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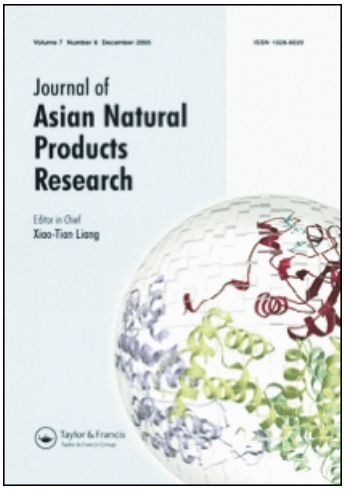

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GLYCOSYL FLAVONOIDS FROM THE ROOTS AND RHIZOMES OF *ASARUM LONGERHIZOMATOSUM*

SHU-XIANG ZHANG[a], TADATO TANI[b], SEIICHI YAMAJI[b], CHAO-MEI MA[a], MIN-CHUAN WANG[a], SHAO-QING CAI[a,*] and YU-YING ZHAO[a]

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Two new glycosyl flavonoids including a glycosyl aurone, together with six known flavonoids were isolated from the roots and rhizomes of *Asarum longerhizomatosum*. The structures of the two new compounds were elucidated as 4,6,4′-trihydroxy-aurone-4,6-di-*O*-β-D-glucopyranoside (**7**, caulesauroneside) and naringenin-7,4′-di-*O*-β-D-glucopyranoside (**8**, caulesnarinside). The six known flavonoids were identified as naringenin (**1**), naringenin-5-*O*-β-D-glucopyranoside (**2**), naringenin-7-*O*-β-D-glucopyranoside (**3**), chalcononaringenin-2′-*O*-β-D-glucopyranoside (**4**), naringenin-5,7-di-*O*-β-D-glucopyranoside (**5**), chalcononaringenin-2′,4′-di-*O*-β-D-glucopyranoside (**6**), respectively. This is the first report of the isolation of aurones in the family Aristolochiaceae.

Keywords: *Asarum longerhizomatosum*; Glycosyl flavonoids; Aurone; Rhizomes

INTRODUCTION

Asarum longerhizomatosum C.F. Liang et C.S. Yang (Aristolochiaceae) is a perennial herbaceous plant native to the Guangxi and Hubei provinces of China. It has been used as a substitute of Xixin [herb Asari: *Asarum heterotropoids* Fr. Schmidt *var. mandshuricum* (Maxim.) Kitag., *A. sieboldii* Miq.var. *seoulense* Nakai, *A. sieboldii* Miq.] in folk medicine for the treatment of colds, coughs, chromatic bronchitis, asthma, gastritis and snakebite. The essential oils of the plant in this genus have been surveyed in detail, but no systematic phytochemical investigations have been done on this plant. We have investigated the roots and rhizomes of *A. longerhizomatosum*, and obtained two new glycosyl flavonoids: 4,6,4′-trihydroxy-aurone-4,6-di-*O*-β-D-glucopyranoside (**7**) and naringenin-7,4′-di-*O*-β-D-glucopyranoside (**8**), along with naringenin (**1**) [1], naringenin-5-*O*-β-D-glucopyranoside (**2**) [2], naringenin-7-*O*-β-D-glucopyranoside (**3**) [3], chalcononaringenin-2′-*O*-β-D-glucopyranoside (**4**), [2], naringenin-5,7-di-*O*-β-D-glucopyranoside (**5**) [2,4], chalcononaringenin-2′,4′-di-*O*-β-D-glucopyranoside (**6**) [5]. In this paper, we report the isolation and characterization of these flavonoids.

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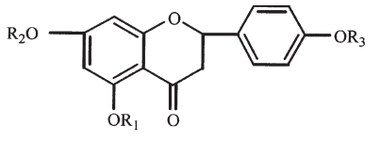
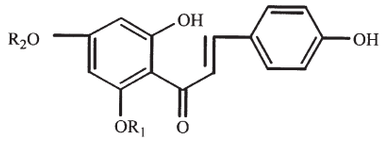
RESULTS AND DISCUSSION

Compound **7** was isolated as a yellow powder, mp 205–207°C, $[\alpha]_D^{20}$ +122.4 (MeOH; *c* 1.16). A molecular formula of $C_{27}H_{30}O_{15}$ for compound **7** was established from its HR-FABMS. It was recognized as a glycosyl flavonoid from a positive test with HCl–Mg powder and Molish reagents. The UV spectrum of **7** in MeOH showed an absorption maximum at 400 nm and a shoulder at 253 nm, revealing the compound to be an aurone [6]. A bathochromic shift of 55 nm in band I with NaOMe (in MeOH) indicated a free hydroxyl group at C-4′. Moreover, since no wavelength shift was observed with NaOAc addition, no free hydroxyl group is present at C-7. Its IR spectrum showed absorptions at IR ν_{max}^{KBr} 3355 cm^{-1} (OH), 1600 cm^{-1} (C=O), 1130–1050 cm^{-1} (glycoside linkage). The ^{1}HNMR spectrum indicated the presence of 6 aromatic proton signals and two glucosyl anomeric proton signals. An olefinic proton at δ 6.67 (1H, s) was correlated to the carbonyl carbon (δ 178.9) and 2′, 6′-carbons (δ 133.1) through $^3J_{CH}$ coupling in HMBC. The AA′BB′ type aromatic protons at δ 7.82 (2H, d, $J = 8.5$ Hz) and 6.88 (2H, d, $J = 8.5$ Hz) each being integrated for two protons, indicated the presence of a symmetrical substitution in ring B. The highfield proton singlets at δ 6.52 and 6.75 were assigned to H-5 and H-7. The substitution patterns were also confirmed by the HMBC spectrum. Two anomeric proton signals at δ 5.18 (1H, d, $J = 7.5$ Hz) and 5.10 (1H, d, $J = 7.2$ Hz) suggested 2 sugar moieties existing in compound **7**, and both of them were identified as glucose by acid hydrolysis and PLC experiment. The coupling constants of the anomeric protons suggested the presence of β-D-glucopyranose. In the HMBC spectrum of compound **7** (Fig. 2), the anomeric protons at



	R_1	R_2	R_3
1	H	H	H
2	glc	H	H
3	H	glc	H
5	glc	glc	H
8	H	glc	glc
9	H	H	glc



	R_1	R_2
4	glc	H
6	glc	glc

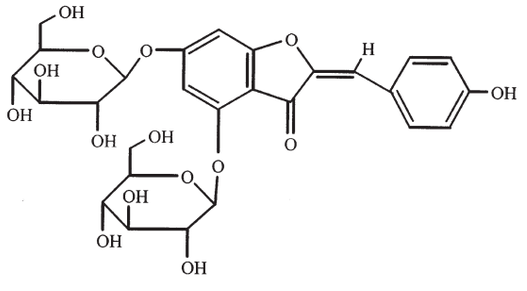


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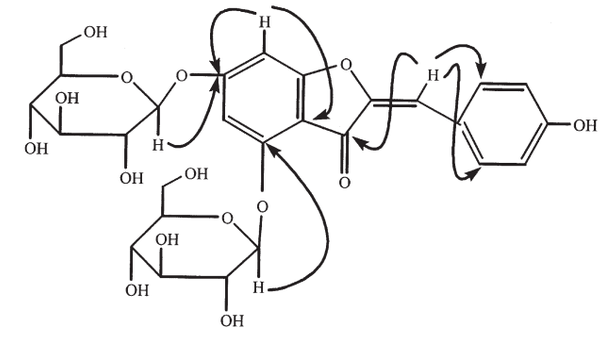
FIGURE 1 Structures of compounds **1**–**9**.

δ 5.18 and 5.10 correlated to the carbons at δ 156.2 (C-4) and 165.7 (C-6), respectively. Therefore, the sugar moieties were determined to be linked to the aglycone via the C-4 and C-6 hydroxy groups. Thus, it was identified as 4,6,4′-trihydroxyaurone-4,6-di-*O*-β-D-glucopyranoside (Fig. 1), and named as caulesauroneside.

Compound **8** was isolated as a yellow powder, mp 208–210°C, $[\alpha]_D^{20}$ +5.31 (MeOH; *c* 0.57). A molecular formula of $C_{27}H_{32}O_{15}$ for compound **8** was established from its HR-FABMS. It was recognized as a glycosyl flavonoid from a positive test with HCl–Mg powder and Molish reagents. The UV spectrum of **8** in MeOH showed an absorption maximum at 283 nm, revealing the compound to be a flavanone [6]. No bathochromic shift was observed in band II with NaOAc (in MeOH), which indicated no free hydroxyl group at C-7. Its IR spectrum showed absorptions at IR ν_{max}^{KBr} 3373 cm^{-1} (OH), 1639 cm^{-1} (C = O), 1135–1050 cm^{-1} (glycoside linkage). In ^{1}HNMR, the AA′BB′ type aromatic protons at δ 7.46 (2H, d, $J = 8.8$ Hz) and 7.08 (2H, d, $J = 8.8$ Hz) showed the presence of a 4′-monosubstituted pattern in ring B. Two anomeric protons signals at δ 4.98 (1H, d, $J = 8.0$ Hz) and 4.91 (1H, d, $J = 7.5$ Hz) suggested the presence of 2 sugar moieties in compound **8**, and they were identified as glucose by acid hydrolysis and PLC experiment. The coupling constants of the anomeric protons suggested the presence of β-D-glucopyranose. In the HMBC spectrum of **8** (Fig. 2), the anomeric protons at δ 4.98 and 4.91 correlated to the carbons at δ 165.3 (C-7) and 157.6 (C-4′), respectively. Therefore, the sugar moieties were determined to be linked to the aglycone via the C-7 and C-4′ hydroxy groups. This was further confirmed by comparing the NMR data of **8** with those of known compounds naringenin-7-*O*-β-D-glucopyranoside [3] and choerospondin (**9**) [7]. A proton signal at δ 12.0 indicated a free hydroxyl group at C-5. Thus, **8** was identified as naringenin-7,4′-di-*O*-β-D-glucopyranoside (Fig. 1), and named as caulesnarinside.



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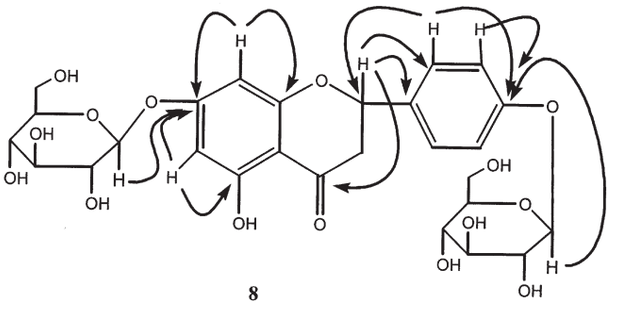

8

FIGURE 2 HMBC correlations for compounds **7** and **8**.

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EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were determined on XT4A apparatus and are uncorrected. IR spectra were taken on a Perkin-Elmer 983 and FAB-mass spectra on a Bruker APEX II with glycerol. ^{13}CNMR and ^{1}HNMR spectra were recorded on a JEOL AL-400 and Bruker 500 MHz using TMS as internal reference.

Plant Material

A. longerhizomatosum C.F. Liang et C.S. Yang was collected in the Guangxi province of China by Professor Shou-Yang Liu and was taxonomically identified by Professor Shao-Qing Cai. The voucher specimen (No. 1255) was deposited in the specimen room of the School of Pharmaceutical Sciences, Peking University.

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EXTRACTION AND ISOLATION

A powder of the roots and rhizomes of *A. longerhizomatosum* was extracted with EtOH. After concentration under reduced pressure, the aqueous residue was partitioned with petroleum ether, EtOAc and *n*-BuOH, respectively. The EtOAc-soluble portion was chromatographed over silica gel and eluted with petroleum ether–EtOAc and $CHCl_3$–MeOH gradient solvent. Combination of similar fractions on the basis of TLC analysis afforded 8 fractions. Fraction 4 was chromatographed over silica gel with $CHC1_3$–Me_2CO to give narigenin (**1**, 14 mg). Fraction 7 was chromatographed over silica gel with $CHCl_3$–MeOH, then purified by ODS column chromatography to afford naringenin-5-*O*-β-D-glucopyranoside (**2**, 22 mg) and naringenin-7-*O*-β-D-glucopyranoside (**3**, 8 mg). Fraction 8 was isolated by the same methods above to afford chalcononaringenin-2′-*O*-β-D-glucopyranoside (**4**, 4 mg). The *n*-BuOH-soluble part was subjected to D101 resin column chromatography and eluted with H_2O, 20% MeOH, 50% MeOH and MeOH, respectively. The 20% MeOH eluted part was subjected to silica gel column chromatography eluted with $CHCl_3$–MeOH–H_2O in gradient manner, then purified by Sephadex LH-20 and RP-18 column chromatography to obtain naringenin-5,7-di-*O*-β-D-glucopyranoside, naringenin-7,4′-di-*O*-β-D-glucopyranoside (**5**, 2.3 g), chalcononaringenin-2′,4′-di-*O*-β-D-glucopyranoside (**6**, 18 mg), 4,6,4′-trihydroxyaurone-4,6-di-*O*-β-D-glucopyranoside (**7**, 12 mg), naringenin-7,4′-di-*O*-β-D-glucopyranoside (**8**, 23 mg) were obtained respectively.

Naringenin (**1**): colorless needles ($CHCl_3$–MeOH), mp 245–247°C. ^{1}HNMR (DMSO) δ: 5.45 (1H, dd, $J = 2.7$, 12.9 Hz, H-2), 2.68 (1H, dd, $J = 3.2$, 17.3 Hz, H-3*cis*), 3.26 (1H, dd, $J = 12.9$, 17.3 Hz, H-3*trans*), 5.87 (2H, s, H-6 and H-8), 7.31 (2H, d, $J = 8.6$ Hz, H-2′, 6′), 6.80 (2H, d, $J = 8.6$ Hz, H-3′, 5′), 12.14 (1H, s, 5-OH), 9.59 (1H, s, 4′-OH). ^{13}CNMR: Table I.

Naringenin-5-*O*-β-D-glucopyranoside (**2**): white powder (MeOH–H_2O), mp 161–163°C. FAB-MS: 435 $[M + 1]^+$. ^{1}HNMR (DMSO) δ: 5.38 (1H, dd, $J = 2.7$, 13.4 Hz, H-2), 2.63 (1H, dd, $J = 2.7$, 17.3 Hz, H-3*cis*), 3.05 (1H, dd, $J = 13.4$, 1.4 Hz, H-3*trans*), 6.93 (1H, d, $J = 2.0$ Hz, H-6), 6.07 (1H, d, $J = 2.2$ Hz, H-8), 7.31 (2H, d, $J = 8.6$ Hz, H-2′, 6′), 6.79 (2H, d, $J = 8.6$ Hz, H-3′, 5′), 9.55 (4′-OH), 5.06 (1H, d, $J = 5.2$, H-1″). ^{13}CNMR: Table I.

Naringenin-7-*O*-β-D-glucopyranoside (**3**): white powder (MeOH–H_2O), mp 147–149°C. FAB-MS: 435 $[M + 1]^+$. ^{1}HNMR (DMSO) δ: 5.51 (1H, dd, $J = 3.0$, H-2), 2.75 (1H, dd, $J = 2.9$, 17.3 Hz, H-3*cis*), 3.36 (1H, overlapping), 6.15 (1H, d, $J = 2.2$ Hz, H-6),

TABLE I ^{13}CNMR spectral data of flavanoids (100 Hz in DMSO-d_6)

C	*1*	*2*	*3*	*9*	*5*	*8**
2	78.4	78.1	78.6	78.1	78.4	78.4
3	41.9	44.5	42.0	42.1	44.5	42.1
4	196.3	190.0	197.2	196.0	190.0	197.1
5	163.4	164.2	165.3	163.0	163.2	162.9
6	94.9	98.8	96.4	95.7	99.2	96.6
7	166.6	164.8	162.9	166.3	163.8	165.3
8	95.8	97.7	95.4	95.0	98.1	95.5
9	162.9	160.6	162.7	162.7	159.8	162.6
10	101.7	105.5	103.2	101.7	106.9	103.3
1′	128.8	129.0	128.6	131.8	128.8	131.6
2′, 6′	128.3	128.2	128.4	128.0	128.3	128.2
3′, 5′	115.1	115.1	115.2	116.1	115.1	116.2
4′	157.7	157.6	157.8	157.4	157.7	157.6
1″		103.4	99.6	100.2	102.5	99.6
2″		73.4	73.0	73.1	73.4	73.0
3″		77.5	77.1	77.0	77.4	77.1
4″		69.6	69.5	69.5	70.0	69.7
5″		75.6	76.3	76.5	75.7	76.3
6″		60.7	60.5	60.6	61.0	60.7
1‴					99.2	100.2
2‴					73.0	73.2
3‴					77.0	77.1
4‴					69.6	69.5
5‴					76.4	76.6
6‴					60.9	60.6

* 125 Hz in DMSO-d_6.

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6.12 (1H, d, $J = 2.2$ Hz, H-8), 7.33 (2H, d, $J = 8.8$ Hz, H-2′, 6′), 6.79 (2H, d, $J = 8.8$ Hz, H-3′, 5′), 12.04 (1H, s, 5-OH), 4.96 (1H, d, $J = 7.6$ Hz, H-1″). ^{13}CNMR: Table I.

Chalcononaringenin-2′-*O*-β-D-glucopyranoside (**4**): yellow powder (MeOH–acetone), mp 151–153°C. ^{1}HNMR (DMSO) δ: 7.99 (1H, d, $J = 15.6$ Hz, H-α), 7.62 (1H, d, $J = 15.6$ Hz, H-β), 7.64 (2H, d, $J = 8.5$ Hz, H-2, 6), 6.82 (2H, d, $J = 8.5$ Hz, H-3, 5), 6.16 (1H, d, $J = 2.0$ Hz, H-3′), 6.16 (1H, d, $J = 2.0$ Hz, H-5′), 5.07 (1H, d, $J = 7.6$ Hz, H-1″). ^{13}CNMR (DMSO) δ: 126.2 (C-1), 130.8 (C-2, 6), 115.9 (C-3, 5), 160.3 (C-4), 124.2 (C-α), 142.9 (C-β), 192.2 (C = O), 105.6 (C-1′), 166.0 (C-2′), 96.9 (C-3′), 164.5 (C-4′), 94.6 (C-5′), 159.9 (C-6′), 100.4 (C-1″), 73.7 (C-2″), 76.8 (C-3″), 69.4 (C-4″), 77.3 (C-5″), 60.5 (C-6″).

Naringenin-5,7-di-*O*-β-D-glucopyranoside (**5**): white powder (MeOH–H_2O), mp 203–205°C. FAB-MS: 597 $[M + H]^+$, $C_{27}H_{32}O_{15}$. ^{1}HNMR (DMSO) δ: 5.44 (1H, dd, $J = 2.72$, 13.2 Hz, H-2), 2.78 (1H, dd, $J = 2.7$, 17.3 Hz, H-3*cis*), 3.37 (1H, unclear), 6.51 (1H, d, $J = 2.4$ Hz, H-6), 6.33 (1H, d, $J = 2.4$ Hz, H-8), 7.33 (2H, d, $J = 8.8$ Hz, H-2′, 6′), 6.79 (2H, d, $J = 8.8$ Hz, H-3′, 5′), 9.58 (1H, s, 4′-OH), 5.09 (1H, d, $J = 7.3$ Hz, H-1″), 5.00 (1H, d, $J = 8.5$ Hz, H-1‴). ^{13}CNMR: Table I.

Chalcononaringenin-2′,4′-di-*O*-β-D-glucopyranoside (**6**): yellow powder MeOH–acetone), mp 183–185°C. FAB-MS: 597 $[M + H]^+$, $C_{27}H_{32}O_{15}$. ^{1}HNMR (DMSO) δ: 7.89 (1H, $J = 15.6$ Hz, H-α), 7.64 (1H, d, $J = 15.6$ Hz, H-β), 7.65 (2H, d, $J = 8.8$ Hz, H-2, 6), 6.82 (2H, d, $J = 8.8$ Hz, H-3, 5), 6.38 (1H, d, $J = 2.4$ Hz, H-3′), 6.19 (1H, d, $J = 2.4$ Hz, H-5′), 5.12 (1H, d, $J = 6.5$ Hz, H-1″), 4.98 (1H, d, $J = 7.3$ Hz, H-1‴). ^{13}CNMR (DMSO) δ: 126.0 (C-1), 131.0 (C-2, 6), 115.9 (C-3, 5), 160.1 (C-4), 124.0 (C-α), 143.7 (C-β), 192.7 (C = O), 107.4 (C-1′), 164.4 (C-2′), 97.6 (C-3′), 162.6 (C-4′), 94.6 (C-5′), 159.3 (C-6′), 100.2 (C-1″), 73.6 (C-2″), 76.8 (C-3″), 69.7 (C-4″), 77.3 (C-5″), 60.8 (C-6″), 99.3 (C-1‴), 73.0 (C-2‴), 76.5 (C-3‴), 69.8 (C-4‴), 77.1 (C-5‴), 60.8 (C-6‴).

4,6,4′-Trihydroxy-aurone-4,6-di-*O*-β-D-glucopyranoside (**7**): yellow powder (MeOH–H_2O), mp 205–207°C, $[\alpha]_D^{20}$ +122.4 (MeOH; *c* 1.16). N-HR-FAB-MS: 593.1514 $[M\text{-}H]^-$ (base, calcd. for $C_{27}H_{30}O_{15}$, *m/z* 593.1512). UV λ_{max}^{MeOH} nm: 253 (weak), 400. IR ν_{max}^{KBr} cm^{-1}: 3353, 1600. ^{1}HNMR (DMSO) δ: 6.52 (1H, s, H-5), 6.75 (1H, s, H-7), 6.67 (1H, s, =CH), 7.82 (2H, d, $J = 8.5$ Hz, H-2′, 6′), 6.89 (2H, d, $J = 8.5$ Hz, H-3′, 50′), 5.18 (1H, d, $J = 7.5$, H-1″), 5.10 (1H, d, $J = 7.5$ Hz). ^{13}CNMR (DMSO) δ: 145.5 (C-2), 178.9 (C-3), 105.3 (C-3a), 156.2 (C-4), 98.0 (C-5), 165.7 (C-6), 93.0 (C-7), 167.0 (C-7a), 110.7 (C-1′), 133.1 (C-2′, 6′), 116.0 (C-3′, 5′), 159.2 (c-4′), 99.7 (C-1″), 73.0 (C-2″), 77.1 (C-3″), 69.9 (C-4″), 76.7 (C-5″), 60.7 (C-6″), 99.3 (C-1‴), 73.0 (C-2‴), 77.1 (C-3‴), 69.6 (C-4‴), 76.5 (C-5‴), 60.7 (C-6‴).

Naringenin-7,4′-di-*O*-β-D-glucopyranoside (**8**): white powder (MeOH–H_2O), mp 208–210°C, $[\alpha]_D^{20}$ +5.31 (MeOH, *c* 0.57). N-HR-FAB-MS: 595.1671 $[M\text{-}H]^{-1}$ (base, calcd. for $C_{27}H_{32}O_{15}$, 595.1668) UV λ_{max}^{MeOH} nm: 283. IR ν_{max}^{KBr} cm^{-1}: 3373, 1639. ^{1}HNMR (DMSO) δ: 5.60 (1H, br.d, $J = 12.5$ Hz, H-2), 2.82 (1H, dd, $J = 3.0$, 17.5 Hz, H-3*cis*), 3.37 (1H, overlapping, H-3*trans*), 12.0 (1H, s, 5-OH), 6.15 (1H, m, H-6), 6.18 (1H, s, H-8), 7.46 (2H, d, $J = 9.0$ Hz, H-2′, 6′), 7.08 (2H, d, $J = 8.8$ Hz, H-3′, 5′), 4.98 (1H, d, $J = 8.0$ Hz, H-1″), 4.91 (1H, dd, $J = 2.0$, 7.5 Hz, H-1‴). ^{13}CNMR: Table I.

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Acknowledgements

The authors are grateful to Professor Shou-Yang Liu of the Guangxi School of Traditional Chinese Medicine for collecting the plant materials.

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