GLYCOSYL FLAVONOIDS FROM THE ROOTS AND RHIZOMES OF ASARUM LONGERHIZOMATOSUM

SHU-XIANG ZHANGa, TADATO TANIb, SEIICHI YAMAJIb, CHAO-MEI MAa, MIN-CHUAN WANGa, SHAO-QING CAIa,* and YU-YING ZHAOa

aDepartment of Natural Medicines, School of Pharmaceutical Sciences, Peking University, Beijing 100083, China; bInstitute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama 930-0194, Japan

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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-7,4'-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, chronic 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.

*Corresponding author. Tel./Fax: +86-10-62091693. E-mail: sqcai@mail.bjmu.edu.cn

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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 $\nu_{\text{max}}$ 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 $\delta$ 6.67 (1H, s) was correlated to the carbonyl carbon ($\delta$ 178.9) and 2', 6'-carbons ($\delta$ 133.1) through $^3$J$_{CH}$ coupling in HMBC. The AA'BB' type aromatic protons at $\delta$ 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 $\delta$ 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 $\delta$ 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 $\beta$-D-glucopyranose. In the HMBC spectrum of compound 7 (Fig. 2), the anomeric protons at
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, [α]D²⁰ +5.31 (MeOH; c 0.57). A molecular formula of C₂₇H₃₂O₁₅ 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 v max 3373 cm⁻¹ (OH), 1639 cm⁻¹ (C=O), 1135–1050 cm⁻¹ (glycoside linkage). In ¹H NMR, 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 anomic 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 anometric protons suggested the presence of β-D-glucopyranose. In the HMBC spectrum of 8 (Fig. 2), the anomic 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.

![FIGURE 2 HMBC correlations for compounds 7 and 8.](image-url)
**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.

**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 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 CHCl$_3$–Me$_2$CO 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-5-0-β-D-glucopyranoside (2, 22 mg) and naringenin-7-0-β-D-glucopyranoside (3, 8 mg). Fraction 8 was isolated by the same methods above to afford chalcononaringenin-2-0-β-D-glucopyranoside (4, 4 mg). The n-BuOH-soluble part was subjected to D101 resin column chromatography and eluted with H$_2$O, 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$_2$O in gradient manner, then purified by Sephadex LH-20 and RP-18 column chromatography to obtain naringenin-5,7-di-0-β-D-glucopyranoside, naringenin-7,4'-di-0-β-D-glucopyranoside (5, 2.3 g), chalcononaringenin-2',4'-di-0-β-D-glucopyranoside (6, 18 mg), 4,6,4'-trihydroxyaurone-4,6-di-0-β-D-glucopyranoside (7, 12 mg), naringenin-7,4'-di-0-β-D-glucopyranoside (8, 23 mg) were obtained respectively.

Naringenin (1): colorless needles (CHCl$_3$–MeOH), mp 245–247°C. $^1$HNMR (DMSO) $\delta$: 5.45 (1H, dd, $J$ = 2.7, 12.9 Hz, H-2), 2.68 (1H, dd, $J$ = 3.2, 17.3 Hz, H-3$^\text{cis}$), 3.26 (1H, dd, $J$ = 12.9, 17.3 Hz, H-3$^\text{trans}$), 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-5-0-β-D-glucopyranoside (2): white powder (MeOH–H$_2$O), mp 161–163°C. FAB-MS: 435 [M + 1]$^+$, $^1$HNMR (DMSO) $\delta$: 5.38 (1H, dd, $J$ = 2.7, 13.4 Hz, H-2), 2.63 (1H, dd, $J$ = 2.7, 17.3 Hz, H-3$^\text{cis}$), 3.05 (1H, dd, $J$ = 13.4, 1.4 Hz, H-3$^\text{trans}$), 6.93 (1H, d, $J$ = 2.0 Hz, H-6), 6.07 (1H, d, $J$ = 2.2 Hz, H-8), 6.31 (1H, 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-7-0-β-D-glucopyranoside (3): white powder (MeOH–H$_2$O), mp 147–149°C. FAB-MS: 435 [M + 1]$^+$. $^1$HNMR (DMSO) $\delta$: 5.51 (1H, dd, $J$ = 3.0, H-2), 2.75 (1H, dd, $J$ = 2.9, 17.3 Hz, H-3$^\text{cis}$), 3.36 (1H, overlapping), 6.15 (1H, d, $J$ = 2.2 Hz, H-6),
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'). 13CNMR: Table I.

Chalcononaringenin-2'-O-\( \beta \)-d-glucopyranoside (4): yellow powder (MeOH–acetone), mp 151–153°C. 1HNMR (DMSO) \( \delta \): 7.99 (1H, d, \( J = 15.6 \) Hz, H-\( a \)), 7.62 (1H, d, \( J = 8.5 \) Hz, H-3'), 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'). 13CNMR (DMSO) \( \delta \): 126.2 (C-1), 130.8 (C-2, 6), 115.9 (C-3, 5), 160.3 (C-4), 124.2 (C-\( a \)), 142.9 (C-\( b \)), 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-4'-), 73.7 (C-2'-), 76.8 (C-3'-), 69.4 (C-5'), 77.3 (C-5'-), 60.5 (C-6').

Naringenin-5,7-di-\( \beta \)-d-glucopyranoside (5): white powder (MeOH–H\(_2\)O), mp 203–205°C. FAB-MS: 597 [M + H]\(^+\). 1HNMR (DMSO) \( \delta \): 5.44 (1H, dd, \( J = 2.7, 17.3 \) Hz, H-3cis), 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''). 11CNMR: Table I.

Chalcononaringenin-2',4'-di-\( \beta \)-d-glucopyranoside (6): yellow powder MeOH–acetone, mp 183–185°C. FAB-MS: 597 [M + H]\(^+\). 1HNMR (DMSO) \( \delta \): 7.89 (1H, J = 15.6 Hz, H-\( a \)), 7.64 (1H, d, J = 15.6 Hz, H-\( \beta \)), 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''). 13CNMR (DMSO) \( \delta \): 126.0 (C-1), 131.0 (C-2, 6), 115.9 (C-3, 5), 160.1 (C-4), 124.0 (C-\( a \)), 143.7 (C-\( b \)), 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-aureone-4,6-di-O-β-D-glucopyranoside (7): yellow powder (MeOH–H₂O), mp 205–207°C, [α]₀²⁰ +122.4 (MeOH; c 1.16). N-HR-FAB-MS: 593.1514 [M-H]⁻ (base, calcd. for C₂₇H₃₀O₁₅, m/z 593.1512). UV λ_max (MeOH) nm: 253 (weak), 6.89 (2H, d, J = 8.5 Hz, H-3', 5'). 1H NMR (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', 5'), 5.18 (1H, d, J = 7.5 Hz, H-1'), 5.10 (1H, d, J = 7.5 Hz). 13C NMR (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), 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'), 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–H₂O), mp 208–210°C, [α]₀²⁰ +5.31 (MeOH; c 0.57). N-HR-FAB-MS: 595.1671 [M-H]⁻ (base, calcd. for C₂₇H₃₂O₁₅, 595.1668) UV λ_max (MeOH) nm: 253 (weak). IR ν_max cm⁻¹: 3373, 1639. 1H NMR (DMSO) δ: 5.60 (1H, br.d, J = 12.5 Hz, H-2), 2.82 (1H, dd, J = 3.0, 17.5 Hz, H-3cis), 3.37 (1H, overlapping, H-Strans), 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''). 13C NMR: Table I.

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References
