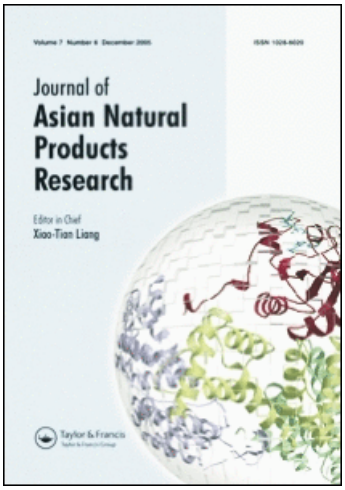

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Novel sesquiterpene and coumarin constituents from the whole herbs of *Crossostephium chinense*

Qi Wu[a]; Lei Zou[b]; Xiu-Wei Yang[a]; De-Xian Fu[b]
[a] State Key Laboratory of Natural and Biomimetic Drugs, Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University, Beijing, China [b] The State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing, China

To cite this Article Wu, Qi , Zou, Lei , Yang, Xiu-Wei and Fu, De-Xian(2009) 'Novel sesquiterpene and coumarin constituents from the whole herbs of *Crossostephium chinense*', Journal of Asian Natural Products Research, 11: 1, 85 — 90
To link to this Article: DOI: 10.1080/10286020802435703
URL: http://dx.doi.org/10.1080/10286020802435703

Journal of Asian Natural Products Research
Vol. 11, No. 1, January 2009, 85–90

Novel sesquiterpene and coumarin constituents from the whole herbs of *Crossostephium chinense*

Qi Wu[a], Lei Zou[b], Xiu-Wei Yang[a]* and De-Xian Fu[b]

[a]*State Key Laboratory of Natural and Biomimetic Drugs, Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University, Beijing, China;* [b]*The State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing, China*

(*Received 5 May 2008; final version received 19 July 2008*)

Novel sesquiterpene, named crossostephin (**1**), and coumarin, named biscopoletin (**2**), together with four known compounds, artesin, tanacetin, scopoletin, and scopolin, were isolated from the 70% ethanolic extract of the whole herbs of *Crossostephium chinense* (L.) Makino. Their structures were determined by spectroscopic methods, including IR, UV, EIMS, HRFTICRMS, 1D and 2D NMR spectral analyses. Both scopoletin and scopolin were isolated from the title plant for the first time.

Keywords: *Crossostephium chinense* (L.) Makino; sesquiterpene; coumarin; crossostephin; biscopoletin

1. Introduction

Crossostephium chinense (L.) Makino is a plant of the genus *Crossostephium* Less. family Compositae, which has been used as a folk herbal drug in southward China and recorded in many Chinese herbal books. The whole herbs of *C. chinense* are used for the treatment of diabetes, wind-cold type of common cold, carbuncle, and furuncle. Previous phytochemical studies on the plant resulted in the isolation of triterpenoids [1,2] and flavonoids [3], and analysis of essential oil [4]. Present studies led to the isolation of a novel sesquiterpene, named crossostephin (**1**), and a coumarin, named biscopoletin (**2**), along with four known compounds, artesin [5], tanacetin [5], scopoletin [6], and scopolin [7], by repeated chromatography. The structures were identified on the basis of spectroscopic methods, including IR, UV, EIMS, HRFTICRMS, 1D and 2D NMR spectral analyses, and comparison with those reported data. The known compounds, scopoletin and scopolin, were isolated from the title plant for the first time.

2. Results and discussion

Compound **1** was obtained as white amorphous powder with $[\alpha]_D^{20}$ +44 (*c* 0.1, MeOH). The EIMS showed a molecular ion peak at *m/z* 296 $[M]^+$ in addition to fragment ion peaks at *m/z* 278 $[M - H_2O]^+$, 264 $[M - H_2O - CH_2]^+$, 246 $[M - 2H_2O - CH_2]^+$, 237 $[M - COOCH_3]^+$, 232 $[M - 2H_2O - 2CH_2]^+$, 228 $[M - 3H_2O - CH_2]^+$, 213 $[M - 3H_2O - 2CH_2 - H]^+$, and 123 (base peak). The molecular formula $C_{16}H_{24}O_5$ was determined by HRFTICRMS. The IR spectrum of **1** showed absorption bands ascribable to hydroxyl (3490 and 3421 cm^{-1}), olefinic (1649 and 1075 cm^{-1}) and α,β-unsaturated ester (1704 and 1627 cm^{-1}) functions. The ^1H and ^{13}C NMR (Table 1) spectra of **1**,

*Corresponding author. Email: xwyang@bjmu.edu.cn; xwyang@hsc.pku.edu.cn

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020802435703
http://www.informaworld.com

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data for **1** and tanacetin (in $CDCl_3$)*.

	1		Tanacetin [5]	
No.	^{1}H (δ_H; *J*/Hz)	^{13}C (δ_C)	^{1}H (δ_H; *J*/Hz)	^{13}C (δ_C)
1	4.10 (1H, dd, 5.0, 11.5)	72.7 d	4.17 (1H, td, 5.0, 12.0)	71.6 d
2	1.85 (1H, dddd, 2.0, 5.0, 13.5, 18.0) 1.56 (1H, dddd, 5.5, 11.5, 13.5, 18.0)	31.0 t	1.84 (1H, dddd, 2.0, 5.0, 14.0, 18.0) 1.56 (1H, dddd, 5.5, 12.0, 14.0, 18.0)	30.3 t
3	2.71 (1H, tddd, 2.0, 5.5, 13.5, 13.5) 2.10 (1H, ddd, 2.0, 5.5, 13.5)	30.2 t	2.66 (1H, tddd, 2.0, 5.0, 14.0, 14.0) 2.18 (1H, ddd, 2.0, 5.0, 14.0)	29.6 t
4		147.4 s		144.5 s
5		78.3 s		76.9 s
6	4.22 (1H, d, 10.5)	71.0 d	4.26 (1H, d, 11.5)	81.8 d
7	2.91 (1H, ddd, 3.5, 10.5, 13.0)	43.1 d	3.34 (1H, tq, 3.5, 11.5, 13.0)	43.1 d
8	1.74 (1H, dq, 3.5, 13.0, 16.0) 1.62 (1H, dddd, 5.5, 8.0, 13.0, 16.0)	25.7 t	2.04 (1H, dq, 3.5, 13.0, 15.8) 1.61 (1H, dddd, 5.5, 7.5, 13.0, 15.8)	21.1 t
9	1.75 (1H, dddd, 5.5, 8.0, 13.0, 16.0) 1.77 (1H, tddd, 3.0, 13.0, 16.0)	29.1 t	1.75 (1H, dddd, 5.5, 7.5, 13.0, 15.8) 1.78 (1H, tddd, 3.5, 13.0, 15.8)	29.7 t
10		43.4 s		44.6 s
11		142.5 s		139.7 s
12		168.6 s		170.7 s
13	5.77 (1H, s) 6.30 (1H, s)	126.1 t	5.42 (1H, d, 3.0) 6.09 (1H, d, 3.0)	117.0 t
14	0.88 (3H, s)	12.7 q	0.88 (3H, s)	13.2 q
15	4.49 (1H, s) 5.00 (1H, s)	109.8 t	5.02 (1H, d, 2.0) 5.05 (1H, d, 2.0)	112.7 t
OCH_3	3.79 (3H, s)	52.1 q		

*All values are in ppm, coupling constants (*J*) in Hz; assignments were made by $^{1}H-^{1}H$ COSY, HSQC, and HMBC spectral data; multiplicity was established from HSQC data; s, C; d, CH; t, CH_2; q, CH_3.

combined with HSQC experiment, showed signals assignable to a tertiary methyl [δ_H 0.88 (3H, s, H_3-14), δ_C 12.7 (C-14)], two methines bearing a hydroxyl group [δ_H 4.10 (1H, dd, $J = 5.0, 11.5$ Hz, H-1); δ_C 72.7 (C-1) and δ_H 4.22 (1H, d, $J = 10.5$ Hz, H-6); δ_C 71.0 (C-6)], two *exo*-methylene [δ_H 5.77, 6.30 (1H each, s each, H_2-13; δ_C 126.1 (C-13) and δ_H 4.49, 5.00 (1H each, s each, H_2-15; δ_C 109.8 (C-15)], together with four methylenes (H_2-2, H_2-3, H_2-8, and H_2-9), one methine [δ_H 2.91 (1H, ddd, $J = 3.5$, 10.5, 13.0 Hz, H-7); δ_C 43.1 (C-7)], one *O*-methyl group [δ_H 3.79 (3H, s, H_3-16; δ_C 52.1 (C-16)], and five quaternary carbons (C-4, C-5, C-10, C-11, and C-12). Comparison of the ^{1}H and ^{13}C NMR spectral data (Table 1) of **1** with those of tanacetin (**3**)[5] suggested that **1** was a derivative of tanacetin cleaved from its five-member lactone ring with an additional *O*-methyl group (Figure 1). The linkage position of the *O*-methyl group to the mother skeleton was established by HMBC spectroscopy in which the signals of H_3-16 at δ_H 3.79 (3H, s) correlated with the carbonyl carbon signal at δ_C 168.6 (C-12; Figure 1). Additionally, the coupling constant $J_{H6,H7} =$ 10.5 Hz implied a *trans*-orientation of configuration for these two protons [5,8]. Furthermore, an optical polarization orientation ($[\alpha]_D^{20}$ +44 (*c* 0.1, MeOH)) of **1** was in agreement with those ($[\alpha]_D^{20}$ +12 (*c* 0.1, MeOH)) of 1β,6α-dihydroxycostic acid [8]. Finally, compound **1** was successfully obtained by methanolysis of tanacetin, whose configuration was established by

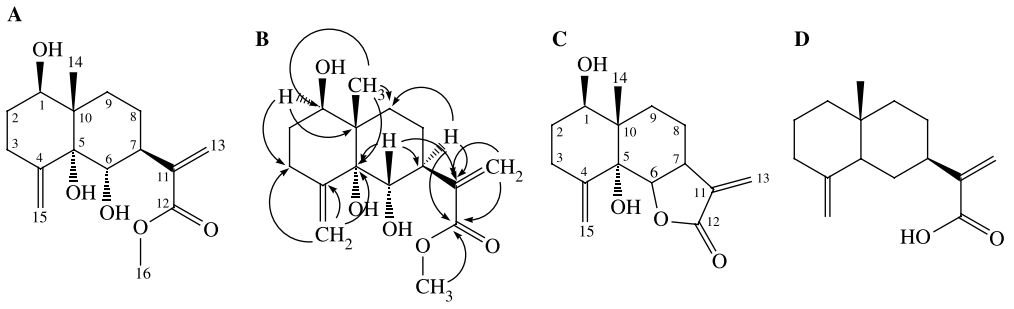

Figure 1. Structures of **1** (A), tanacetin (C), costic acid (D), and key HMBC correlations of **1** (B) (from H to C).

single crystal X-ray diffraction analysis [5], using $NaOCH_3$ reagent in MeOH solution (Figure 2). Therefore, the structure of **1** was determined as 1β,5α,6α-trihydroxy-costic acid methyl ester, and named crossostephin.

Compound **2** was isolated as pale yellow powder and analyzed for $C_{20}H_{14}O_8$ from its HRFTICRMS. Its UV spectrum [λ_{max} = 238, 269, and 333 nm (log ε 4.33, 4.34, and 4.38, respectively)] showed typical data for a 7-oxygenated coumarin [9]. The IR spectrum revealed strong absorption bands at 3472 (—OH), 1717 (lactonic carbonyl), and 1629 (aromatic nucleus) cm^{-1}. The ^1H NMR spectrum displayed signals of four singlets proton [δ_H 10.45 (OH), 8.29 (CH), 7.29 (CH), 6.83 (CH)] and a methoxyl singlet at δ_H 3.83, suggesting the presence of a 7-hydroxy-6,3-disubstituted coumarin nucleus, by comparison with those of scopoletin (Table 2). The ^{13}C NMR and HSQC experiments exhibited the presence of three methines (CH), one methoxyl (OCH_3), and six quaternary (C) carbon atoms. The quaternary carbon atoms were deduced by subtracting these from the HSQC spectrum. From above mention, $C_{10}H_7O_4$ moiety could be identified in molecular structure of **2**. In combination with $C_{20}H_{14}O_8$ of molecular formula from HRFTICRMS and using the ^{13}C shift values of scopoletin as reference data, it could be firmly deduced that a linear linked dimeric scopoletin unit must be present and this inconsistency suggested that the 10 carbon signals were doubled. The ^1H and ^{13}C NMR spectral patterns were similar to those of scopoletin except for the absence of the signal at δ_H 6.23 (1H, d, $J = 9.6$ Hz; H-3 of scopoletin) and the singlet signal at δ_H 8.29 (s, H-4 of **2**) instead of the doublet signal at δ_H 7.92 (1H, d, $J = 9.6$ Hz; H-4 of scopoletin) in the ^1H NMR spectrum. $^1H-^{13}C$ HMQC and HMBC experiments were not only useful for the attribution of the chemical shifts of several protonated and non-protonated carbon atoms, but also helpful for confirming the above assignments. The methoxyl protons at δ_H 3.83 (3H, s) showed cross peak to carbon at δ_C 145.6. Likewise, the proton at δ_H 8.29 (H-4, 4′) showed cross peaks to carbons at δ_C

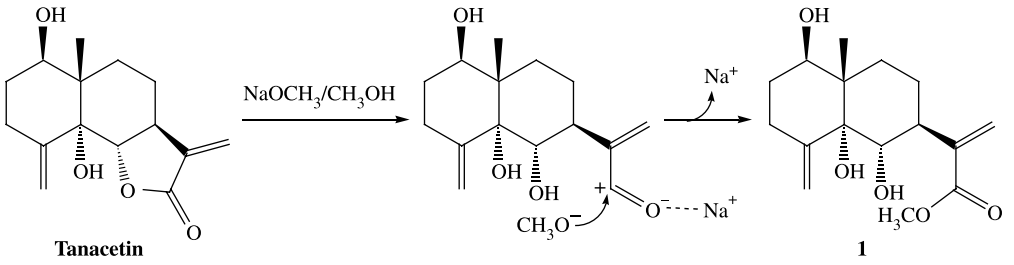

Figure 2. Chemical conversion of tanacetin to compound **1**.

Table 2. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectral data for biscopoletin and scopoletin (in DMSO-d_6)*.

	Biscopoletin			Scopoletin	
No.	^{1}H (δ_H; *J*/Hz)	^{13}C (δ_C)	No.	^{1}H (δ_H; *J*/Hz)	^{13}C (δ_C)
2, 2′		159.7 s; 159.7 s	2		160.7 s
3, 3′		117.0 s; 117.0 s	3	6.23 (1H, d, 9.6)	111.7 d
4, 4′	8.29 (2H, s)	143.1 d; 143.1 d	4	7.92 (1H, d, 9.6)	144.5 d
4a, 4′a		110.6 s; 110.6 s	4a		110.5 s
5, 5′	7.29 (2H, s)	109.7 d; 109.7 d	5	7.23 (1H, s)	109.6 d
6, 6′		145.6 s; 145.6 s	6		145.2 s
7, 7′		151.6 s; 151.6 s	7		151.1 s
8, 8′	6.83 (2H, s)	102.5 d; 102.5 d	8	6.79 (1H, s)	102.8 d
8a, 8′a		149.0 s; 149.0 s	8a		149.5 s
C_6—OMe C_6'—OMe	3.83 (6H, s)	56.1 q; 56.1 q	C_6—OMe	3.83 (3H, s)	56.0 q
C_7—OH C_7'—OH	10.45 (2H, s)		C_7—OH	10.33 (1H, s)	

* All values are in ppm, coupling constants (*J*) in Hz; assignments were made by $^{1}H-^{1}H$ COSY, HSQC, and HMBC spectral data; multiplicity was established from HSQC data; s, C; d, CH; t, CH_2; q, CH_3.

117.0 (C-3, C-3′), 159.7 (C-2, C-2′), 149.0 (C-8a, C-8′a), and 109.7 (C-5, C-5′) in Figure 3. In this way, it was possible to work around the dicoumarin skeleton and to assign the skeletal structure and signals of the carbon atoms. These results led to the structure of **2** as 7-hydroxy-6-methoxy-3-[2-oxo-2H-1-benzopyran-7-hydroxy-6-methoxy-3-yl]-2H-1-benzopyran-2-one as shown in Figure 3. It was a novel compound and named biscopoletin.

3. Experimental

3.1 General experimental procedures

Optical rotation was measured on an Autopol III polarimeter (Rudolph Research Analytical, Flanders, NJ, USA) with MeOH as solvent. UV spectra were obtained on a Varian Cary-300 UV–vis photometer in MeOH solution. IR spectra were recorded on a Thermo Nicolet Nexus 470 FT-IR spectrometer. Mass spectra were recorded on a TRACE 2000 GC–MS [for electronic impact mass spectrum (EIMS)] and an APEX II FTICRMS [for high-resolution Fourier transform ion cyclotron resonance mass spectrum (HRFTICRMS)] spectrometer. 1D and 2D NMR spectra were recorded on a JEOL AL-300 OV (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR) and a Varian Inova-500 (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) using TMS as internal standard. Preparative high-performance liquid chromatography (HPLC) system consisted of a LabTech P600 pump, UV 600 Ultra-violet Visible Detector, and LC Workstation 2005 (LabTech Inc., Beijing, China), equipped with a Phenomenex PRODIGY ODS column (250 mm × 21.2 mm, 10 μm) at a flow rate of 5.0 ml/min. The mobile phase consisted of H_2O–MeOH (40:60, v/v).

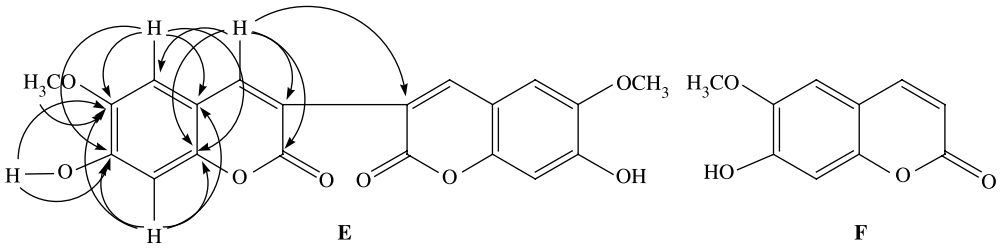

Figure 3. Structures of compound **2** (E), scopoletin (F), and its key HMBC correlations (from H to C).

The UV detection was set at a single wavelength of 210 nm. Open column chromatography was carried out using silica gel (200–300 mesh, Qingdao Marine Chemical Co., Qingdao, China) as a stationary phase. TLC was conducted on silica gel GF_{254} plates (Merck, Whitehouse Station, NJ, USA) for qualitative analysis. HPLC grade MeOH was purchased from Tianjin Xihua Chemicals Co. (Tianjin, China). Sodium methoxide ($NaOCH_3$) was purchased from Beijing Qingshengda Chemical Technology Co., Ltd. (Beijing, China).

3.2 Plant material

The whole herbs of *C. chinense* were collected from Guangzhou City, Guangdong Province, China, in July 2003, and authenticated by Prof. Zhong-chen Guo of Institute of Botany, the Chinese Academy of Sciences. The voucher specimen (no. 2003081001) of this plant has been deposited in the Herbarium of the State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences.

3.3 Extraction and isolation

Dried whole herb powders of *C. chinense* (8.8 kg) were extracted with 70% aqueous EtOH. After evaporation of the solvent under reduced pressure, the residue was suspended in H_2O and extracted successively with cyclohexane (CHX), ethyl acetate (EtOAc), and normal butanol (*n*-BuOH) saturated with H_2O. Each solvent was evaporated *in vacuo* to yield the CHX extract (350 g, yield 3.97%), EtOAc extract (470 g, 5.34%), *n*-BuOH extract (160 g, 1.81%), and a water-soluble material as described in previous report [2,5].

A portion of the CHX extract (250 g) was subjected to a silica gel column and eluted with CHX–EtOAc mixture of increasing polarity (10:0 → 9:1 → 4:1 → 1:1 → 0:10) to give eight fractions. Fraction 6 was chromatographed on a silica gel column, successively eluted with CHX–EtOAc (4:1) and $CHCl_3$–EtOAc (9:1), to yield artesin (50 mg)[5] and a mixture, respectively. The mixture was recrystallized with acetone to give tanacetin (100 mg)[5]. The mother liquors were then further purified using a C_{18} Sep-Pak® followed by separation using C_{18} RP-HPLC to afford compound **1** (200 mg). Fraction 7 was chromatographed on a silica gel column and eluted with petroleum ether–acetone (9:1) to afford scopoletin (50 mg).

A portion of the EtOAc extract (350 g) was subjected to a silica gel column and successively eluted with CHX–EtOAc mixture of increasing polarity (9:1 → 4:1 → 1:1 → 0:10) and EtOAc–MeOH mixture of increasing polarity (15:1 → 9:1) to give seven fractions. Fraction 5 was chromatographed on a silica gel column and eluted with $CHCl_3$–acetone mixture of increasing polarity (9:1 → 4:1) to afford compound **2** (30 mg). Fraction 6 was chromatographed on a silica gel column and eluted with $CHCl_3$–MeOH (5:1) to yield scopolin (200 mg).

3.3.1 *Crossostephin (**1**)*

White amorphous powder; $[\alpha]_D^{20}$ +44 (*c* 0.1, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3490, 3421, 2934, 1704, 1649, 1627, 1439, 1330, 1288, 1265, 1155, 1075, 1045, 1007, 967, 908, 893, 680; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) spectral data were shown in Table 1; EIMS *m/z*: 296 $[M]^+$, 278 $[M - H_2O]^+$, 264 $[M - H_2O - CH_2]^+$, 246 $[M - 2H_2O - CH_2]^+$, 237 $[M - COOCH_3]^+$, 232 $[M - 2H_2O - 2CH_2]^+$, 228 $[M - 3H_2O - CH_2]^+$, 213 $[M - 3H_2O - 2CH_2 - H]^+$, 200, 182, 150, 123 (base peak); positive HRFTICRMS *m/z*: 319.1514 $[M + Na]^+$ (calculated for $C_{16}H_{24}NaO_5$, 319.1516), 615.3142 $[2M + Na]^+$ (calculated for $C_{32}H_{48}NaO_{10}$, 615.3140).

3.3.2 *Biscopoletin (**2**)*

Pale yellow powder; UV (MeOH) λ_{max} nm (log ε): 212 (4.61), 238 (4.33), 269 (4.34), 333 (4.38); IR (KBr) ν max (cm^{-1}): 3472, 2923, 1717, 1629, 1611, 1574, 1509, 1402,

1348, 1308, 1273, 1216, 1164, 1123, 1006, 947, 928, 865, 763, 666, 595; ^{1}H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (75 MHz, DMSO-d_6) spectral data were shown in Table 2; EIMS *m/z*: 382 $[M]^+$, 367 $[M - CH_3]^+$, 354 $[M - CO]^+$, 339, 324, 310, 296, 268, 240, 212, 199, 178, 155, 127, 112, 99, 69; negative HRFTICRMS *m/z*: 381.0621 $[M - H]^-$ (calculated for $C_{20}H_{13}O_8$, 381.0615).

3.3.3 *Scopoletin (**3**)*

Colorless needles [$CHCl_3$–hexane]; mp 204–205°C; IR, UV, and EIMS data were in agreement with those reported for scopoletin [6,9]; ^{1}H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (75 MHz, DMSO-d_6) spectral data, in Table 2, which were in good agreement with those reported for scopoletin [6].

3.3.4 *Scopolin (**4**)*

Colorless needles (MeOH); mp 223–224°C; UV (MeOH) λ_{max} nm (log ε): 227 (3.81), 249sh (3.42), 257 (2.60), 291 (3.53), 341 (3.89); IR, EI-MS, ^{1}H NMR (300 MHz, DMSO-d_6), and ^{13}C NMR (75 MHz, DMSO-d_6) spectral data were in good agreement with those reported for scopolin [7].

3.3.5 *Conversion of tanacetin to compound **1***

Tanacetin (100 mg) was treated with $NaOCH_3$ (0.9 mg) in 100 ml of MeOH at 65°C for 1 h. The reaction mixtures were concentrated to dryness, dissolved in 100 ml of distilled H_2O, and then extracted with 300 ml of $CHCl_3$ for five times. The $CHCl_3$ extract was combined, washed with H_2O, and then evaporated to dryness *in vacuo* to give a white residue. The residue was dissolved in 4 ml of MeOH and purified by preparative HPLC [Phenomenex PRODIGY ODS column (250 mm × 21.2 mm, 10 μm); mobile phase, H_2O–MeOH (40:60, v/v); flow rate, 5.0 ml/min; detection, UV at 210 nm] to give target compound (20.1 mg, yield 20.1%) and starting material (50.5 mg). The EIMS, NMR spectral, and $[\alpha]_D^{20}$ data of target compound were in agreement with those of compound **1**.

Acknowledgements

This work was supported in party by National High Technology Research and Development Program of China (2001CB51008).

References

[1] S. Sasaki, S. Aoyagi, and H.Y. Hsu, *Chem. Pharm. Bull.* **13**, 87 (1965).
[2] X.W. Yang, L. Zou, Q. Wu, and D.X. Fu, *China J. Chin. Mater. Med.* **33**, 905 (2008).
[3] D.X. Fu, L. Zou, and X.W. Yang, *Nat. Prod. Res. Dev.* **20**, 250 (2008).
[4] L. Zou, D.X. Fu, X.W. Yang, and R.J. Kang, *Nat. Prod. Res. Dev.* **19**, 250 (2007).
[5] X.W. Yang, Q. Wu, L. Zou, D.X. Fu, Y. Chang, Y. Lu, and Q.T. Zheng, *Chin. J. Magn. Reson.* **25**, 117 (2008).
[6] S.J. Piao, G.X. Qu, and F. Qiu, *Chin. J. Med. Chem.* **16**, 40 (2006).
[7] T. Hiroki, H. Sueo, and N. Sansei, *Chem. Pharm. Bull.* **33**, 396 (1985).
[8] F. Hilmi and O. Sticher, J. Heilmann. *Planta Med.* **69**, 462 (2003).
[9] L. Huang and D.Q. Yu, *The Application of UV Spectroscopy in Organic Chemistry*, 2, (Science Press, Beijing, 2000), pp. 296–304.