three different solvent systems that exhibited one spot both under UV radiation and visualized with H_2SO_4, and NMR spectra with the smooth baseline and no impurity peaks.

Swermacrolactone A (**1**): colorless oil; UV (MeOH) λ_{max} (log ε): 229 (3.86) nm; IR (KBr) ν_{max}: 3429, 2938, 1701, 1062 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; HRESIMS *m/z* 193.0846 ($[M + Na]^+$, $C_9H_{14}O_3Na$, calcd for 193.0840).

Swermacrolactone B (**2**): pale yellow gum; $[\alpha]_D^{17.7}$: −104.13 (*c* 0.33, $CHCl_3$–MeOH 1:1, *v/v*); IR (KBr) ν_{max}: 3366, 3275, 1676, 1601, 1055 cm^{-1}; UV (MeOH) λ_{max} (log ε): 248 (3.88), 215 (3.71) nm; ^1H NMR and ^{13}C NMR data, see Table 1; HREIMS *m/z* 214.0834 ($[M]^+$, $C_{10}H_{14}O_5$, calcd for 214.0841).

Swermacrolactone C (**3**): colorless oil; $[\alpha]_D^{24.5}$: +64.48 (*c* 0.41, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε): 240 (3.49) nm; IR (KBr) ν_{max}: 3430, 1705, 1066 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 199.0975 ($[M + H]^+$, $C_{10}H_{15}O_4$, calcd for 199.0970).

2.4. Anti-HBV assay on Hep G 2.2.15 cell line in vitro

The anti-HBV assay was performed according to our previous report [9]. Tenofovir, purchased from Jiangxi Chenyang Pharmaceutical Co. Ltd, China (purity > 97.6%, No. 200904009) was used as the positive control.

3. Results and discussion

Swermacrolactone A (**1**) was obtained as colorless oil and its molecular formula was determined to be $C_9H_{14}O_3$ by the

Table 1
^1H NMR and ^{13}C NMR data for compounds **1** (600/150 MHz, in $CDCl_3$), **2** (400/100 MHz, in C_5D_5N) and **3** (400/100 MHz, in $CDCl_3$).

Position	1		2		3	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	3.80 (2H, t, 6.5)	60.6 (t)	5.00 (1H, m)	80.5 (d)	3.88 (1H, m)	70.3 (d)
3	2.68 (2H, t, 6.5)	36.6 (t)	8.03 (1H, s)	155.1 (d)	4.29 (1H, overlapped) 4.37 (1H, overlapped)	61.9 (t)
4	–	130.7 (s)	–	105.3 (s)	–	149.9 (s)
5	–	149.9 (s)	2.76 (1H, m)	30.0 (d)	–	125.0 (s)
6	2.43 (2H, t, 6.2)	28.9 (t)	1.39 (1H, m), 2.01 (1H, m)	28.0 (t)	2.70 (1H, m) 2.28 (1H, m)	26.2 (t)
7	4.28 (2H, t, 6.2)	65.5 (t)	4.21 (1H, m) 4.29 (1H, m)	68.2 (t)	4.44 (2H, m)	65.9 (t)
8	1.08 (3H, t, 7.8)	14.3 (q)	3.94 (1H, m) 4.06 (1H, m)	60.3 (t)	3.79 (1H, dd, 11.0, 2.8) 3.88 (1H, overlapped)	60.8 (t)
9	2.37 (2H, q, 7.4)	20.4 (t)	1.99 (1H, m)	42.1 (d)	1.83 (1H, m)	46.5 (d)
10		165.7 (s)	4.06 (1H, m) 4.21 (1H, m)	60.5 (t)	1.31 (3H, d, 6.4)	18.8 (q)
11	–	–	–	165.4 (s)	–	163.5 (s)

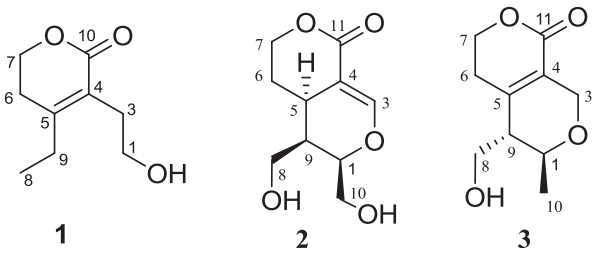

Fig. 1. Structures of compounds **1**–**3**.

positive HRESIMS (*m/z* 193.0846 $[M + Na]^+$), indicating three degrees of unsaturation. The presence of hydroxyl (3429 cm^{-1}) and carbonyl (1701 cm^{-1}) groups was deduced from the IR spectrum. The ^{13}C NMR (DEPT) spectrum displayed 9 carbon resonances, assigned to three quaternary carbons, five methylenes (two oxygenated) and one methyl.

In the ^1H and ^{13}C NMR spectra, the carbons observed at δ_C 165.7 (s, C-10), 130.7 (s, C-4), 149.9 (s, C-5), 28.9 (t, C-6) and 65.5 (t, C-7), in combination with proton resonances at δ_H 2.43 (2H, t, $J = 6.2$ Hz, H-6) and 4.28 (2H, t, $J = 6.2$ Hz, H-7), indicated an α, β-unsaturated δ-lactone fragment, which was further confirmed by $^1H-^1H$ COSY (H-6/H-7) and HMBC (H-6/C-4, C-7 and H-7/C-5, C-10) experiments. An additional ethyl group was proposed at C-5 on the basis of the HMBC correlations of H-8 with C-5; and H-9 with C-4, C-6, in combination with $^1H-^1H$ COSY correlation of H-8 to H-9. Accordingly, the residual hydroxyethyl group was located at C-4 by the HMBC correlations from H-1 to C-4 and H-3 to C-5, C-10. Thus, the structure of compound **1** was determined as swermacrolactone A.

Swermacrolactone B (**2**), pale yellow gum, was assigned to have a molecular formula of $C_{10}H_{14}O_5$ based on HREIMS (*m/z* 214.0834 $[M]^+$). The IR spectrum displayed absorption attributable to hydroxyl (3366, 3275 cm^{-1}) and carbonyl (1676 cm^{-1}). The ^1H and ^{13}C NMR spectra of compound **2** (Table 1) displayed 10 carbon signals due to four methylenes, four methines and two quaternary carbons, of which one carbonyl and two double carbons were characterized. The careful analyses of ^1H NMR and ^{13}C NMR spectroscopic data suggested that the structure of **2** was similar to metabolite A [16] except for one more hydroxymethyl moiety instead of the methyl at C-1, which was confirmed by the correlations of H-10 to C-1 and C-9 in the HMBC spectrum (Fig. 2). The relative configuration of compound **2** was defined by the ROESY experiment (Fig. 3) in which the correlations between H-1 and H-9, H-5 and H-9 were observed, indicating the structure of compound **2** was characterized as shown in Fig. 1.

Swermacrolactone C (**3**), colorless oil, had a molecular formula of $C_{10}H_{14}O_4$ as determined by the positive HRESIMS (*m/z* 199.0975 $[M + H]^+$), indicating four degrees of unsaturation. The presence of hydroxyl (3430 cm^{-1}) and carbonyl (1705 cm^{-1}) groups was deduced from the IR spectrum. The ^{13}C-NMR (DEPT) exhibited 10 carbon resonances attributed to three quaternary carbons, two methines, four methylenes (three oxygenated), and one methyl (Table 1). Comparison of its NMR data with those of metabolite A [16] indicated that they possessed a similar skeleton and the main difference was that the position of the double bond located in between C-4 and C-5 in compound **3**, which was further confirmed by HMBC (Fig. 2) correlations from H-7 to C-5, C-11; H-6 to C-4, C-5, C-9; and H-9 to C-4, C-5. In the ROESY experiment (Fig. 3), the correlation between H-1 and H-8 suggested that H-1 and the hydroxymethyl fragment were cofacial. Therefore, the relative configuration of swermacrolactone B was determined as shown in Fig. 1.

The other fourteen compounds were determined as *epi*-eustomoside (**4**) [17], 7-*epi*-vogeloside (**5**) [18], desacetylcentapicrin (**6**) [19], sweroside (**7**) [20], swertiaside A (**8**) [21], luteolin (**9**) [22], isovitexin (**10**) [23], (−)-berchmol (**11**) [24], *erythro*-guaiacylglycerol-β-coniferyl aldehyde ether (**12**) [25], *threo*-guaiacylglycerol-β-coniferyl aldehyde ether (**13**) [25], *erythro*-1,2-bis(4-hydroxy-3-methoxyphenyl)-1,3-propanediol (**14**) [26], erythrocentaurin (**15**) [27], balanophpnin (**16**) [28] and coniferyl alcohol (**17**) [29], by comparison of their spectroscopic data with those reported.

In order to evaluate their anti-HBV activities, compounds **1**, **6**, **9**, **12**–**14**, **16**, **17** were assayed on the Hep G 2.2.15 cell line *in vitro* (some other compounds with anti-HBV activities had been reported in our previous study [14,30]). The anti-HBV assay was performed according to the previous report [9] by using tenofovir as the positive control (Table 2). The most active compound, luteolin (**9**) exhibited inhibitory activity on the secretion of HBsAg (IC_{50} 0.02 mM) and HBeAg (IC_{50} 0.02 mM). Compound **16** also showed activities against the secretion of HBsAg (IC_{50} 2.00 mM) and HBeAg (2.41 mM). Compound **17** only displayed inhibitory activity on HBsAg with an IC_{50} value of 0.62 mM, but no inhibitory activity on HBeAg. Based on our previous results, it was showed that compound **9** improved anti-HBV activity compared with *epi*-eustomoside (**4**) [14], 7-*epi*-vogeloside (**5**) [30] and erythrocentaurin (**15**) [14], and compounds **16**

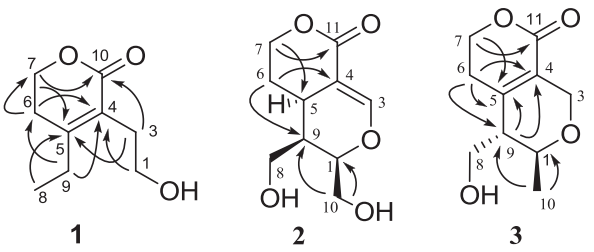

Fig. 2. Selected HMBC correlations of compounds **1**–**3**.

Table 2
Anti-HBV activities of compounds **1**, **6**, **9**, **12**–**14**, **16**, **17**.[a]

No.	CC_{50} (mM) and the cell destroy rate (%)[b]	HBsAg[c]		HBeAg[d]	
		IC_{50} (mM) or the inhibitory rate (%)[b]	SI	IC_{50} (mM) or the inhibitory rate (%)[b]	SI
1	>5.10 (39.4%)	>5.10 (2.3%)	–	>5.10 (3.6%)	–
6	>0.47 (43.7%)	>0.47 (27.2%)	–	>0.47 (32.1%)	–
9	0.08 (0.07–0.09)	0.02 (0.01–0.03)	4.00	0.02 (0.01–0.03)	4.00
12	>3.52 (−28.3%)	>3.52 (10.4%)	–	>3.52 (−20.3%)	–
13	>3.25 (−18.9%)	>3.25 (46.4%)	–	>3.25 (−36.1%)	–
14	>2.68 (28.2%)	>2.68 (26.7%)	–	>2.68 (−9.4%)	–
16	>3.02 (28.6%)	2.00 (1.96–2.04)	>1.51	2.41 (2.38–2.44)	>1.25
17	1.41 (1.38–1.44)	0.62 (0.59–0.65)	>2.27	>1.52 (20.8%)	–
Tenofovir[e]	>4.25 (3.8%)	4.02 (3.99–4.05)	>1.06	3.02 (3.00–3.04)	>1.41

[a] All values are the mean of two independent experiments.
[b] CC_{50} = 50% cytotoxic concentration, IC_{50} = 50% inhibition concentration, if the CC_{50} or IC_{50} was not reached at the tested concentration, the cell destroy or inhibitory rate was given; SI = CC_{50}/IC_{50}.
[c] HBsAg: HBV surface antigen.
[d] HBeAg: HBV e antigen;
[e] Tenofovir, an antiviral agent used as the positive control.

and **17** displayed similar activity with 7-*epi*-vogeloside (**5**) [14] and erythrocentaurin (**15**) [14], but enhanced anti-HBV activity compared to *epi*-eustomoside (**4**) [30].

In conclusion, it is well-known that secoiridoids widely coexist in the Gentianaceae family, of which secoiridoid glycosides are the main constituents. Therefore, the isolation, structural elucidation and anti-HBV properties of three new secoiridoids, swermacrolactones A–C, not only enrich the amount of secoiridoids, but also provide information for the comprehensive identification of the active constituents of *S. macrosperma*.

Conflict of interest

There are no conflicts of interests among all authors in this manuscript.

Acknowledgments

This work was supported by the National Natural Science Foundation of China for Distinguished Young Scholars (No. 81025023), the National Natural Science Foundation of China and Yunnan Province (Grant No. U0832603), the West Light Foundation of the Chinese Academy of Sciences, and the International Foundation for Science, Stockholm, Sweden, through a grant (No. F/5202-1 to Chang-An Geng). We are grateful to the staff of the analytical group of the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

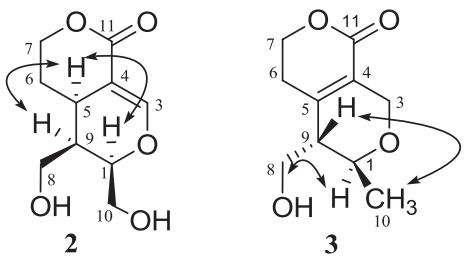

Fig. 3. Selected ROESY correlations of compounds **2**–**3**.

Appendix A. Supplementary data

1D and 2D NMR, HRESIMS, HREIMS, IR, and UV spectra of compounds **1**–**3** as well as the procedure for anti-HBV assay and the anti-HBV data from two independent experiments are available as supporting information. Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2013.06.002.

References

[1] Ho TN. Flora of China, vol. 62. Beijing: Science Press; 1988 344.
[2] Yang YC. Zangyaozhi. Qinghai: People of Qinghai Press; 1992 104.
[3] Tian LT, Ma L, Du NS. Pharmacological review of oleanolic acid. Zhongguo Zhongyao Zazhi 2002;27:884–6.
[4] Hase K, Li J, Basnet P, Xiong Q, Takamura S, Namba T, et al. Hepatoprotective principles of *Swertia japonica* Makino on D-galactosamine/lipopolysaccharide-induced liver injury in mice. Chem Pharm Bull 1997;45:1823–7.
[5] Li JC, Feng L, Sun BH, Ikeda T, Nohara T. Hepatoprotective activity of the constituents in *Swertia pseudochinensis*. Biol Pharm Bull 2005;28:534–7.
[6] Geng CA, Jiang ZY, Ma YB, Luo J, Zhang XM, Wang HL, et al. Swerilactones A and B, anti-HBV new lactones from a traditional Chinese herb: *Swertia mileensis*. Org Lett 2009;11:4120–3.
[7] Geng CA, Zhang XM, Ma YB, Shen Y, Zuo AX, Liu JF, et al. Swerilactones C and D, anti-HBV new lactones from a traditional Chinese herb: *Swertia mileensis*. Org Lett 2009;11:4838–41.
[8] Geng CA, Zhang XM, Ma YB, Luo J, Zhou J, Wang HL, et al. Swerilactones E–G, three unusual lactones from *Swertia mileensis*. Tetrahedron Lett 2010;51:2483–5.
[9] Geng CA, Wang LJ, Zhang XM, Ma YB, Luo J, Guo RH, et al. Anti-hepatitis B virus active lactones from the traditional Chinese herb: *Swertia mileensis*. Chem Eur J 2011;17:3893–903.
[10] Geng CA, Zhang XM, Ma YB, Luo J, Chen JJ. Swerilactones L–O, secoiridoids with C_{12} and C_{13} skeletons from *Swertia mileensis*. J Nat Prod 2011;74:1822–5.
[11] He K, Ma YB, Geng CA, Zhang XM, Cao TW, Jiang FQ, et al. Anti-hepatitis B virus active secoiridoids from *Swertia kouitchensis*. Nat Prod Bioprospect 2011;1:48–51.
[12] Wang HL, Geng CA, Zhang XM, Ma YB, Jiang ZY, Chen JJ. Chemical constituents of *Swertia macrosperma*. Zhongguo Yaoxue Zazhi 2010;35:3161–4.
[13] Jiang FQ, Zhang XM, Ma YB, Geng CA, Jiang ZY, Chen JJ. Chemical constituents of *Swertia hispidicalyx*. Zhongguo Zhongyao Zazhi 2011;36:2215–8.

[14] He K, Ma YB, Cao TW, Wang HL, Jiang FQ, Geng CA, et al. Seven new secoiridoids with anti-hepatitis B virus activity from *Swertia angustifolia*. Planta Med 2012;78:814–20.
[15] Wang HL, He K, Geng CA, Zhang XM, Ma YB, Luo J, et al. Gentiocrucines A–E, five unusual lactonic enamino ketones from *Swertia macrosperma* and *Swertia angustifolia*. Planta Med 2012;78:1867–72.
[16] El-Sedawy AI, Hattori M, Kobashi K, Namby T. Metabolism of sweroside from *Swertia japonica* by human intestinal bacteria. Shoyakugaku Zasshi 1990;44:122–6.
[17] Luo LH, Nie RL. Iridoid glycosides from *Swertia angustifolia*. Yao Xue Xue Bao 1992;27:125–9.
[18] Bi YF, Tian Y, Pei SS, Liu HM. Secoiridoid glycosides from *Flos lonicerae*. Zhongcaoyao 2008;39:18–21.
[19] Van der Sluis WG, Labadie RP. Secoiridoids and xanthones in the genus *Centaurium*. Planta Med 1981;41:150–60.
[20] Inouye H, Ueda S, Nakamura Y. Structure of sweroside, a new glucoside from *Swertia japonica*. Tetrahedron Lett 1966;43:5229–34.
[21] Ikeshino Y, Tomita Y. A new iridoid glycoside of *Swertia japonica*. Planta Med 1984;50:485–8.
[22] Feng J, Yang XW. Constituents from the leaves of *Aquilaria sinensis*. Zhongguo Zhongyao Zazhi 2012;37:230–4.
[23] Li W, Chen FK, Yin XG, Liu XQ. Chemical constituents of *Folium isatidis*. Shenyang Yaoke Daxue Xuebao 2005;22:15–6.
[24] Sakurai N, Nagashima SI, Kawai K, Takao I. A new lignan, (−)-berchmol, from *Berchemia racemosa*. Chem Pharm Bull 1989;37:3311–5.
[25] Gao GC, Tao SH, Zhang S, Li QX. Study on lignans from *Catunaregam spinosa*. Zhongguo Yaoxue Zazhi 2010;45:650–3.
[26] Luo XJ, Yang SL, Pei YH. Chemical constituents from *Acanthopanax senticosus*. Zhongcaoyao 2011;42:852–5.
[27] Wei SH, Yu CY, Lu LM, Chen G. Nor-iridoid constituents from roots of *Gentiana straminea*. Zhongcaoyao 2011;41:1242–5.
[28] Li XS, Jiang ZY, Wang FS, Ma YB, Zhang XM, Chen JJ. Chemical constituents from herbs of *Swertia mileensis*. Zhongguo Zhongyao Zazhi 2008;33:2790–3.
[29] Tao HM, Wang LS, Zhao DQ, Zhu QH, Liu YH. Phenolic compounds from roots of *Asparagus filicinus*. Zhongcaoyao 2011;42:2181–5.
[30] Wang HL, Chen H, Geng CA, Zhang XM, Ma YB, Jiang ZY, et al. Chemical constituents of *Halenia elliptica*. Zhongguo Zhongyao Zazhi 2011;36:1454–7.