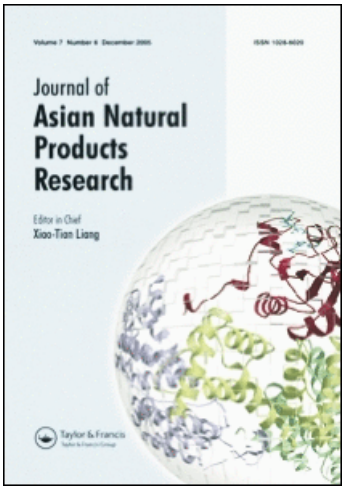

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Secoiridoids and xanthones from *Tylophora secamonoides* Tsiang

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A new secoiridoid, secamonoide A (**1**), and a new xanthone glycoside, secamonoide B (**2**), together with nine known compounds, were isolated from the aerial parts of *Tylophora secamonoides* Tsiang. Their structures were elucidated on the basis of spectroscopic methods. An antimicrobial bioassay showed that secamonoides A and B exhibited weak activities (MIC values greater than 100 mg/ml) against some hospital bacteria *in vitro*.

Keywords: *Tylophora secamonoides* Tsiang; Asclepiadaceae; secamonoide A; secamonoide B; antimicrobial activity

1. Introduction

Tylophora secamonoides Tsiang (Asclepiadaceae) is widely distributed in Guangdong, Guangxi, and Hainan provinces as erect shrublets. The roots of this species have long been used as a folk medicine for the treatment of cough in Guangxi province. Chemical investigations on some *Tylophora* species have led to the isolation of phenanthroindolizidine alkaloids,[1–4] which were regarded to be the characteristic constituents for this genus. However, no studies have been reported as yet on *T. secamonoides*. In the present paper, we describe the isolation and characterization of two new compounds, secamonoides A and B (**1** and **2**), and nine known compounds from the aerial parts of the title plant. Their structures were elucidated by spectral analysis, as well as by comparison of the spectroscopic data with those reported in the literature. Nine known compounds were identified as desacelylcentapicrin[5] (**3**), 1,3,6-trihydroxyxanthone[6] (**4**), bellidifolin[7] (**5**), 3,8-dimethoxy-1,7-dihydroxyxanthone[8] (**6**), demethylbellidifolin[9] (**7**), 1-hydroxy-3,5,8-trimethoxyxanthone[10] (**8**), isobellidifolin[11] (**9**), 1,3,5,6-tetrahydroxyxanthone[12] (**10**), and gentisin[13] (**11**). The isolated compounds were tested for their *in vitro* antimicrobial activities on some hospital bacteria.

2. Results and discussion

Compound **1** was isolated as a light yellow powder. The molecular formula was established as $C_{30}H_{30}O_{13}$ by the quasi-molecular ion at *m/z* 621.1595 $[M + Na]^+$ in the HR-ESI-MS. The IR spectrum indicated the presence of phenolic hydroxyl (3429 cm^{-1}), carbonyl (1693 cm^{-1}), double bond (1619 and 1456 cm^{-1}), and benzene ring (1566 cm^{-1}). The ^1H NMR spectrum displayed eight aromatic protons [δ_H 7.54 (1H, dd, $J = 7.7$, 2.7 Hz), 7.48 (1H, dd, $J = 2.7$, 1.2 Hz), 7.23 (1H, dd, $J = 8.2$, 7.7 Hz), and 7.05 (1H, dd, $J = 8.2$, 1.2 Hz); δ_H 7.46 (1H, dd, $J = 8.2$, 2.6 Hz), 7.42 (1H, dd, $J = 2.6$, 1.5 Hz), 7.30 (1H, dd, $J = 8.2$, 7.6 Hz), and

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7.02 (1H, dd, $J = 7.6$, 1.5 Hz)], which were ascribed to two *m*-substituted benzene rings (Table 1). Correspondingly, the ^{13}C NMR spectrum showed two sets of almost superimposable carbon signals (δ_C 159.4, 132.6, 131.3, 122.2, 121.9, and 117.6; 159.4, 133.0, 131.2, 122.2, 121.9, and 117.6) (Table 1). Considering two carbonyl signals (δ_C 168.4 and 168.0), these data suggested the presence of two *m*-hydroxybenzoyl groups in the structure. This elucidation was further supported by the HMBC correlations from the proton signals at δ_H 7.42 (7.48) and 7.46 (7.54) to the carbon at δ_C 168.0 (168.4), and from the proton at δ_H 7.30 (7.23) to the carbon at δ_C 159.4. In addition to these aromatic signals, the remaining resonances in the ^1H and ^{13}C NMR spectra were accordant with the spectroscopic data of the known compound, sweroside.[5] Thus, the same structural skeleton as sweroside was revealed in the structure of **1**. Furthermore, the two *m*-hydroxybenzoyl groups were assigned to be attached to C-2′ and C-6′ of the sugar moiety by the HMBC correlations between C-7‴ (δ_C 168.4) and H-6′ (δ_H 4.57 and 4.68), and C-7″ (δ_C 168.0) and H-2′ (δ_H 5.03). Therefore, the structure of **1** was established as 2′,6′-*O*-di-(3-hydroxybenzoyl)-sweroside (Figure 1).

Compound **2** was isolated as a yellow powder. Its molecular formula $C_{20}H_{20}O_{10}$ was established by HR-ESI-MS. The IR

Table 1. ^1H NMR and ^{13}C NMR spectral data for compound **1** (in MeOH-d_4).

No.	δ_H	δ_C	HMBC (H → C)
1	5.34 (1H, d, $J = 1.5$ Hz)	98.2 d	C-1′, C-3, C-5
3	7.31 (1H, s)	153.8 d	C-1, C-4, C-5, C-11
4		106.4 s	
5	2.68 (1H, m)	29.2 d	C-8, C-9
6	1.64 (1H, m), 1.52 (1H, m)	26.1 t	C-4, C-5, C-7, C-9
7	4.27 (1H, m), 3.89 (1H, m)	69.9 t	C-5, C-6, C-11
8	5.41 (1H, ddd, $J = 10.3$, 6.8, 2.7 Hz)	133.1 d	C-1, C-9, C-10
9	2.63 (1H, m)	43.7 d	C-1, C-4, C-8, C-10
10	5.25 (1H, d, $J = 1.6$ Hz)	121.6 t	C-8, C-9
	5.21 (1H, ddd, $J = 10.3$, 2.7, 1.6 Hz)		
11		167.8 s	
1′	5.07 (1H, d, $J = 8.6$ Hz)	97.7 d	C-1, C-2′
2′	5.03 (1H, dd, $J = 8.6$, 9.3 Hz)	75.7 d	C-1′, C-3′, C-7″
3′	3.81 (1H, t, $J = 9.3$ Hz)	75.8 d	C-2′, C-4′
4′	5.04 (1H, t, $J = 9.3$ Hz)	72.2 d	C-3′, C-5′
5′	3.80 (1H, ddd, $J = 9.3$, 5.2, 1.8 Hz)	76.4 d	C-1′, C-4′, C-6′
6′	4.68 (1H, dd, $J = 11.4$, 1.8 Hz)	65.1 t	C-5′, C-7‴
	4.57 (1H,dd, $J = 11.4$, 5.2 Hz)		
1″		133.0 s	
2″	7.42 (1H, dd, $J = 2.6$, 1.5 Hz)	117.6 d	C-3″, C-6″, C-7″
3″		159.4 s	
4″	7.02 (1H, dd, $J = 7.6$, 1.5 Hz)	122.2 d	C-2″, C-3″, C-6″
5″	7.30 (1H, dd, $J = 8.2$, 7.6 Hz)	131.2 d	C-1″, C-3″
6″	7.46 (1H, dd, $J = 8.2$, 2.6 Hz)	121.9 d	C-2″, C-4″, C-7″
7″		168.0 s	
1‴		132.6 s	
2‴	7.48 (1H, dd, $J = 2.7$, 1.2 Hz)	117.6 d	C-3‴, C-6‴, C-7‴
3‴		159.4 s	
4‴	7.05 (1H, dd, $J = 8.2$, 1.2 Hz)	121.9 d	C-2‴, C-3‴, C-6‴
5‴	7.23 (1H, dd, $J = 8.2$, 7.7 Hz)	131.3 d	C-1‴, C-3‴
6‴	7.54 (1H, dd, $J = 7.7$, 2.7 Hz)	122.2 d	C-2‴, C-4‴, C-7‴
7‴		168.4 s	

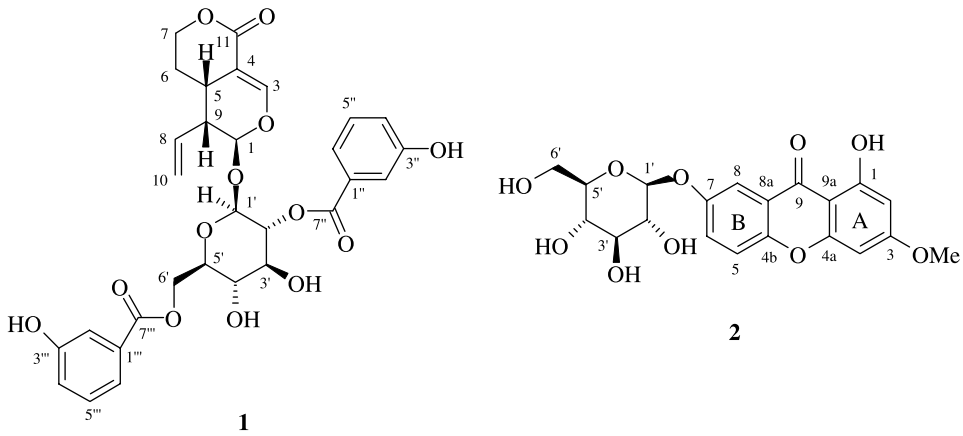

Figure 1. Structures of compounds **1** and **2**.

spectrum showed the presence of the hydroxyl group (3425 cm^{-1}), carbonyl groups (1652 cm^{-1}), and benzene rings (1610 and 1481 cm^{-1}). A detailed analysis of its ^{1}H and ^{13}C NMR spectral data revealed the existence of a sugar moiety and a basic xanthone nucleus (Table 2). A mild acid hydrolysis of **2** yielded β-glucose, which was identified by co-TLC analysis with the authentic sample. The ^{13}C NMR spectroscopic data of the sugar moiety were in good agreement with the literature data.[14–17] Except for the sugar signals, the ^{1}H NMR spectrum of **2** displayed signals of one methoxy [δ_H 3.88 (3H, s)], two *meta*-coupled aromatic protons [δ_H 6.64 (1H, d, $J = 2.0$ Hz) and 6.40 (1H, d, $J = 2.0$ Hz)] and three aromatic protons in an ABX system [δ 7.69

Table 2. ^{1}H NMR and ^{13}C NMR spectral data for compound **2** (in DMSO-d_6).

No.	δ_H	δ_C	HMBC (H → C)
1		157.5 s	
2	6.40 (1H, d, $J = 2.0$ Hz)	92.9 d	C-3, C-9a
3		166.7 s	
4	6.64 (1H, d, $J = 2.0$ Hz)	97.2 d	C-9a
4a		162.3 s	
4b		151.0 s	
5	7.60 (1H, d, $J = 9.1$ Hz)	126.0 d	C-4b, C-7, C-8a
6	7.57 (1H, dd, $J = 9.1$, 2.7 Hz)	119.3 d	C-4b, C-7
7		154.0 s	
8	7.69 (1H, d, $J = 2.7$ Hz)	110.3 d	C-9, C-4b
8a		120.5 s	
9		180.0 s	
9a		103.1 s	
1′	4.95 (1H, d, $J = 7.3$ Hz)	101.3 d	C-7
2′	3.27 (1H, dd, $J = 9.2$, 7.3 Hz)	73.2 d	C-1′, C-3′
3′	3.31 (1H, dd $J = 9.2$, 9.0 Hz)	76.3 d	C-2′, C-4′
4′	3.19 (1H,dd, $J = 9.2$, 9.0 Hz)	69.6 d	
5′	3.38 (1H, m)	77.2 d	C-6′
6′	3.50 (1H, m)	60.7 t	C-5′
	3.70 (1H, d, $J = 11.7$ Hz)		
—OMe	3.88 (3H, s)	56.3 q	C-3

(1H, d, $J = 2.7$ Hz), 7.60 (1H, d, $J = 9.1$ Hz), and 7.57 (1H, dd, $J = 9.1$, 2.7 Hz)]. The ^{13}C NMR spectrum of **2** correspondingly showed 12 aromatic carbons, 1 carbonyl carbon, and 1 methoxy carbon (Table 2). These data revealed a xanthone nucleus with a 1,3-dioxygenated ring A and a 7-oxygenated ring B. The HMBC correlation between H-8 (δ_H 7.69) and C-9 (δ_C 180.0) supported the above conclusion. The attachment of the sugar moiety to C-7 of the xanthone nucleus was revealed by the key HMBC cross-peak between the anomeric proton H-1′ [δ_H 4.95 (1H, d, $J = 7.3$ Hz)] and C-7 (δ_C 154.0). Accordingly, **2** was designated as 1,7-dihydroxy-3-methoxyxanthone-7-*O*-β-glucopyranoside (Figure 1).

The antimicrobial tests of the isolated compounds against *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (from Huashan Hospital), *Bacillus pumilus* (from Huashan Hospital), *Bacillus cereus* (from Huashan Hospital), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Cryptococcus neoformans* (from Changzheng Hospital), *Candida albicans* (ATCC 64550), *Torulopsis glabrata* (from Huashan Hospital), and *Candida sake* (from Huashan Hospital) were carried out according to the protocols described in the literature.[18] The results showed that secamonoides A and B exhibited a weak activity (MIC values greater than 100 mg/ml) against the above-mentioned bacteria *in vitro*.

In conclusion, the first phytochemical investigation on *T. secamonoides* revealed the occurrence of secoiridoids and xanthones. This result was not consistent with the previous reports on other *Tylophora* species. According to the literatures, phenanthroindolizidine alkaloids existed widely in *Tylophora* species, and non-alkaloid ingredients, such as pentacyclic triterpenes[19] and glycosides of 14,15-*seco*-type pregnanes,[20–22] were also reported. Since no characteristic phenanthroindolizidine alkaloids and non-alkaloidal constituents were detected in our study, from a chemotaxonomic viewpoint, *T. secamonoides* was probably completely different from other *Tylophora* species. Our study could supply evidences for the reconsideration of *T. secamonoides* in phytotaxonomy.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured using a Perkin-Elmer 241MC polarimeter or a Perkin-Elmer 341 polarimeter. UV spectra were recorded using a Beckman DU-7 spectrometer. IR spectra were recorded using a Perkin-Elmer 577 Spectrometer. ESI-MS were measured using a Finnigan LCQ-DECA mass spectrometer, and HR-ESI-MS were obtained on a Q-TOF Micro LC-MS-MS spectrometer. NMR spectra were run on a Bruker AM-400 spectrometer or a Bruker AM-600 spectrometer with TMS as the internal standard. Column chromatographic separations were carried out by using silica gel H60 (300–400 mesh, Qingdao Haiyang Chemical Group Corporation, China), MCI GEL CHP20P (75–150 μm, Mitsubishi Chemical Industries), and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) as packing materials. HSGF254 silica gel TLC plates (Yantai Chemical Industrial Institute, China) were used for analytical TLC. Analytical HPLC was preformed on a Waters 2690 separations module with an Alltech ELSD 2000 detector. Preparative HPLC was carried out on a Varian Pro-star solvent delivery module with a Varian Pro-star UV–Vis detector.

3.2 Plant material

The aerial parts of *Tylophora secamonoides* Tsiang were collected from Xichou county, Yunnan province, China, in April 2004 and identified by Jingui Shen, Shanghai Institute of Materia Medica, Chinese Academy of Sciences. A voucher specimen (20040401) has been deposited at the Herbarium of Shanghai Institute of Materia Medica.

3.3 Extraction and isolation

The air-dried aerial parts of *Tylophora secamonoides* Tsiang (4.7 kg) were ground and repeatedly extracted with 95% EtOH (201 × 3) at room temperature. The extract was concentrated under reduced pressure to give a black syrup. The syrup was suspended in water and partitioned with petroleum ether, EtOAc, and *n*-BuOH successively to give-petroleum ether fractions (70 g), EtOAc (95 g), and *n*-BuOH (200 g), respectively. The EtOAc fraction (95 g) was subjected to column chromatography (CC) over silica gel and eluted with a gradient of $CHCl_3$/MeOH to yield fractions I–VIII. About 5.5 g of fraction II was subjected to CC over silica gel and eluted with petroleum ether/EtOAc gradually to yield subfractions IIa–IIg. Subfraction IIe (360 mg) was separated by Sephadex LH-20 ($CHCl_3$:MeOH = 6:4) to give compounds **6** (27 mg) and **11** (22 mg). Subfraction IIf (100 mg) was purified by Sephadex LH-20 ($CHCl_3$:MeOH = 6:4) to afford a mixture of the two compounds. This mixture was separated further by pre-HPLC (40% of CH_3CN in H_2O, 15 ml/min, 330 nm) to yield compounds **5** (3 mg) and **9** (7 mg). Subfraction IIg (400 mg) was subjected to CC over silica gel and eluted with $CHCl_3$ to yield compound **8** (29 mg). Compound **7** (1.7 g) was obtained from fraction IV by recrystallization in a solvent mixture of $CHCl_3$/MeOH. Fraction V (5.5 g) was subjected to an MCI column and eluted with MeOH in H_2O (30–100%) to obtain subfractions Va–Vd. Subfraction Vb (400 mg) was subjected to CC on silica gel eluted with $CHCl_3$/MeOH (100:4) and further purified by Sephadex LH-20 ($CHCl_3$:MeOH = 6:4) to yield compound **10** (15 mg). Subfractions Va (230 mg) and Vd (420 mg) were separated by Sephadex LH-20 (MeOH:H_2O = 3:1), respectively, to give compounds **1** (39 mg), **3** (175 mg), and **4** (8 mg). Compound **2** (16 mg) was obtained from fraction VI (5.5 g) by CC over silica gel ($CHCl_3$:MeOH = 100:7) and further purified by Pre-HPLC (10–40% CH_3CN in H_2O, 15 ml/min, 260 nm).

3.3.1 Secamonoide A (*1*)

Light yellow powder, $[\alpha]_D^{20}$ − 155 (*c* 0.009, MeOH); IR (KBr): ν_{max} (cm^{-1}) 3429 (br), 2921, 1693, 1619, 1566, 1456, 987, 754; ^1H NMR and ^{13}C NMR spectral data are given in Table 1; ESI MS *m/z*: 597.2 $[M - H]^-$; HR-ESI-MS *m/z*: 621.1595 $[M + Na]^+$ (calcd for $C_{30}H_{30}O_{13}Na$, 621.1584).

3.3.2 Secamonoide B (*2*)

Yellow powder, $[\alpha]_D^{20}$ − 42 (*c* 0.0065, MeOH); IR (KBr): ν_{max} (cm^{-1}) 3245 (br), 2923, 1652, 1610, 1481; ^1H NMR and ^{13}C NMR spectral data are given in Table 2; ESI-MS *m/z*: 421.0 $[M + H]^+$; HR-ESI-MS *m/z*: 443.0968 $[M + Na]^+$ (calcd for $C_{20}H_{20}O_{10}Na$, 443.0954).

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