JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

New Flavan-3-ol Dimer from Green Tea Produced from *Camellia taliensis* in the Ai-Lao Mountains of Southwest China

Li-Fang Zhu,[†,‡] Min Xu,[†] Hong-Tao Zhu,[†] Dong Wang,[†] Shi-Xiong Yang,[§] Chong-Ren Yang,[†,⊥] and Ying-Jun Zhang*,[†]

[†]State Key Laboratory of Phytochemistry and Plant Resources of West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China

[‡]University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

[§]Key Lab of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Science, Kunming 650201, People's Republic of China

[⊥]Weihe Biotech Laboratory, Yuxi 653100, People's Republic of China

Supporting Information

ABSTRACT: *Camellia taliensis* (W. W. Smith) Melchior, belonging to the genus *Camellia* sect. *Thea* (Theaceae), is an endemic species distributed from the west and southwest of Yunnan province, China, to the north of Myanmar. Known as a wild tea tree, its leaves have been used commonly for producing tea beverages by the local people of its growing area. One new flavan-3-ol dimer, talienbisflavan A (**1**), was isolated from green tea prepared from the leaves of *C. taliensis* collected from the east side of the Ai-Lao mountains, Yuanjiang county of Yunnan province, China. In addition, five hydrolyzable tannins (**2**−**6**), five flavonols and flavonol glycosides (**9**−**13**), three flavan-3-ols (**14**−**16**), nine simple phenolic compounds and glycosides (**7**, **8**, and **17**−**23**), and caffeine (**24**) were identified. Their structures were determined by detailed spectroscopic analysis. All of the isolated phenolic compounds were tested for their antioxidant activities by DPPH and $ABTS^+$ radical scavenging assays. The contents of its main chemical compositions were also compared with those collected from the Lincang area of Yunnan province by high-performance liquid chromatography analysis.

KEYWORDS: *Camellia taliensis, green tea, talienbisflavan A, antioxidant activities, DPPH, $ABTS^+$, HPLC analysis*

■ INTRODUCTION

Tea is one of the most popular beverages consumed in the world. It is normally produced from the leaves of two widely cultivated *Camellia* plants (Theaceae), *Camellia sinensis* var. *sinensis* (L.) O. Kuntze and *Camellia sinensis* var. *assamica* (Masters) Kitamura. In addition, some species from the same genus *Camellia* section *Thea* have also been used for making tea by the local people of their growing areas. Of them, *Camellia taliensis* (W. W. Smith) Melchior, called wild tea tree, is the most closely related wild tea species to the widely cultivated tea plants (*C. sinensis* var. *sinensis* and *C. sinensis* var. *assamica*), and its leaves have been used widely to make tea beverages by the local people in Yunnan province, China.

Many chemical studies have been carried out on tea and fresh tea leaves. A series of flavan-3-ols and hydrolyzable tannins from green tea,[1,2] 8-*C*-ascorbyl-(−)-epigallocatechin 3-*O*-gallate and novel flavan-3-ol derivatives from oolong tea,[3−5] benzotropolone type pigments from black tea,[6,7] and 8-C-substituted flavan-3-ols from Pu-er tea,[8] were reported. In addition, new chalcan-flavan dimers, flavan-3-ols, and proanthocyanidins were also identified from the fresh leaves of *C. sinenesis* var. *assamica*,[9] among which flavanoids and hydrolyzable tannins were suggested to be the major bioactive constituents in tea due to their stronger antioxidative properties.[10−12]

C. taliensis, an evergreen tree about 10−20 m high, is endemic from the west and southwest of Yunnan province, China, to the north of Myanmar and growing in the mountain evergreen forest with altitudes of 1500−2400 m.[13] In China, it is mainly distributed in the areas on the west side of the Ai-Lao mountains of Yunnan province, such as Dali, Baoshan, Dehong, Lincang, Puer, and Xishuangbanna areas, while Yuanjiang is the only county located on the east side of the Ai-Lao mountains, where *C. taliensis* is distributed.[14,15]

A previous study on the leaves of *C. taliensis* collected from Lincang, one of the regions located on the west side of the Ai-Lao mountains, Yunnan province, China, suggested that this plant contained rich flavan-3-ols and caffeine, which were similar to those of the cultivated tea plants, *C. sinensis* var. *sinensis* and *C. sinensis* var. *assamica*. In addition, abundant hydrolyzable tannins were found in *C. taliensis*.[16] Of them, 1,2-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-*β*-D-glucopyranose (**6**) was considered as a mark hydrolyzable tannin of *C. taliensis*, of which the content of **6** in the dried leaves reached 2.44%.[16] As a part of our continuing research on tea and its original plants,[8,16−19] the leaves of *C. taliensis* collected from Yuanjiang county, the only area on the east side of the Ai-Lao mountains where we found the titled plant, were studied chemically. This led to the isolation of a new flavan-3-ol dimer,

Received: July 4, 2012
Revised: November 12, 2012
Accepted: November 12, 2012
Published: November 21, 2012

ACS Publications © 2012 American Chemical Society

Table 1. NMR Spectroscopic Data for Compound 1 and (−)-ECG (CD_3OD)

	1[a]		ECG[b]	
no.	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
2	79.0, CH	4.81, 2H, br s	78.8, CH	4.99, 1H, br s
3	69.5, CH	5.40, 2H, br s	70.1, CH	5.52, 1H, br s
4	26.7, CH_2	2.93, 2H, dd, 17.2, 4.4	27.0, CH_2	2.99, 1H, dd, 17.4, 4.6
		2.77, 2H, dd, 17.2, 2.2		2.84, 1H, dd, 17.4, 2.1
5	155.3, C		157.4, C	
6	96.9, CH	6.01, 2H, s	96.6, CH	5.95, 1H, d, 2.3
7	155.7, C		158.0, C	
8	106.6, C		96.0, CH	5.94, 1H, d, 2.3
9	153.7, C		158.0, C	
10	99.9, C		99.5, C	
1′	130.9, C		131.6, C	
2′	115.2, CH	6.84, 2H, s	115.2, CH	
3′	146.1, C		146.1, C	
4′	145.9, C		146.0, C	
5′	116.1, CH	6.67, 2H, m	116.1, CH	
6′	119.7, CH	6.67, 2H, m	119.5, CH	
1″	121.4, C		121.5, C	
2″,6″	110.3, CH	6.91, 4H, s	110.3, CH	6.94, 2H, s
3″,5″	146.2, CH		146.5, CH	
4″	139.8, C		139.9, C	
7″	167.5, C		167.7, C	
CH_2	16.7, CH_2	3.92, 2H, s		

[a]Measured at 400 MHz. [b]Measured at 500 MHz.

talienbisflavan A (**1**), together with 22 known phenolic compounds (**2**−**23**) and caffeine (**24**). The isolated compounds were tested by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and $ABTS^+$ radical scavenging assays to evaluate the antioxidant activities of the wild tea from Yuanjiang. Moreover, the contents of main chemical compositions in the leaves of *C. taliensis* collected from Yuanjiang and Lincang were compared by high-performance liquid chromatography (HPLC) analysis.

■ MATERIALS AND METHODS

General Procedure. Optical rotations were measured with a Horiba SEPA-300 high-sensitive polarimeter. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. UV spectra were recorded on a UV 210A Shimadzu spectrometer. One- and two-dimensional NMR spectra were recorded in CD_3OD or acetone-d_6 with Bruker AM-400 and DRX-500 spectrometers operating at 400 and 500 MHz for ^1H and at 100 and 125 MHz for ^{13}C, respectively. Coupling constants were expressed in Hertz, and chemical shifts were given on a ppm scale with tetramethylsilane as the internal standard. ESIMS were recorded on a VG Auto Spec-300 spectrometer with glycerol as the matrix. HRESIMS were recorded on an API QSTAR Pular-1 mass spectrometer. DPPH and $ABTS^+$ radical scavenging assays were performed on an Emax precision microplate reader.

Chemicals and Reagents. Column chromatography was carried out over 25−100 μm Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd. Uppsala, Sweden), 75−100 μm MCI-gel CHP20P (Mitsubishi Chemical Co., Ltd. Tokyo, Japan), and 37−70 μm Toyopearl HW-40F (Tosoh Co., Ltd. Tokyo, Japan). Thin-layer chromatography (TLC) was carried on precoated 0.2−0.25 mm thick silica gel H plates (Qingdao Hailang Chemical Co., Qingdao, China), with benzene/ethyl formate/formic acid (3:6:1, 2:7:1, or 1:7:1, v/v/v) and chloroform/methanol/water (7:3:0.5 or 8:2:0.2, v/v/v), and spots were detected by spraying with 2% ethanolic $FeCl_3$ or anisaldehyde−H_2SO_4 reagent followed by heating. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), trolox, ABTS, and potassium persulfate were purchased from Sigma-Aldrich Chemicals (Steinheim, Germany), and ascorbic acid was obtained from Xinxing Chemical Industrial Reagent Institute (Shanghai, China); acetonitrile (chromatographic grade) and phosphoric acid (reagent grade) were purchased from Merck (Darmstadt, Germany). Water was purified with a Milli-Q apparatus (Millipore, Bedford, MA). Authentic samples, 1-*O*- (**5**), 1,2-di-*O*- (**6**) galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-β-D-glucopyranose, theogallin (**18**), and caffeine (**24**) used for HPLC analysis were isolated from *C. taliensis* in the present study. EGCG and epicatechin-3-*O*-gallate (ECG) were purchased from Sigma-Aldrich Chemicals.

Plant Materials. The green tea used in this study was produced from the leaves of *C. taliensis* (W. W. Smith) Melchior, collected at Yangchajie, Yuanjiang County, Yuxi City, Yunnan Province of China, in April of 2010. The plant material was identified by one of the authors, Dr. Shi-Xiong Yang, from Kunming Institute of Botany (KIB), Chinese Academy of Sciences (CAS). A voucher specimen (no. KUN_56275) was deposited in Kunming Herbarium, KIB, CAS. The green tea sample used for comparison study was the same as our previous study[4] and was produced from the leaves of *C. taliensis*, collected at Dazongshan Mountain, Yunxian County, Lincang City, Yunnan Province, China.

Extraction and Isolation. Green tea (1.9 kg) produced from the leaves of *C. taliensis* collected in Yuanjiang county was extracted three times with 70% aqueous ethanol at room temperature (each 7 days × 5 L). Then, the filtrates were combined and concentrated under reduced pressure for removal of the organic solvent. The concentrated aqueous fraction was extracted with chloroform, to give a chloroform fraction (15.5 g) in which caffeine is the main constituent, and water fraction (544.5 g).

The water fraction (544.5 g) was subjected to a Sephadex LH-20 column, eluting with water−methanol (1:0−0:1), to give seven fractions (1−7). Fraction 1 (52 g) was further applied to repeated column chromatography (CC) over MCI-gel CHP20P, Sephadex LH-20, and Toyopearl HW-40F, eluting with methanol−water (0:1−1:0), to afford compounds **7** (12 mg), **17** (13 mg), **23** (10 mg), and **24** (700 mg). Fraction 2 (150 g) was separated by MCI-gel CHP20P (methanol−water, 1:9−6:4) and Sephadex LH-20 (methanol−water, 3:7−9:1) CC, to give compounds **2** (500 mg), **3** (52 mg), **8** (13 mg), **16** (800 mg), and **22** (300 mg). Repeated CC over MCI-gel CHP20P, Sephadex LH-20, and Toyopearl HW-40F, eluting with methanol−water (0:1−7:3), led to the isolation of compounds **9** (10 mg), **10** (12

mg), **11** (6 mg), and **14** (13 mg) from fraction 3 (15 g), and compounds **12** (43 mg) and **13** (49 mg) from fraction 4 (12 g), respectively. Fractions 5 (74.8 g) and 6 (136.7 g) were separately chromatographed over MCI-gel CHP20P, Sephadex LH-20, and Toyopearl HW-40F, eluting with methanol−water (3:7−8:2), to yield compounds **1** (10 mg), **5** (345 mg), and **15** (26 mg) from fraction 5 and compounds **4** (250 mg), **6** (450 mg), **18** (10 mg), and **19** (25 mg) from fraction 6, respectively. Fraction 7 (15 g) was subjected to CC over MCI-gel CHP20P, Sephadex LH-20, and Toyopearl HW-40F, eluting with methanol−water (3:7−9:1), to give compounds **20** (80 mg) and **21** (36 mg).

Talienbisflavan A (1). Brownish yellow amorphous powder; $[\alpha]_D^{14}$ −105.1° (*c* 0.1, methanol). ESI-MS: *m/z* 895 [M − H]$^-$. HRESIMS ($C_{45}H_{36}O_{20}$) *m/z* 895.1732 [M − H]$^-$ (calculated for 895.1722). IR (KBr): ν_{max} 3407, 1695, 1614, 1451, 1231, 1036, 766. UV λ_{max} (methanol) (log ε): 208 (4.42), 230 (4.36), 294 (4.09), 317 (4.09) nm. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR (CD_3OD), see Table 1.

DPPH Radical Scavenging Assay. The DPPH assay was performed as described in our previous paper,[16−18] and ascorbic acid was used as a positive control. Briefly, reaction mixtures containing a methanol solution (100 μL) of DPPH (100 μM) and 2-fold serial dilutions of each sample (100 μL in methanol, with amounts of sample ranging from 2 to 1000 μg/mL) were placed in a 96-well microplate and incubated at room temperature for 15 min. After incubation, the absorbance was read at 490 nm, and the scavenging activity was determined according to the following equation: percentage of DPPH reduction (%) = $[A_{control} - A_{sample}]/A_{control} \times 100$. Then, a linear plot of percentage of DPPH reduction and sample concentration was made (correlation coefficient R^2 = 0.90−1). The antioxidant activity was evaluated by SC_{50} values (the concentration of sample required to scavenge 50% of DPPH radicals), which were obtained through extrapolation from the linear plot. In this assay, each sample was evaluated in triplicate, and the data presented were means ± SDs of three determinations.

$ABTS^+$ Radical Scavenging Assay. As described in the literature,[20,21] $ABTS^+$ was prepared by reacting ABTS (7 mM, Sigma Chemical Co.) water solution (5 mL) with potassium persulphate (140 mM, 88 μL) in a ratio of 1:0.35, and the mixture was kept in the dark at room temperature for 12−16 h before use. Prior to assay, $ABTS^+$ stock solution was diluted with methanol (ratio 1:88) to give an absorbance at 734 nm of 0.70 ± 0.02 and was equilibrated to 30 °C. The $ABTS^+$ solution (200 μL) was added to a 96-well microplate containing 10 μL of each sample and incubated at room temperature for 6−8 min, and the absorbance at 405 nm was immediately recorded. The scavenging activity was determined according to the following equation: percentage of $ABTS^+$ reduction (%) = $[A_{control} - A_{sample}]/A_{control} \times 100$. Then, a linear plot of the percentage of $ABTS^+$ reduction and sample concentration was made (correlation coefficient R^2 = 0.90−1). The antioxidant activity was evaluated by SC_{50} values (the concentration of sample required to scavenge 50% of $ABTS^+$ radicals), which were obtained through extrapolation from the linear plot. In this assay, each sample was evaluated in triplicate, and the data presented were means ± SDs of three determinations.

HPLC Analysis. Green tea (2.5 g) produced from the leaves of *C. taliensis* in a volumetric flask was saturated with 70% aqueous methanol (100 mL) for 12 h at room temperature, during which an ultrasonic bath was carried out twice, each time for 15 min. Authentic samples were prepared as methanolic solutions, respectively. All sample solutions were filtered through a 0.45 μm (Millipore) filter before injection into the HPLC analysis, which was performed on an Agilent 1260 (Agilent, United States) equipped with a G4212B diode array detector. The optimal mobile phase for analysis was a binary gradient elution system consisting of solvent A (water containing 0.34% phosphoric acid) and solvent B (acetonitrile). The gradient was programed as 4% (solvent B) to 96% (solvent A) at 0 min and being changed to 40% (solvent B) to 60% (solvent A) in 45 min. The column was a Zorbax SB-C18 column (250 mm × 4.6 mm, i.d., 5 μm) (Agilent). The flow rate was 1 mL/min, and the column temperature was set at 30 °C. The injection volume was 10 μL. The UV detection wavelength was monitored at 280 nm. The peaks were confirmed by the UV absorptions and retention times of authentic samples. All of the experiments were performed in triplicate, and the results are expressed as mean values.

LC-MS Analysis. LC-MS analysis was carried out to confirm the main peaks of *C. taliensis*. The 70% aqueous methanol extract of *C. taliensis* was analyzed on a Bruker HCT LC/Esquire spectrometer equipped with a 250 mm × 4.6 mm, i.d., 5 μm, Zorbax SB-C18 column (Agilent). The mobile phases were solvent B (acetonitrile) and solvent C (water containing 0.1% formic acid). The gradient program used was from 4 to 50% of solvent B in solvent C in 50 min at a flow rate of 1 mL/min. The column temperature was set at 30 °C, and the effluent was monitored at 280 nm.

Quantification of the Main Constitutes. Good linearity was established for all of the tested authentic samples, **5**, **6**, **18**, **24**, EGCG, and ECG. Gradient amounts of 1−20 μL of authentic sample solutions (1 mg/mL) were injected to examine the linear relations. Then, calibration curves were made accordingly to yield the following regression equations and ranges for quantificational analysis: $y = 2038219x - 566703$ (r^2 = 0.9969, 1.02−14.30 μg) (**5**); $y = 1389476x + 6321$ (r^2 = 0.9999, 1.00−16.03 μg) (**6**); $y = 1451455x - 209074$ (r^2 = 0.9996, 0.43−7.65 μg) (**18**); $y = 2596636x - 1207851$ (r^2 = 0.9992, 1.06−10.56 μg) (**24**); $y = 1322516x - 45583$ (r^2 = 0.9998, 1.19−11.9 μg) (EGCG); and $y = 1898199x + 277563$ (r^2 = 0.9997, 1.09−10.90 μg) (ECG). Their retention times were 17.8 (**5**), 19.5 (**6**), 6.7 (**18**), 15.9 (**24**), 18.9 (EGCG), and 24.6 (ECG) min, respectively.

RESULTS AND DISCUSSION

Isolation and Characterization. Repeated column chromatography over Sephadex LH-20, MCI-gel CHP20P, and Toyopearl HW-40F of the 70% aqueous ethanol extract of the leaves of *C. taliensis* collected from Yuanjiang county of Yunnan Province, China, led to isolation of one new flavan-3-ol dimer, talienbisflavan A (**1**), together with 23 known compounds. The known ones were identified as five hydrolyzable tannins, 1-*O*-galloyl- (**2**),[22] 1,3-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl- (**3**),[23] 1,2,4,6-tetra-*O*-galloyl- (**4**),[24] 1-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl- (**5**),[2] and 1,2-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl- (**6**)[12] β-D-glucopyranose; five flavonoids, quercetin (**9**),[19] myricetin (**10**),[25] kaempferol (**11**),[25] quercetin 3-*O*-β-D- galactopyranoside (**12**),[26] and quercetin 3-*O*-α-L-rhamnopyranoside (**13**);[25] three flavan-3-ols, (−)-epiafzelechin-3-*O*-gallate (**14**),[27] (−)-epigallocatechin (**15**),[28] and (−)-epicatechin (**16**);[28] four quinic acid derivatives, chlorogenic acid (**17**),[19] theogallin (**18**),[29] 3,4-di-*O*-galloyl-quinic acid (**19**),[29] and 4-*O*-caffeoyl-quinic acid (**20**);[30] five simple phenolic compounds, gentisic acid 5-*O*-(6′-*O*-galloyl)-β-D-glucopyranoside (**7**),[31] gallic acid 3-*O*-(6′-*O*-galloyl)-β-D-glucopyranoside (**8**),[32] coniferin (**21**),[33] gallic acid (**22**),[19] and 3,4-dihydroxybenzoic acid (**23**);[34] in addition to caffeine (**24**) (Figure 1), by comparison with authentic samples and of their spectroscopic and physical data with those previously reported in literatures. The known hydrolyzable tannins **3** and **4** and phenolic glycosides **7** and **8** have been isolated from tea and its original tea plants for the first time.

Compound **1** was obtained as a brownish yellow powder, and its molecular formula was determined as $C_{45}H_{36}O_{20}$ by the negative HRESIMS (*m/z* 895.1732 [M − H]$^-$, calcd for 895.1722). The IR spectrum showed absorption bands for hydroxyl group (3407 cm^{-1}), aromatic rings (1614 and 1451 cm^{-1}), and carbonyl group (1695 cm^{-1}). The structure of compound **1** was further elucidated by detailed analysis of ^{1}H and ^{13}C NMR chemical shifts and by HSQC and HMBC experiments.

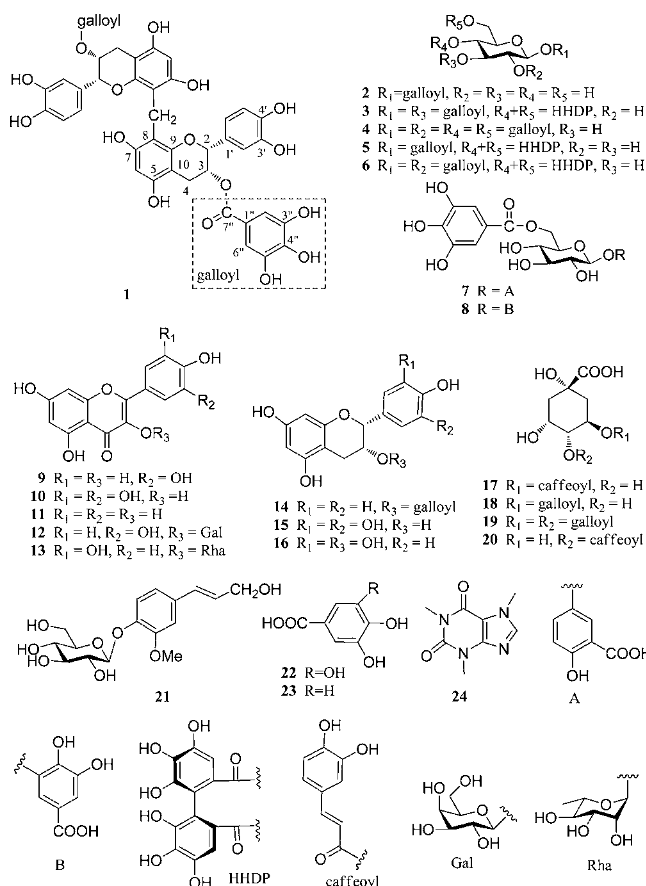

Figure 1. Compounds **1**−**24** isolated from *C. taliensis*.

The ^{1}H, ^{13}C NMR, and DEPT spectra of compound **1** showed the characteristic signals of galloyl group [δ_H 6.91 (s), δ_C 121.4 (C-1), 110.3 (C-2,6), 146.2 (C-3,5), 139.8 (C-4), 167.5 (COO)] and C-ring of flavan-3-ol [δ_H 4.81 (br s, H-2), 5.40 (br s, H-3), 2.93 (dd, J = 17.2, 4.4 Hz, H-4a), and 2.77 (dd, J = 17.2, 2.2 Hz, H-4b), δ_C 26.7 (C-4), 69.5 (C-3), 79.0 (C-2)]. In addition, the 1,3,4-tri-substituted B ring signals at δ_H 6.84 (s, H-2′), 6.67 (m, H-5′, H-6′), and δ_C 130.9 (C-1′), 115.2 (C-2′), 146.1 (C-3′), 145.9 (C-4′), 116.1 (C-5′), and 119.7 (C-6′) were observed, together with the A ring carbon signals [δ_C 99.9 (C-10), 96.9 (C-6), 155.7, 155.3 (C-5,7), 106.6 (C-8), 153.7 (C-9)]. The aforementioned NMR data were closely related to those of ECG. According to the chemical shifts of C-2/C-3 [δ_C 78.9/70.2 for (−)-ECG and δ_C 82.6/68.2 for (+)-catechin] and the coupling constants of H-3/H-4 ($J_{3,4a}$ = 4.6 Hz, $J_{3,4b}$ = 2.2 Hz for (−)-ECG, and $J_{3,4a}$ = 5.4 Hz, $J_{3,4b}$ = 8.4 Hz for (+)-catechin), the configurations of C-2 and C-3 in **1** (δ_C 79.0 (C-2)/69.5 (C-3) and $J_{3,4a}$ = 4.4 Hz/$J_{3,4b}$ = 2.2 Hz) were concluded to be the same of those in ECG. However, instead of the two A ring aromatic methines (CH-6, CH-8) in ECG, the NMR spectra of compound **1** displayed only one aromatic methine (δ_C 96.9, CH) for the A ring and an additional quarternary aromatic carbon at δ_C 106.6 (C) as well as an additional benzylic methylene signals [δ_H 3.92 (s), δ_C 16.7 (CH_2)]. This indicated that compound **1** is comprised symmetrically of two ECG unites connecting between C-6 or C-8 positions through a methylene bridge. In the HMBC spectrum (Figure 2), δ_C 153.7 was assignable to C-9 on the basis of its correlation with H-2 (δ_H 4.81). The CH_2 proton signal at δ_H 3.92 showing HMBC correlations with δ_C 153.7

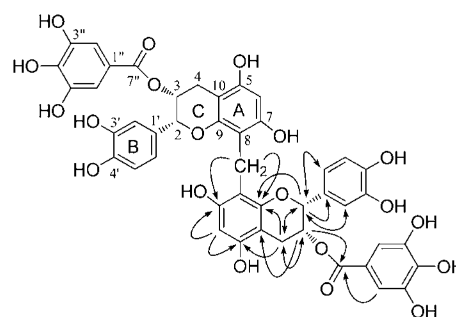

Figure 2. Key HMBC correlations of compound **1**.

(C-9), 155.7 (C-7), and 106.6 (C-8) revealed that the two ECG units in **1** were linked between their C-8 positions through a methylene bridge. Furthermore, HMBC correlations from H-4 (δ_H 2.93, 2.77) to C-2 (δ_C 79.0), C-3 (δ_C 69.5), C-5 (δ_C 155.3), C-9, and C-10 (δ_C 99.9) and from H-6 (δ_H 6.01) to C-5 and C-7 confirmed the partial structures of C ring in **1**, while HMBC correlations from H-2 to C-1′ (δ_C 130.9), C-2′ (δ_C 115.2), C-6′ (δ_C 119.7), and C-4 (δ_C 26.7) and from both H-3 (δ_H 5.40) and the galloyl proton at δ_H 6.91 to the galloyl carbonyl carbon (δ_C 167.5) confirmed the linkages of the B ring and the galloyl group to the C ring of **1**. Therefore, the structure of compound **1** was assigned as two ECG unites connecting between their C-8 positions through a methylene bridge and named talienbisflavan A (Figure 1).

Oligomeric or polymeric catechin derivatives normally exist as C-4/C-6 or C-4/C-8 linkages. To date, only two C-8(6)/CH_2/C-8(6) linked flavan-3-ol dimers, oolonghomobisflavans A and B, were reported from oolong tea, which were produced from the leaves of *C. sinensis* var. *sinensis* by condensation of formaldehyde with two molecules of catechins during a partial fermented process.[5] Because such a flavan-3-ol dimer had not been found from green tea (nonfermented) or black tea (fully fermented), it was of great interest from the viewpoint of both the chemotaxonomy of tea plants and the formation of formaldehyde in tea leaves.[5] Talienbisflavan A (**1**) was not only the third flavan-3-ol dimer with C-8(6)/CH_2/C-8(6) linkage but also the first one found in green tea produced from the leaves of *C. taliensis*. It may occur in the original fresh tea leaf of *C. taliensis*, in which formaldehyde was formed and condensed with catechins.

Free Radical Scavenging Activities of Isolated Compounds. The antioxidant activities of phenolic compounds were evaluated by DPPH and $ABTS^+$ radical scavenging assays, and the results are shown in Table 2.

Most of the isolated phenolic compounds displayed potent DPPH and $ABTS^+$ radical scavenging activities, which were comparable to those of the positive control (ascorbic acid and trolox). Among them, the hydrolyzable tannins (**2**−**6**) and flavan-3-ols (**14**−**16**) exhibited stronger DPPH and $ABTS^+$ radical scavenging activities than those of the other types of compounds. The new flavan-3-ol dimer, talienbisflavan A (**1**), was also proved to possess higher activity. It was in accordance with the previously reported data and showed that more catechol and/or pyrogallol groups attached to the molecules led to stronger radical scavenging activities.[16−18] All of the flavonol glycosides (**12** and **13**) displayed moderate DPPH and $ABTS^+$ radical scavenging activities since the *O*-glycosylation at the C-3 position in the C ring of the flavonols could be a hindrance in the free radical reactions.

Table 2. Antioxidant Activity of Compounds from *C. talliensis*

sample	SC_{50} (μM)^a DPPH^b	ABTS^b
talienbisflavan A (**1**)	3.0 ± 0.1	21.2 ± 0.9
1-*O*-galloyl-*β*-D-glucopyranose (**2**)	16.2 ± 3.3	51.6 ± 0.7
1,3-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxy diphenoyl-*β*-D-glucopyranose (**3**)	4.7 ± 0.2	19.8 ± 0.9
1,2,4,6-tetra-*O*-galloyl-*β*-D-glucopyranose (**4**)	3.5 ± 0.09	12.1 ± 0.9
1-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoyl-*β*-D- glucopyranose (**5**)	5.3 ± 0.3	26.3 ± 2.8
1,2-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxy diphenoyl-*β*-D-glucopyranose (**6**)	6.9 ± 1.7	16.8 ± 1.4
gentisic acid 5-*O*-*β*-D-(6′-*O*-galloyl) glucopyranoside (**7**)	46.8 ± 14.9	209.4 ± 13.2
gallic acid 3-*O*-*β*-D-(6′-*O*-galloyl) glucopyranoside (**8**)	6.7 ± 0.2	67.9 ± 2.2
quercetin (**9**)	9.0 ± 0.3	76.2 ± 1.4
myricetin (**10**)	7.1 ± 0.4	73.2 ± 1.0
quercetin-3-*O*-*β*-D-galactopyranoside (**12**)	12.3 ± 2.6	185.7 ± 3.1
quercetin-3-*O*-*α*-L-rhamnopyranoside (**13**)	15.2 ± 1.5	156.3 ± 4.6
(−)-epiafzelechin-3-*O*-gallate (**14**)	2.9 ± 0.2	33.5 ± 0.8
(−)-epigallocatechin (**15**)	11.1 ± 2.4	27.6 ± 1.2
(−)-epicatechin (**16**)	8.5 ± 0.4	32.4 ± 2.5
chlorogenic acid (**17**)	19.4 ± 0.5	198.6 ± 18.5
theogallin (**18**)	17.8 ± 1.2	4.6 ± 4.0
3,4-di-*O*-galloyl-quinic acid (**19**)	11.8 ± 1.8	61.1 ± 0.9
4-*O*-caffeoyl-quinic acid (**20**)	33.9 ± 2.6	206.3 ± 6.7
gallic acid (**22**)	3.5 ± 0.6	48.7 ± 2.3
3,4-dihydroxybenzoic acid (**23**)	25.8 ± 1.4	379.8 ± 20.2
ascorbic acid (positive control)	32.7 ± 0.3	121.9 ± 5.6
trolox (positive control)		140.8 ± 6.7

^{a}Values represent means ± SDs (n = 3). ^{b}SC_{50} = concentration in micromolar required to scavenge 50% of DPPH and $ABTS^+$ radical.

HPLC and LC-MS Analysis. The main compounds in the leaves of *C. taliensis* from Yuanjiang County were confirmed by LC-MS and co-HPLC analysis with authentic samples. These were two hydrolyzable tannins, 1-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-*β*-D-glucopyranose (**5**) (*m*/*z* 633 [M − H]$^-$, 1267 [2M − H]$^-$) and 1,2-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-*β*-D-glucopyranose (**6**) (*m*/*z* 785 [M − H]$^-$, 169 [gallic acid − H]$^-$), three flavan-3-ols, EGC (**15**) (*m*/*z* 305 [M − H]$^-$, 611 [2M − H]$^-$), (−)-epigallocatechin-3-*O*-gallate (EGCG, *m*/*z* 457 [M − H]$^-$, 169 [gallic acid − H]$^-$) (peak a) and ECG (*m*/*z* 441 [M − H]$^-$, 289 [M − 153 (galloyl)]$^-$) (peak b), two quinic acid derivatives, chlorogenic acid (**17**) (*m*/*z* 353 [M − H]$^-$, 191 [M − 163(caffeoyl)]$^-$) and theogallin (**18**) (*m*/*z* 343 [M − H]$^-$, 191 [M − 153 (galloyl)]$^-$), as well as caffeine (**24**). The results are shown in Figure 3A and Table 3.

HPLC Quantitative Analysis. The contents of the main compounds, **5**, **6**, **18**, **24**, EGCG (peak a), and ECG (peak b), in the leaves of *C. taliensis* collected from Yuanjiang county (east side of the Ai-Lao mountains) were compared with those collected from Lincang area (west side of the Ai-Lao mountains) by HPLC analysis. As shown in Figure 3, both samples contained rich flavan-3-ols (EGCG and ECG), hydrolyzable tannins (**5** and **6**), and theogallin (**18**), in addition to caffeine (**24**); however, their contents were different. The content of EGCG (4.38%) in *C. taliensis* from Yuanjiang was a little higher than that from Lincang (4.03%),

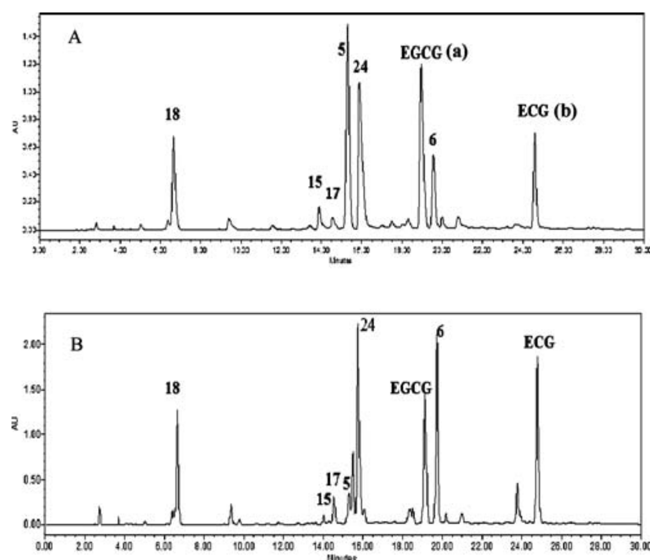

Figure 3. HPLC chromatograms of green tea produced from *C. taliensis* from two different areas [A, Yuanjiang; B, Lincang; peak 5, 1-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-*β*-D-glucopyranose; peak 6, 1,2-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-*β*-D-glucopyranose; peak a, (−)-epigallocatechin-3-*O*-gallate (EGCG); peak b, ECG; peak 17, chlorogenic acid; peak 18, theogallin; and peak 24, caffeine].

Table 3. UV and ESI-MS Data of the Main Compounds in the Leaves of *C. taliensis* from Yuanjiang

chemical constituents	UV (nm)	ESI-MS (negative) (*m*/*z*)
1-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoyl-*β*-D-glucopyranose (**5**)	218/270	633 [M − H]$^-$, 1267 [2M − H]$^-$
1,2-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxy-diphenoyl-*β*-D-glucopyranose (**6**)	217/276	785 [M − H]$^-$, 169 [gallic acid − H]$^-$
EGC (**15**)	207/270	305 [M − H]$^-$, 611 [2M − H]$^-$
chlorogenic acid (**17**)	217/241/327	353 [M − H]$^-$, 191 [M − 163 (caffeoyl)]$^-$
theogallin (**18**)	215/273	343 [M − H]$^-$, 191 [M − 153 (galloyl)]$^-$
EGCG (**a**)	209/275	457 [M − H]$^-$, 169 [gallic acid − H]$^-$
ECG (**b**)	204/277	441 [M − H]$^-$, 289 [M − 153 (galloyl)]$^-$

while the contents of ECG (1.57%), theogallin (**18**) (2.01%), and caffeine (**24**) (2.67%) were on the contrary lower than that from Lincang (3.39, 2.81, and 3.32%, respectively). What's more, the content of 1,2-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-*β*-D-glucopyranose (**6**, 1.57%), which was considered as one of the major and characteristic constituents in *C. taliensis*,[16] was lower than that from Lincang (2.44%). It is noted that another hydrolyzable tannin, 1-*O*-galloyl-4,6-*O*-(*S*)-hexa-hydroxyldiphenoyl-*β*-D-glucopyranose (**5**), one of the minor compounds in *C. taliensis* from Lincang county (0.69%), appeared to be the predominant major hydrolyzable tannin in the one collected from Yuanjiang county (3.27%). The two hydrolyzable tannins (**5** and **6**) and their contents in the leaves may be considered as indicators to differentiate *C. taliensis* from different areas, Yuanjiang and Lincang.

In the present study, one new flavan-3-ol dimer with C-8/C-8 linkage through methylene bridge, namely, talienbisflavan A (**1**), was isolated from the leaves of *C. taliensis* collected from

Yuanjiang county, together with 22 known phenolic compounds, including hydrolyzable tannins (**2–6**), flavonols and flavonol glycosides (**9–13**), flavan-3-ols (**14–16**), and simple phenolic compounds (**7**, **8**, and **17–23**), in addition to caffeine (**24**). The hydrolyzable tannins and flavan-3-ols exhibited stronger antioxidant activities than the other types of compounds, by DPPH and $ABTS^+$ assays. The new compound, talienbisflavan A (**1**), also showed stronger potential antioxidant activity than the positive controls. This may provide the scientific support for why the local people use this plant to produce tea beverages. Moreover, LC-MS and HPLC analyses suggested that hydrolyzable tannins, 1-*O*- (**5**) and 1,2-di-*O*- (**6**) galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-*β*-D-glucopyranose, could be considered to be the characteristic marker to differentiate *C. taliensis* from different collecting areas. The difference of secondary metabolites from different regions may be caused by the differences of soil, climate, altitude, etc. To give more evidence, numbers of *C. taliensis* samples from different regions and environments were collected, and detailed comparison and analysis for their polyphenols are now in progress.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H, ^{13}C, HMQC, and HMBC spectra of talienbisflavan A (**1**) in CD_3OD. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Corresponding author. Tel: + 86 871 522 3235; Fax: + 86 871 5223235. *E-mail address*: zhangyj@mail.kib.ac.cn.

Funding

This work was supported by the NSFC 30970287.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to the staffs of the analytical group at State Key Laboratory of Phytochemistry & Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, for measuring the spectroscopic data.

■ REFERENCES

(1) Nonaka, G.; Kawahara, O.; Nishioka, I. Tannins and related compounds. XV. A new class of dimeric flavan-3-ol gallates, theasinensins A and B, and proanthocyanidin gallates from green tea leaf. *Chem. Pharm. Bull.* **1983**, *31*, 3906–3914.

(2) Nonaka, G.; Sakai, R.; Nishioka, I. Hydrolyzable tannins and proanthocyanidins from green tea. *Phytochemistry* **1984**, *23*, 1753–1755.

(3) Hashimoto, F.; Nonaka, G.; Nishioka, I. Tannins and related compounds. LVI. Isolation of four new acylated flavan-3-ols from oolong tea (1). *Chem. Pharm. Bull.* **1987**, *35*, 611–616.

(4) Hashimoto, F.; Nonaka, G.; Nishioka, I. Tannins and related compounds. LXIX. Isolation and structure elucidation of B, B′-linked bisflavanoids, theasinensins D-G and oolongtheanin from oolong tea (2). *Chem. Pharm. Bull.* **1988**, *36*, 1676–1684.

(5) Hashimoto, F.; Nonaka, G.; Nishioka, I. Tannins and related compounds. XC. 8-C-ascorbyl (−)-epigallocatechin 3-*O*-gallate and novel dimeric flavan-3-ols, oolonghomobisflavans A and B, from oolong tea (3). *Chem. Pharm. Bull.* **1989**, *37*, 3255–3263.

(6) Nonaka, G.; Hashimoto, F.; Nishioka, I. Tannins and related compounds. XXXVI. Isolation and structures of theaflagallins, new red pigments from black tea. *Chem. Pharm. Bull.* **1986**, *34*, 61–65.

(7) Hashimoto, F.; Nonaka, G.; Nishioka, I. Tannins and related compounds. CXIV. Structure of novel fermentation products, theogallinin, theaflavonin and desgalloyl theaflavonin from black tea, and changes of tea leaf polyphenols during fermentation. *Chem. Pharm. Bull.* **1992**, *40*, 1383–1389.

(8) Zhou, Z. H.; Zhang, Y. J.; Xu, M.; Yang, C. R. Puerins A and B, tow new 8-C substituted flavan-3-ols from Pu-er tea. *J. Agric. Food Chem.* **2005**, *53*, 8614–8617.

(9) Hashimoto, F.; Nonaka, G. I.; Nishioka, I. Tannins and related compounds. LXXVII. Novel chalcan-flavan dimers, assamicains A, B and C, and a new flavan-3-ol and proanthocyanidins from the fresh leaves of *Camellia sinensis* L. var. *assamica* Kitamura. *Chem. Pharm. Bull.* **1989**, *37*, 77–85.

(10) Hanasaki, Y.; Ogawa, S.; Fukui, S. The correlation between active oxygens scavenging and antioxidative effects of flavonoids. *Free Radical Biol. Med.* **1994**, *16*, 845–850.

(11) Sichel, G.; Corsaro, C.; Scalia, M.; Di Bilio, A. J.; Bonomo, R. O. In vitro scavenger activity of some flavonoids and melanin against $O_2^{-\bullet}$. *Free Radical Biol. Med.* **1991**, *11*, 1–8.

(12) Fukuda, T.; Ito, H.; Yoshida, T. Antioxidative polyphenols from walnuts (*Juglans regia* L.). *Phytochemistry* **2003**, *63*, 795–801.

(13) Min, T. L. A revision of Camellia sect. *Acta Bot. Yunnan* **1992**, *14*, 115–132.

(14) Jiang, H. B.; Wang, Y. G.; Tang, Y. C.; Song, W. X.; Li, Y. Y.; Ji, P. Z.; Huang, X. Q. Investigation of Wild Tea Plant (*Camellia taliensis*) Germ plasm R esource from Yunnan, China. *Southwest China J. Agric. Sci.* **2009**, *22*, 1153–1157.

(15) Yang, C. R.; Zhang, Y. J.; Gao, D. F.; Chen, K. K.; Jiang, H. J. The evaluation of *Camllia taliensis* germplasm resources and the origin of the cultivated tea. *Tea Sci. Technol.* **2008**, 1–4.

(16) Gao, D. F.; Zhang, Y. J.; Yang, C. R.; Chen, K. K.; Jiang, H. J. Phenolic antioxidants from green tea produced from *Camellia taliensis*. *J. Agric. Food Chem.* **2008**, *56*, 7517–7521.

(17) Gao, D. F.; Xu, M.; Yang, C. R.; Xu, M.; Zhang, Y. J. Phenolic antioxidants from the leaves of *Camellia pachyandra* Hu. *J. Agric. Food Chem.* **2010**, *58*, 8820–8824.

(18) Liu, Q.; Zhang, Y. J.; Yang, C. R.; Xu, M. Phenolic antioxidants from green tea produced from *Camellia crassicolumna* Var. *multiplex*. *J. Agric. Food Chem.* **2009**, *57*, 586–590.

(19) Zhou, Z. H.; Yang, C. R. Chemical constituents of crude green tea, the material of pu-er tea in Yunnan. *Acta Bot. Yunnan* **2000**, *22*, 343–350.

(20) Chun, S. S.; Vattem, D. A.; Lin, Y. T.; Shetty, K. Phenolic antioxidants from clonal oregano (Origanum vulgare) with antimicrobial activity against *Helicobacter pylori*. *Process Biochem.* **2005**, *40*, 809–816.

(21) Zhu, F.; Cai, Y. Z.; Sun, M.; Ke, J. X.; Lu, D. Y.; Corke, H. Comparison of major phenolic constituents and in vitro antioxidant activity of diverse Kudingcha genotypes from *Ilex kudingcha*, *Ilex cornuta*, and *Ligustrum robustum*. *J. Agric. Food Chem.* **2009**, *57*, 6082–6089.

(22) Saijo, R.; Nonaka, G. I.; Nishioka, I. Gallic acid esters of bergenin and norbergenin from *Mallotus japonicus*. *Phytochemistry* **1990**, *29*, 267–270.

(23) Orabi, M. A. A.; Taniguchi, S.; Hatano, T. Monomeric and dimeric hydrolysable tannins of *Tamarix nilotica*. *Phytochemistry* **2009**, *70*, 1286–1293.

(24) Sugimoto, K.; Nakagawa, K.; Hayashi, S.; Amakura, Y.; Yoshimura, M.; Yoshida, T.; Yamaji, R.; Nakano, Y.; Inui, H. Hydrolyzable tannins as antioxidants in the leaf extract of *Eucalyptus globulus* possessing tyrosinase and hyaluronidase inhibitory activities. *Food Sci. Technol. Res.* **2009**, *15*, 331–336.

(25) Li, G. C.; Chen, C. X.; Luo, J. L.; Wang, D. Y. Chemical constituents in hypoglycemic active fraction in leaves of *Myrica rubra*. *Chin. Tradit. Herb. Drugs* **2011**, *42*, 863–865.

(26) Yasukawa, K.; Takido, M. A flavonol glycoside from *Lysimachia Mauritiana*. *Phytochemistry* **1987**, *26*, 1224−1226.
(27) Wan, S. B.; Chan, T. H. Enantioselective synthesis of afzelechin and epiafzelechin. *Tetrahedron* **2004**, *60*, 8207−8211.
(28) Lee, M. W.; Morimoto, S.; Nonaka, G. I.; Nishioka, I. Flavan-3-ol gallates and proanthocyanidins from *Pithecellobium lobatum*. *Phytochemistry* **1992**, *31*, 2117.
(29) Nishimura, H.; Nonaka, G.; Nishioka, I. Seven quinic acid gallates from *quercus stenophylla*. *Phytochemistry* **1984**, *23*, 2621−2623.
(30) Lin, Y. L.; Lu, C. K.; Huang, Y. J.; Chen, H. J. Antioxidative caffeoylquinic acids and flavonoids from *Hemerocallis fulva* flowers. *J. Agric. Food Chem.* **2011**, *59*, 8789−8795.
(31) Ishimaru, K.; Nonaka, G.; Nishoka, I. Phenolic glucoside gallates from *Quercus mongolica* and *Q. acutissima*. *Phytochemistry* **1987**, *26*, 1147−1152.
(32) Nonaka, G.; Nishioka, I. Tannins and related compounds. X. Rhubarb (2): Isolation and structures of a glycerol gallate, gallic acid glucoside gallates, galloylglucoses and isolindleyin. *Chem. Pharm. Bull.* **1983**, *31*, 1652−1658.
(33) Sun, L.; Yan, Y. B. Chemical constituents of *Senecio laetus*. *Zhongguo Yaoye* **2011**, *20*, 34.
(34) Kang, J. M.; Ouyang, S.; Xiao, B. K.; Yang, J. Y.; Huang, R. Q. Isolation and identification of chemical constituents from *Hypericum sampsonii* Hance. *Shizhen Guoyi Guoyao* **2011**, *22*, 2641−2642.