Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 66 (2005) 2752–2758
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Phenolic glycosides and ionone glycoside from the stem of *Sargentodoxa cuneata*

Jun Chang *, Ryan Case

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, IL 60612, USA

Received 22 March 2005; received in revised form 16 August 2005
Available online 4 November 2005

Abstract

Four phenolic glycosides, cuneatasides A–D (**1**–**4**), and one ionone glycoside cuneataside E (**5**), together with seven known phenolic compounds (**6**–**12**) were isolated from the water-soluble constituents of the stem of *Sargentodoxa cuneata* (Sargentodoxaceae). Their structures were elucidated by spectroscopic analysis. In vitro tests for antimicrobial activity showed compounds **1** and **2** to possess significant activity against two Gram-positive organisms, *Staphylococcus aureus* and *Micrococcus epidermidis*.

Keywords: *Sargentodoxa cuneata*; Sargentodoxaceae; Phenolic glycosides; Ionone glycoside; Cuneatasides A–E; Antimicrobial activity

1. Introduction

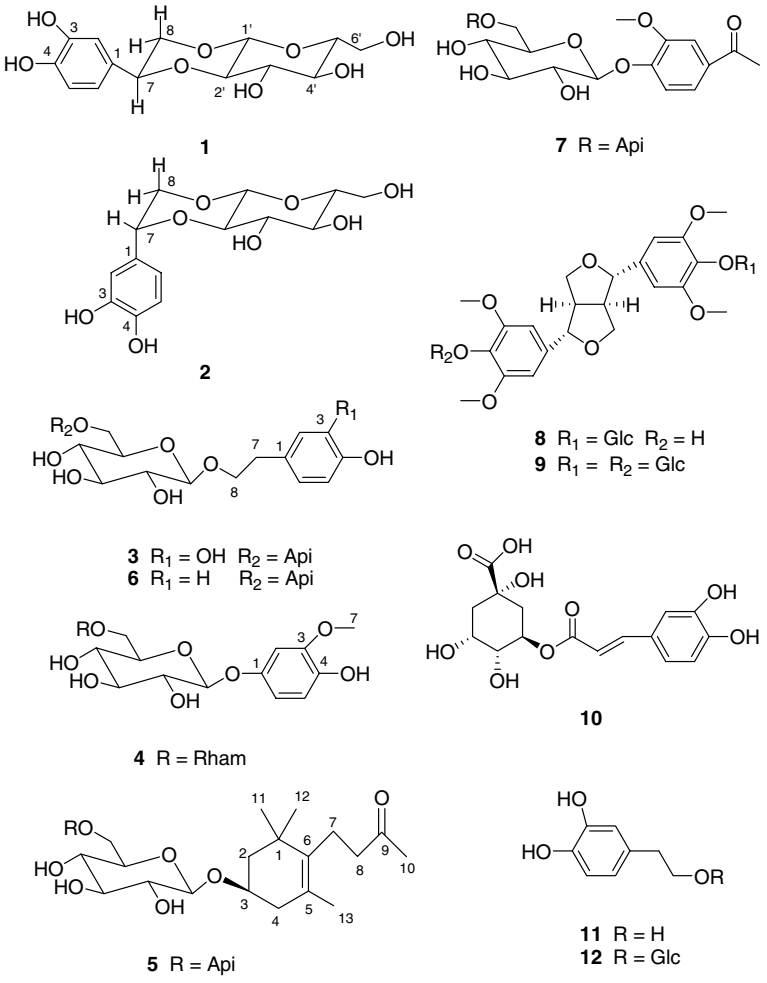
Sargentodoxa cuneata Rehd. Et Wils (Sargentodoxaceae) is a deciduous climber, widely spread over forests in mountainous regions from East Asia to central China (Jiangsu New Medical College, 1986). The stem of *S. cuneata*, is used in Chinese folk medicine for the treatment of rheumatic arthritis, abdominal pain, acute appendicitis, dysmenorrhea, amenorrhea, trauma and painful menstruation (National Bureau of Chinese Traditional Medicine Editorial Committee, 1998). Several kinds of compounds including phenolic acids, sterols, phenolic glycosides, sugar, triterpene saponins, polysaccharide, anthraquinones, lignans, flavonoids, and polycyclic phenolic compound have been previously isolated from *S. cuneata* (Damu et al., 2003; Ge et al., 2002; Han et al., 1986; Li et al., 1984, 1988; Miao et al., 1995; Ruecker et al., 1991; Sakakibara et al., 1995; Wang et al., 1982; Zhang et al., 1988). Ruecker et al. (1991) reported that rosamultin and kajiichigoside F1 isolated from *S. cuneata* showed hemolytic and in vitro antiviral activity. Sakakibara et al. (1994) reported that two phenolic glycosides isolated from *S. cuneata* stem, significantly inhibited the activity of prostaglandin synthetase from sheep's seminal vesicle, indicating antiinflammatory effect. The water extract of the plant was reported to inhibit the growth of many kinds of human pathogenic bacilli in vitro (Jiangsu New Medical College, 1986). As part of our investigation on hydrophilic biologically active constituents from Chinese Traditional Medicines (Chang et al., 2001, 2002), the water-soluble constituents of the stem of *S. cuneata* were systematically studied.

2. Results and discussion

The water-soluble fraction from the 60% aqueous acetone extract of the stem of *S. cuneata* was subjected to column chromatography on MCI gel CHP 20P, Cosmosil 75 C_{18}-OPN and TSK gel Toyopearl HW-40F to afford four new phenolic glycosides, cuneatasides A–D (**1**–**4**), one new ionone glycoside cuneataside E (**5**), and seven known phenolic compounds (**6**–**12**).

* Corresponding author. Tel.: +1 312 996 5607; fax: +1 312 355 2693.
E-mail address: jch1@uic.edu (J. Chang).

0031-9422/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2005.09.018



Compound **1** was obtained as a white amorphous powder. The positive and negative ESI-MS showed quasimolecular ion peaks at *m/z* 337 $[M + Na]^+$ and 313 $[M - H]^+$, respectively, indicating a molecular weight of 314. According to the HR-ESI-MS, ^1H and ^{13}C NMR spectroscopic data, the molecular formula of **1** was determined to be $C_{14}H_{18}O_8$. The IR absorption at 3405 cm^{-1} for hydroxyl groups and 1597 and 1521 cm^{-1} for an aromatic ring suggested that compound **1** was a phenolic compound, and the UV absorption also indicated the presence of an aromatic moiety (223.1, 278.7 nm). In the ^1H NMR spectrum (Table 1), the signals at δ 6.91 (1H, *br d*, $J = 8.0$ Hz, H-6) and δ 6.96 (1H, *d*, $J = 8.0$ Hz, H-5) and 7.00 (1H, *br s*, H-2) defined a 1,3,4-trisubstituted aromatic ring, which was further confirmed in the ^{13}C NMR spectrum by three quaternary aromatic carbons at δ 130.9 (C-1), 146.3 (C-3) and 146.8 (C-4) and three methine peaks at δ 117.2 (C-2), 118.5 (C-5) and 122.0 (C-6). Apart from six typical signals of hexose, the ^{13}C NMR spectrum showed the presence of one methine and one methylene. The linkage between the ethyl group and aromatic ring was established unambiguously by an HMBC experiment. Moreover, the down-field shift of methine and methylene signals (δ 79.3 and 72.9) indicated etherification of them. $^3J_{C-H}$ correlations were observed not only between the anomeric proton at δ 4.63 (H-1′) and the aliphatic carbon at δ 72.9 (C-8), but also between δ 3.33 (H-2′) and δ 79.3 (C-7), which indicated the double linkage. The down-field shifting of δ 99.6 (C-1′) and 75.1 (C-3′) compared with usual values could readily be explained by the β-effects due to etherification of positions 1′ and 2′ at the same time. The C-1′ and C-3′ signals were shifted to higher-field, while the C-2′ signal was shifted to lower-field. The glucosyl moiety was identified by acid hydrolysis of **1** with comparison to an authentic sample, and the β-stereochemistry of the anomeric carbon was determined by the coupling constant ($J = 7.7$ Hz) of the anomeric proton (Agrawal, 1992). The relative configuration at C-7 was also determined to be β-stereochemistry by the coupling constant ($J_{7-8\beta} = 9.5$). Consequently, compound **1** was identified as 7β-(3,4-dihydroxyphenyl)-ethane 7,8-(2′,1′-*O*-β-D-glucopyranosyl)-7,8-diol, and given the trivial name cuneataside A.

Compound **2** was obtained as a white amorphous powder. Based on the HR-ESI-MS, ^1H and ^{13}C NMR spectroscopic data, the molecular formula of **2** was determined as $C_{14}H_{18}O_8$, same as that of **1**. The IR and UV absorptions indicated that **2** was also a phenolic glycoside. Comparison of the ^1H and ^{13}C NMR spectroscopic data (Table 1) of **2**

Table 1
NMR spectroscopic data for compounds **1** and **2**

Position	**1**			**2**	
	^{1}H[a]	^{13}C[b]	HMBC (C → H)	^{1}H[a]	^{13}C[b]
1		130.9 *s*	H-5, H-7, H-8β		130.8 *s*
2	7.00 *br s*	117.2 *d*	H-6, H-7	7.12 *br s*	117.0 *d*
3		146.3 *s*	H-5		146.1 *s*
4		146.8 *s*	H-2, H-6		145.9 *s*
5	6.96 *d* (8.0)	118.5 *d*		6.90 *d* (8.0)	117.4 *d*
6	6.91 *br d* (8.0)	122.0 *d*	H-2, H-7	7.02 *br d* (8.0)	121.5 *d*
7	4.70 *d* (9.5)	79.3 *d*	H-2, H-6, H-8β, H-2′	4.92 *d* (0.7)	73.8 *d*
8α	4.07 *d* (13.6)	72.9 *t*	H-7, H-1′	4.65 *d* (12.8)	68.1 *t*
8β	3.84 *dd* (13.6, 9.5)			4.29 *dd* (12.8, 0.7)	
1′	4.63 *d* (7.7)	99.6 *d*	H-8α, H-2′	4.68 *d* (7.9)	99.5 *d*
2′	3.33 *dd* (9.3, 7.7)	81.1 *d*	H-7, H-1′, H-3′	3.22 *dd* (9.7, 7.9)	73.4 *d*
3′	3.75 *dd* (9.3, 9.2)	75.1 *d*	H-2′, H-4′	3.68 *dd* (9.7, 9.2)	74.4 *d*
4′	3.56 *dd* (9.3, 9.2)	72.3 *d*	H-3′, H-5′, H-6′a	3.39 *dd* (9.3, 9.2)	71.4 *d*
5′	3.64 *m*	80.0 *d*	H-1′, H-4′, H-6′b	3.61 *ddd* (9.3, 5.8, 2.2)	78.8 *d*
6′a	3.97 *br d* (12.1)	62.8 *t*	H-4′, H-5′	3.94 *dd* (12.4, 2.2)	62.2 *t*
6′b	3.80 *dd* (12.1, 5.4)			3.75 *dd* (12.4, 5.8)	

[a] 400 MHz, D_2O; chemical shifts in ppm relative to TMS; coupling constant (*J*) in Hz.
[b] 100 MHz, D_2O; multiplicity was established from DEPT data.

with those of **1** indicated the existence of the same skeleton and sugar moiety, which was also established unambiguously by an HMBC and HMQC experiments. The difference is the relative configuration at C-7 that was determined to be α-stereochemistry by the coupling constant ($J_{7-8\beta} = 0.7$). Consequently, compound **2** was identified as 7α-(3,4-dihydroxyphenyl)-ethane 7,8-(2′,1′-*O*-β-D-glucopyranosyl)-7,8-diol, and given the trivial name cuneataside B.

Compound **3** was obtained as a white amorphous powder. The ESI-MS showed quasimolecular ion peaks at *m*/*z* 471 $[M + Na]^+$ and 447 $[M - H]^+$, indicating a molecular weight of 448. According to the HR-ESI-MS, ^{1}H and ^{13}C NMR spectroscopic data, the molecular formula of **3** should be $C_{19}H_{28}O_{12}$. In the ^{1}H NMR spectrum (Table 2), the signals at δ 6.79 (1H, *dd*, $J = 8.0$, 1.6 Hz, H-6) and δ 6.90 (1H, *d*, $J = 8.0$ Hz, H-5) and 6.88 (1H, *d*, $J = 1.6$ Hz, H-2) defined a 1,3,4-trisubstituted aromatic ring. In the sugar part

Table 2
NMR spectroscopic data for compounds **3** and **4**[a,b,c]

Position	**3**		**4**		
	^{1}H	^{13}C	^{1}H	^{13}C	HMBC (C → H)
1		131.5 *s*		153.3 *s*	H-2, H-5, H-6, H-1′
2	6.88 *d* (1.6)	116.9 *d*	6.79 *d* (2.3)	105.9 *d*	H-6
3		146.1 *s*		150.7 *s*	H-2, H-5, H-7
4		146.4 *s*		143.4 *s*	H-2, H-5, H-6
5	6.90 *d* (8.0)	118.1 *d*	6.87 *d* (8.7)	118.3 *d*	H-6
6	6.79 *dd* (8.0, 1.6)	121.3 *d*	6.64 *dd* (8.7, 2.3)	111.7 *d*	H-2, H-5
7	2.85 *t* (6.8)	37.0 *t*	3.89 *s*	58.7 *q*	
8a	4.10 *m*	73.2 *t*			
8b	3.89 *m*				
1′	4.49 *d* (8.0)	104.5 *d*	5.00 *d* (7.5)	103.9 *d*	H-2′
2′	3.28 *dd* (9.0, 8.0)	75.2 *d*	3.53 *dd* (9.0, 7.5)	75.7 *d*	H-3′
3′	3.48 *dd* (9.1, 9.0)	78.0 *d*	3.59 *dd* (9.1, 9.0)	78.3 *d*	H-4′, H-5′
4′	3.42 *dd* (9.4, 9.1)	71.9 *d*	3.48 *dd* (9.4, 9.1)	72.4 *d*	H-6′
5′	3.59 *m*	77.2 *d*	3.72 *m*	77.6 *d*	H-3′, H-6′
6′a	4.03 *br d* (11.5)	69.8 *t*	4.02 *br d* (9.7)	69.2 *t*	H-4′, H-5′, H-1″
6′b	3.75 *dd* (11.5, 6.2)		3.68 *m*		
1″	5.12 *d* (3.3)	111.0 *d*	4.90 *d* (1.3)	103.1 *d*	H-6′, H-2″
2″	4.01 *d* (3.3)	79.1 *d*	3.92 *dd* (3.2, 1.3)	72.8 *d*	H-1″, H-3″
3″		81.2 *s*	3.76 *dd* (9.7, 3.2)	72.9 *d*	H-1″, H-4″, H-5″
4″	4.07, 3.90 *d* (10.1)	75.8 *t*	3.40 *dd* (9.7, 9.6)	74.7 *d*	H-6″
5″	3.68 *s*	65.9 *t*	3.65 *m*	71.4 *d*	H-3″, H-6″
6″			1.20 *d* (6.1)	19.2 *q*	H-4″, H-5″

[a] 400 MHz, D_2O; chemical shifts in ppm relative to TMS; coupling constant (*J*) in Hz.
[b] 100 MHz, D_2O; multiplicity was established from DEPT data.
[c] Assignments were made by ^{1}H–^{1}H COSY, HMBC and HMQC data.

Table 3
NMR spectroscopic data for compound **5**

Position	^1H[a]	^{13}C[b]	HMBC (C → H)	NOESY
1		39.8 *s*	H-2β, H-7, H-11, H-12	
2α	1.80 *dd* (12.4, 5.0)	47.8 *t*	H-4α, H-11, H-12	H-2β, H-3, H-12
2β	1.50 *dd* (12.4, 12.2)			H-2α, H-4β, H-11
3	4.11 *m*	75.6 *d*	H-2β, H-4α, H-4β, H-1′	H-2α, H-4α, H-12, H-1′
4α	2.38 *dd* (16.0, 5.0)	40.4 *t*	H-13	H-3, H-4β
4β	2.00 *dd* (16.0, 10.1)			H-2β, H-4α, H-13
5		126.8 *s*	H-4α, H-4β, H-7, H-13	
6		138.0 *s*	H-4β, H-7, H-8, H-11, H-12, H-13	
7	2.25 *m*	24.0 *t*		H-8, H-12
8	2.65 *t* (8.0)	46.0 *t*	H-7, H-10	H-7, H-10
9		219.2 *s*	H-7, H-8, H-10	
10	2.20 *s*	31.6 *q*	H-8	H-8
11	1.01 *s*	30.0 *q*	H-2β, H-12	H-2β, H-12
12	1.02 *s*	31.2 *q*	H-2β, H-11	H-2α, H-3, H-7, H-11
13	1.60 *s*	21.4 *q*		H-4β
1′	4.55 *d* (8.0)	101.3 *d*	H-3, H-2′	H-3, H-3′, H-5′
2′	3.24 *dd* (9.3, 8.0)	75.2 *d*	H-3′	H-4′
3′	3.48 *dd* (9.3, 9.2)	78.0 *d*	H-2′, H-4′	H-1′, H-5′
4′	3.40 *dd* (9.6, 9.2)	72.2 *d*	H-3′	H-2′
5′	3.58 *ddd* (9.6, 5.4, 1.2)	76.8 *d*	H-6′b	H-1′, H-3′
6′a	4.03 *dd* (12.4, 1.2)	70.0 *t*	H-4′	H-6′b, H-1″
6′b	3.70 *dd* (12.4, 5.4)			H-6′a, H-1″
1″	5.12 *d* (3.1)	110.4 *d*	H-6′b, H-2″, H-4″	H-6′a, H-6′b, H-4″
2″	3.98 *d* (3.1)	78.8 *d*	H-4″, H-5″	H-5″
3″		81.0 *s*	H-4″, H-5″	
4″	4.05, 3.90 *d* (10.0)	75.8 *t*	H-1″, H-5″	H-1″, H-5″
5″	3.63 *s*	66.0 *t*	H-4″	H-2″, H-4″

[a] 400 MHz, D_2O; chemical shifts in ppm relative to TMS; coupling constant (*J*) in Hz.
[b] 100 MHz, D_2O; multiplicity was established from DEPT data.

of ^1H NMR spectrum, the anomeric proton δ 4.49 (1H, *d*, $J = 8.0$ Hz) indicated the presence of glucose, while δ 5.12 (1H, *d*, $J = 3.3$ Hz) indicated the presence of apiose. These observations were confirmed by acid hydrolysis and comparison with authentic samples. The stereochemistry of the anomeric carbon of glucose was determined as β according to the coupling constant of the anomeric proton and the chemical shift of C-1′ (104.5). The anomeric carbon of the apiosyl residue was determined to be β from the ^{13}C NMR chemical shift of C-1″ (111.0) (Sugiyama and Kikuchi, 1993). The down-field shift of the glucose C-6′ signal (δ 69.8) indicated that glucose and apiose were linked through a 1 → 6 glycosidic bond. The glycosidic site was unambiguously established by a HMBC experiment in which the long-range correlation between H-1′ (δ 4.49) and C-8 (δ 73.2) was observed. Consequently, the structure of compound **3** was established as 2-(3,4-dihydroxyphenyl) ethyl-*O*-β-D-apiofuranosyl-(1″ → 6′)-β-D-glucopyranoside, and given the trivial name cuneataside C.

Compound **4** was obtained as a white amorphous powder. The ESI-MS showed quasimolecular ion peaks at *m/z* 471 $[M + Na]^+$ and 447 $[M - H]^+$, indicating a molecular weight of 448. According to the HR-ESI-MS, ^1H and ^{13}C NMR spectroscopic data, the molecular formula of **4** was determined to be $C_{19}H_{28}O_{12}$. The IR and UV absorptions indicated that **4** was also a phenolic glycoside. In the sugar part of ^1H NMR spectrum, the anomeric proton δ 5.00 (1H, *d*, $J = 7.5$ Hz) indicated the presence of glucose, while the signal at δ 1.20 (3H, *d*, $J = 6.1$ Hz) indicated the presence of rhamnose. These observations were confirmed by acid hydrolysis and comparison with authentic samples. The stereochemistry of the anomeric carbon of glucose was determined as β according to the coupling constant of the anomeric proton and the chemical shift of C-1′ (103.9). The anomeric carbon of the rhamnosyl residue was determined to be α from the ^{13}C NMR chemical shifts of C-3″ and C-5″ (Kasai et al., 1979). The down-field shift of the glucose C-6′ signal (δ 69.2) indicated that glucose and rhamnose were linked through a 1 → 6 glycosidic bond. The glycosidic site was unambiguously established by a HMBC experiment in which the long-range correlation between H-1′ (δ 5.00) and C-1 (δ 153.3) was observed. Consequently, the structure of compound **4** was established as 3-methoxy-4-hydroxylphenyl-1-*O*-α-L-rhamnopyranosyl-(1″ → 6′)-β-D-glucopyranoside, and given the trivial name cuneataside D.

Compound **5** was obtained as a white amorphous powder. The ESI-MS showed quasimolecular ion peaks at *m/z* 527 $[M + Na]^+$ and 503 $[M - H]^+$, indicating a molecular weight of 504. According to the HR-ESI-MS, ^1H and ^{13}C NMR spectroscopic data, the molecular formula of **5** was determined to be $C_{24}H_{40}O_{11}$. IR spectroscopy showed absorption at 3415 cm^{-1} for hydroxyl groups, and 1702 cm^{-1} for the carbonyl group which was supported by

a ^{13}C NMR signal δ 219.2. The ^{1}H NMR spectrum (Table 3), showed the presence of four methyl groups attached to the quaternary carbon at δ 2.20 (3H, *s*), 1.60 (3H, *s*), 1.02 (3H, *s*), and 1.01 (3H, *s*). The ^{13}C NMR spectrum exhibited four methyls (δ 21.4, 30.0, 31.2, 31.6), four methylenes (δ 24.0, 40.4, 46.0, 47.8), one methine (δ 75.6), and four quarternary carbons (δ 39.8, 126.8, 138.0, 219.2), in addition to the sugar part. Further analysis of ^{1}H–^{1}H COSY, HMBC, and HMQC spectra of **5** established the ionone-type skeleton. In the sugar part of the ^{1}H NMR spectrum, the anomeric proton δ 4.55 (1H, *d*, $J = 8.0$ Hz) indicated the presence of glucose, while the signal at δ 5.12 (1H, *d*, $J = 3.1$ Hz) indicated the presence of apiose. These observations were confirmed by acid hydrolysis and comparison with an authentic sample. The stereochemistry of the anomeric carbon of glucose was determined as β according to the coupling constant of the anomeric proton and the chemical shift of C-1′ (101.3). The anomeric carbon of the apiosyl residue was determined to be β from the ^{13}C NMR chemical shifts of C-1″ (110.4) (Sugiyama and Kikuchi, 1993). The down-field shift of the glucose C-6′ signal (δ 70.0) indicated that glucose and apiose were linked through a 1 → 6 glycosidic bond. The glycosidic site was established unambiguously by a HMBC experiment in which long-range correlation between H-1′ (δ 4.55) and C-3 (δ 75.6) was observed. The relative configuration was deduced from the results of the NOESY spectrum, in which NOE signals were observed between H-3 (δ 4.11) and H-2α (δ 1.80), H-4α (δ 2.38), H-12 (δ 1.02). The relative configuration at C-3 was also determined to be β-stereochemistry by the coupling constant ($J_{3-4\beta} = 10.1$ and $J_{3-2\beta} = 12.2$). Consequently, the structure of compound **5** was established as 4-[4β-*O*-β-D-apiofuranosyl-(1″ → 6′)-β-D-glucopyranosyl-2,6,6-trimethyl-1-cyclohexen-1-yl]-butan-2-one, and given the trivial name cuneataside E.

2-(4-hydroxyphenyl) ethyl-*O*-β-D-apiofuranosyl-(1″ → 6′)- β-D-glucopyranoside (osmanthuside H) (**6**) (Sugiyama and Kikuchi, 1993), 2-methoxy-4-acetylphenyl-1-*O*-β-D-apiofuranosyl-(1″ → 6′)-β-glucopyranoside (**7**) (Sakakibara et al., 1994), (+)-Syringaresinol 4′-*O*-β-glucopyranoside (eleutheroside E1) (**8**) (Yoshizawa et al., 1990), (+)-Syringaresinol di-*O*-β-glucopyranoside (liriodendrin) (**9**) (Deyama, 1983), chlorogenic acid (**10**) (Xiang et al., 2001), 2-(3,4-dihydroxyphenyl) ethanol (**11**) (Shen et al., 1990), and 2-(3,4-dihydroxyphenyl) ethyl-*O*-β-D-glucopyranoside (**12**) (Greca et al., 1998) were also identified by comparison of their spectroscopic data with the values in literature. Among the known compounds, four compounds (**6, 8**, **10**, **11**) were isolated for the first time from the *S. cuneata*. There were six phenylethanoids, two lignans, one phenylpropanoids, one phenylglycoside, one phenolic ketone glycoside, and one ionone glycoside isolated from this plant. These phenylethanoids proved to be closely related biosynthetically, and phenylethanoid glycosides have been reported to possess antimicrobial activities (Ersoz et al., 2002). Based on the interesting pharmacological activities of the the stem of *S. cuneata*, compounds **1**–**12** were tested in an antimicrobial assay (Demetzos et al., 1999), in which only compounds **1** (MIC 30.0 and 20.0 μM, respectively) and **2** (MIC 20.0 and 20.0 μM, respectively) showed significant activity against two Gram-positive organisms, *Staphylococcus aureus* and *Micrococcus epidermidis*, using bakuchiol as a positive control (MIC 25.0 and 15.0 μM, respectively).

3. Experimental

3.1. General

Optical rotation data were obtained on a Perkin–Elmer 241 automatic digital polarimeter. UV spectroscopic data were measured on a Shimadzu UV-260 instrument. IR spectroscopic data were measured on a Perkin–Elmer 599B instrument with KBr disks. ^{1}H, ^{13}C NMR, ^{1}H–^{1}H COSY, HMQC, HMBC and NOESY spectra were recorded on a Bruker DRX-400 spectrometer (^{1}H 400 MHz and ^{13}C 100 MHz). The carbon multiplicities were obtained by DEPT experiment. ESI-MS data were measured on a Quattro instrument. Reversed-phase chromatography utilized TSK gel Toyopearl HW-40F (30–60 μm, Toso Co., Ltd.), MCI gel CHP 20P (75–150 μm, Mitsubishi Chemical Industries Co., Ltd.) and Cosmosil 75 C_{18}-OPN (42–105 μm, Nacalai Tesque Inc.) columns. TLC was performed using precoated silica gel 60 F_{254} plates (0.2 mm, Merck).

3.2. Plant material

The stem of *S. cuneata* Rehd. Et Wils was purchased from Shanghai Medicine Materia Corporation, People's Republic of China in 2004, and authenticated by Dr. Chang J. A voucher specimen has been deposited in the Herbarium of our lab (DMP 2004036).

3.3. Extraction and isolation

The stem of dried *S. cuneata* Rehd. Et Wils (2 kg) was extracted three times with aqueous acetone (4:6) at room temperature (3 × 10 L). The solvent was evaporated under reduced pressure to 1 L and filtered using celite. The filtrate was concentrated in vacuo to yield 70 g of a gummy residue. The extract was dissolved in 400 mL water, and subjected to MCI gel CHP 20P (8 × 60 cm) eluting with MeOH/H_2O gradient with a flow rate of 15 mL/min to obtain fraction 1 [1.0 L, H_2O], fraction 2 [0.6 L, MeOH/H_2O (10:90)], fraction 3 [0.6 L, MeOH/H_2O (20:80)], fraction 4 [0.6 L, MeOH/H_2O (30:70)], fraction 5 [0.7 L, MeOH/H_2O (40:60)], fraction 6 [0.7 L, MeOH/H_2O (50:50)], fraction 7 [0.7 L, MeOH/H_2O (60:40)], fraction 8 [1.0 L, MeOH/H_2O (70:30)], and fraction 9 [2.0 L, MeOH]. Fraction 2 (7.4 g) was subjected to Toyopearl HW-40F (6 × 60 cm) chromatography using water as an eluent to obtain six fractions 2A–2F (eluent volume: 200 mL/fraction). Fraction 2C (0.2 g) was further purified

by MCI gel CHP 20P (5 × 40 cm, eluted with H_2O → 10% MeOH) and Cosmosil 75 C_{18}-OPN (4 × 30 cm, eluted with H_2O → 10% MeOH) to give **3** (8 mg) and **4** (11 mg). Fraction 3 (5.2 g) was applied to a Toyopearl HW-40F (6 × 60 cm) column using water as eluent to obtain five fractions 3A–3E (eluent volume: 200 mL/fraction). Fraction 3C (0.4 g) was further purified by MCI gel CHP 20P (5 × 40 cm, eluted with 10–20% MeOH) and Cosmosil 75 C_{18}-OPN (4 × 30 cm, eluted with H_2O → 20% MeOH) to give **6** (6 mg), **8** (10 mg), and **12** (15 mg). Fraction 4 (6.4 g) was subjected to Toyopearl HW-40F (6 × 60 cm) chromatography using water as eluent to obtain six fractions 4A–4F (eluent volume: 200 mL/fraction). Fraction 4C (0.2 g) was further purified by MCI gel CHP 20P (5 × 40 cm, eluted with 20–30% MeOH) and Cosmosil 75 C_{18}-OPN (4 × 30 cm, eluted with H_2O → 30% MeOH) to give **5** (9 mg) and **9** (11 mg). Fraction 5 (4.1 g) was applied to a Toyopearl HW-40F (6 × 60 cm) column using water as eluent to obtain six fractions 5A–5F (eluent volume: 200 mL/fraction). Fraction 5C (0.3 g) was further purified by MCI gel CHP 20P (5 × 40 cm, eluted with 30–40% MeOH) and Cosmosil 75 C_{18}-OPN (4 × 30 cm, eluted with H_2O → 40% MeOH) to give **1** (8 mg) and **2** (7 mg). Fraction 6 (6.2 g) was subjected to Toyopearl HW-40F (6 × 60 cm) chromatography using water as eluent to obtain five fractions 6A–6E (eluent volume: 200 mL/fraction). Fraction 6C (0.1 g) was further purified by MCI gel CHP 20P (5 × 40 cm, eluted with 40–50% MeOH) and Cosmosil 75 C_{18}-OPN (4 × 30 cm, eluted with H_2O → 50% MeOH) to give **7** (10 mg). Fraction 7 (7.8 g) was subjected to Toyopearl HW-40F (6 × 60 cm) chromatography using water as eluent to obtain six fractions 7A–7F (eluent volume: 200 mL/fraction). Fraction 7C (0.4 g) was further purified by MCI gel CHP 20P (5 × 40 cm, eluted with 50–60% MeOH) and Cosmosil 75 C_{18}-OPN (4 × 30 cm, eluted with H_2O → 60% MeOH) to give **10** (25 mg) and **11** (21 mg). Experiments were monitored using TLC developed in benzene/formic acid/ethyl acetate (3:2:5), and detected by spraying with sulfuric acid/ethanol (1:4) reagent followed by heating.

3.4. Characterization

3.4.1. Cuneataside A (*1*)

White amorphous powder; $[\alpha]_D^{20}$ +45.0° (*c* 0.10 H_2O); UV (MeOH) λ_{max} (log ε) 223.1 (3.89), 278.7 (3.14) nm; IR (KBr) ν_{max} 3405, 1597, 1521, 1425, 1272, 1227, 1071 cm^{-1}; ESI-MS *m/z* 337 $[M + Na]^+$, 313 $[M - H]^+$; HR-ESI-MS *m/z* 337.0904 $[M + Na]^+$ (calcd. for $C_{14}H_{18}O_8Na$, 337.0899); for ^1H and ^{13}C NMR spectra, see Table 1.

3.4.2. Cuneataside B (*2*)

White amorphous powder; $[\alpha]_D^{20}$ +65.8° (*c* 0.10 H_2O); UV (MeOH) λ_{max} (log ε) 223.4 (3.93), 278.0 (3.15) nm; IR (KBr) ν_{max} 3400, 1600, 1522, 1430, 1273, 1229, 1077 cm^{-1}; ESI-MS *m/z* 337 $[M + Na]^+$, 313 $[M - H]^+$; HR-ESI-MS *m/z* 337.0906 $[M + Na]^+$ (calcd. for $C_{14}H_{18}O_8Na$, 337.0899); for ^1H and ^{13}C NMR spectra, see Table 1.

3.4.3. Cuneataside C (*3*)

White amorphous powder; $[\alpha]_D^{20}$ −70.1° (*c* 0.10 H_2O); UV (MeOH) λ_{max} (log ε) 222.9 (3.87), 279.1 (3.25) nm; IR (KBr) ν_{max} 3400, 1602, 1525, 1431, 1274, 1226, 1071 cm^{-1}; ESI-MS *m/z* 471 $[M + Na]^+$, 447 $[M - H]^+$; HR-ESI-MS *m/z* 471.1483 $[M + Na]^+$ (calcd. for $C_{19}H_{28}O_{12}Na$, 471.1478); for ^1H and ^{13}C NMR spectra, see Table 2.

3.4.4. Cuneataside (*4*)

White amorphous powder; $[\alpha]_D^{20}$ −60.3° (*c* 0.10 H_2O); UV (MeOH) λ_{max} (log ε) 202.6 (3.79), 227.0 (3.60), 285.4 (3.36) nm; IR (KBr) ν_{max} 3400, 1618, 1514, 1441, 1250, 1203, 1055 cm^{-1}; ESI-MS *m/z* 471 $[M + Na]^+$, 447 $[M - H]^+$; HR-ESI-MS *m/z* 471.1481 $[M + Na]^+$ (calcd. for $C_{19}H_{28}O_{12}Na$, 471.1478); for ^1H and ^{13}C NMR spectra, see Table 2.

3.4.5. Cuneataside E (*5*)

White amorphous powder; $[\alpha]_D^{20}$ −81.7° (*c* 0.10 H_2O); IR (KBr) ν_{max} 3415, 2929, 1702, 1633, 1454, 1373, 1074, 1032 cm^{-1}; ESI-MS *m/z* 527 $[M + Na]^+$, 503 $[M - H]^+$; HR-ESI-MS *m/z* 527.2470 $[M + Na]^+$ (calcd. for $C_{24}H_{40}O_{11}Na$, 527.2468); For ^1H and ^{13}C NMR spectra, see Table 3.

3.5. Acid hydrolysis

A solution of compounds **1**–**5** (4 mg each) in 5% HCl (0.5 mL) was heated (90 °C) for 2 h. After removing HCl by evaporation in vacuum, the mixture was diluted with H_2O and extracted with EtOAc. The aqueous layer was neutralized with 0.1 M NaOH and sugars were detected by TLC analysis with authentic sugars: glucose from **1** and **2**, glucose and apiose from **3** and **5**, glucose and rhamnose from **4**. TLC conditions: Kieselgel 60 F_{254} plate (Merck) [using $CHCl_3$–MeOH–H_2O (14:6:1)], R_f 0.13 (glucose), R_f 0.31 (apiose), R_f 0.24 (rhamnose); cellulose 60 F plate [using *n*-BuOH–pyridine–H_2O (6:4:3)], R_f 0.37 (glucose), R_f 0.55 (apiose), R_f 0.47 (rhamnose) (Çalış and Kırmıbekmez, 2004).

3.6. Antimicrobial tests

The microbial strains were from the American Type Culture Collection, and the antimicrobial susceptibility tests were carried out by a microdilution assay. The microbial cells were suspended in Mueller-Hinton broth to form a final density of 5×10^{-5}–10^{-6} CFU/mL and incubated at 37 °C for 18 h under aerobic conditions with the respective compounds dissolved in DMSO. The blank controls of microbial culture were incubated with a limited amount of DMSO under the same conditions. DMSO was determined

not to be toxic under these experimental conditions (Demetzos et al., 1999).

References

Agrawal, P.K., 1992. NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides. Phytochemistry 31, 3307–3330.

Çalış, İ, Kırmızıbekmez, H., 2004. Glycosides from *Phlomis lunariifolia*. Phytochemistry 65, 2619–2625.

Chang, J., Xuan, L.J., Xu, Y.M., 2001. Three new phenolic glycosides from the fertile sprouts of *Equisetum arvence*. Acta Bot. Sin. 43, 193–197.

Chang, J., Xuan, L.J., Xu, Y.M., Zhang, J.S., 2002. Cytotoxic terpenoid and immunosuppressive phenolic glycosides from the root bark of *Dictamnus dasycarpus*. Planta Med. 68, 425–429.

Damu, A.G., Kuo, P.C., Shi, L.S., Hu, C.Q., Wu, T.S., 2003. Chemical constituents of the stem of *Sargentodoxa cuneata*. Heterocycles 60, 1645–1652.

Demetzos, C., Stahl, B., Anastassaki, M., Gazouli, M., Tzouvelekis, L.S., Rallis, M., 1999. Chemical analysis and antimicrobial activity of the resin Ladano, of its essential oil and of the isolated compounds. Planta Med. 65, 76–78.

Deyama, T., 1983. The constituents of *Eucommia ulmoides* Oliv.I. Isolation of (+)-medioresinol di-*O*-β-D-glucopyranoside. Chem. Pharm. Bull. 31, 2993–2997.

Ersoz, T., Ozalp, M., Ekizoglu, M., Calis, I., 2002. Antimicrobial activities of the phenylethanoid glycosides from *Scutellaria galericulata*. Hacettepe Universitesi Eczacilik Fakultesi Dergisi 22, 1–8.

Ge, M.J., Jin, Z.X., Li, J.M., Zhong, Z.C., Zhang, L.L., 2002. Flavonoids from *Sargentodoxa cuneata*. Zhejiang Linxueyuan Xuebao 19, 382–386.

Greca, M.D., Ferrara, M., Fiorentino, A., Monaca, P., Previtera, L., 1998. Antialgal compounds from *Zantedeschia aethiopica*. Phytochemistry 49, 1299–1304.

Han, G.Q., Chang, M.N., Hwang, S.B., 1986. The investigation of lignans from *Sargentodoxa cuneata* (Oliv) Rehd et Wils. Acta Pharm. Sin. 21, 68–70.

Jiangsu New Medical College, 1986. The Dictionary of Chinese Traditional Medicine. Shanghai Press of Science and Technology, Shanghai, pp. 268–270.

Kasai, R., Okihara, M., Asakawa, J., Mizutani, K., Tanaka, O., 1979. ^{13}C NMR study of α- and β-anomeric pairs of D-mannopyranosides and L-rhamnopyranosides. Tetrahedron 35, 1427–1432.

Li, Z.L., Liang, G.J., Xu, G.Y., 1984. Studies on the chemical constituents of *Sargent gloryvine* (*Sargentodoxa cuneata*). Zhongcaoyao 15, 297–299.

Li, Z.L., Chao, Z.M., Chen, K., 1988. Isolation and identification of the ethereal constituents of *Sargentodoxa cuneata*. Shanghai Yike Daxue Xuebao 15, 68–70.

Miao, K.L., Zhang, J.Z., Wang, F.Y., Qin, Y.J., 1995. Chemical constituents of *Sargent gloryvine* (*Sargentodoxa cuneata*). Zhongcaoyao 26, 171–173.

National Bureau of Chinese Traditional Medicine Editorial Committee, 1998. Zhonghua Bencao. Shanghai Press of Science and Technology, Shanghai, pp. 571–573.

Ruecker, G., Mayer, R., Shin-Kim, J.S., 1991. Triterpene saponins from the Chinese drug "Daxueteng" (*Caulis sargentodoxae*). Planta Med. 57, 468–470.

Sakakibara, I., Yoshida, M., Hayashi, K., Maruno, M., 1994. Antiinflammatory activities of glycosides from *Sargentodoxa cuneata* stems. Jpn Kokai Tokkyo Koho JP 06199885.

Sakakibara, I., Hayashi, K., Shimoda, Y., 1995. Extraction of vasodilating polycyclic phenolic compound from *Sargentodoxa cuneata* stems. Jpn Kokai Tokkyo Koho JP 07149630.

Shen, Y.C., Lin, C.Y., Chen, C.H., 1990. Secoiridoid glycosides from *Jasminum multiflorum*. Phytochemistry 29, 2905–2912.

Sugiyama, M., Kikuchi, M., 1993. Phenylethanoid glycosides from *Osmanthus asiaticus*. Phytochemistry 32, 1553–1555.

Wang, Z.Q., Wang, X.R., Yang, Z.H., 1982. Studies on the chemical constituents of Hong Teng (*Sargentodoxa cuneata*). Zhongcaoyao 13, 7–9.

Xiang, T., Xiong, Q.B., Ketut, A.I., Tezuka, Y., Nagaoka, T., Wu, L.J., Kadota, S., 2001. Studies on the hepatocyte protective activity and the structure-activity relationships of quinic acid and caffeic acid derivatives from the flower buds of *Lonicera bournei*. Planta Med. 67, 322–325.

Yoshizawa, F., Deyama, T., Takizawa, N., Usmanghani, K., Ahmad, M., 1990. The constituents of *Cistanche tubulosa* (Schrenk) hook. f. II. Isolation and structures of a new phenylethanoid glycoside and a new neolignan glycoside. Chem. Pharm. Bull. 38, 1927–1930.

Zhang, P., Yan, S.Q., Shao, Y.D., Li, Z.L., 1988. Effect of some water-soluble substances of *Sargentodoxa cuneata* on myocardial ischemia. Shanghai Yike Daxue Xuebao 15, 191–194.