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Chalcone and flavonol glycosides from *Asarum canadense* (Aristolochiaceae)

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Abstract

Two chalcone glycosides were isolated, together with seven known flavonol glycosides, from the leaves of *Asarum canadense*. The structures of the chalcone glycosides were established as chalcononaringenin 2′,4′-di-*O*-glucoside and chalcononaringenin 2′-*O*-glucoside-4′-*O*-gentiobioside by chemical, UV, FAB MS, ^{1}H and ^{13}C NMR evidence. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Asarum canadense*; Aristolochiaceae; Chalcononaringenin 2′,4′-di-*O*-glucoside; Chalcononaringenin 2′-*O*-glucoside-4′-*O*-gentiobioside

1. Introduction

The genus *Asarum sensu lato*, comprising ca. 70 species, is native to the temperate zone of the northern hemisphere (Satake and Momiyama, 1982). The genus has been surveyed in detail for essential oils (Nagasawa, 1961; Fujita, 1966; Saiki et al., 1967a, b, c; Hayashi et al., 1980). However, a few flavonoid compounds have also been reported. These include peonidin 3-*O*-*p*-coumaroylgentiobioside, peonidin 3-*O*-caffeoylgentiobioside, cyanidin 3-*O*-*p*-coumaroylgentiobioside and cyanidin 3-*O*-caffeoylgentiobioside from the flowers of *Asarum asaroides* (Morren & Decaisne) Makino (Ishikura, 1971) and isorhamnetin 3-*O*-glucosyl-(1→6)-galactoside-7-*O*-glucoside from *Asarum asperum* (F. Maek.) F. Maek. (= *Heterotropa aspera* F. Maek.) as an oviposition stimulant of the zeryntiine swallowtail butterfly (Nishida, 1994).

In a chemotaxonomical study of the *Asarum sensu lato* species, we investigated the flavonoids of *Asarum canadense* L., which is native to North America. The flavonoids of *A. canadense* have been partially characterized as kaempferol 3,7-di-*O*-glycoside, quercetin 3,7-di-*O*-glycoside and quercetin 3-methyl ether 7-*O*-glycoside (Saunders and McClure, 1976). We report here the isolation and characterization of two chalcone glycosides from the leaves of *A. canadense*. This is the first report of the isolation of chalcones in Aristolochiaceae.

2. Results and discussion

Flavonoid **1** was isolated as a yellow powder. The UV spectrum of **1** in MeOH showed an absorption maximum at 368 nm and a shoulder at 243 nm, revealing the compound to be a chalcone. A colorless compound and glucose were obtained by acid hydrolysis with an accompanying cyclization of chalcone to flavanone (Shimokoriyama, 1980). This compound was identified as (±)-naringenin by UV spectra and direct PC and HPLC comparison with an authentic specimen, showing the original compound to be chalcononaringenin (4,2′,4′,6′-tetrahydroxychalcone) glucoside. The FAB mass spectrum of **1** exhibited a molecular ion peak at m/z 597 $[M+H]^{+}$ showing the attachment of 2 mol glucose to chalcononaringenin. The ^{1}H NMR spectrum indicated the presence of eight aromatic proton signals and two glucosyl anomeric proton signals (Table 1). As reported by Shimokoriyama (1957), the phloroglucinol-type chalcone glycoside **1** was readily converted to the corresponding flavanone glycoside (**3**) without release of

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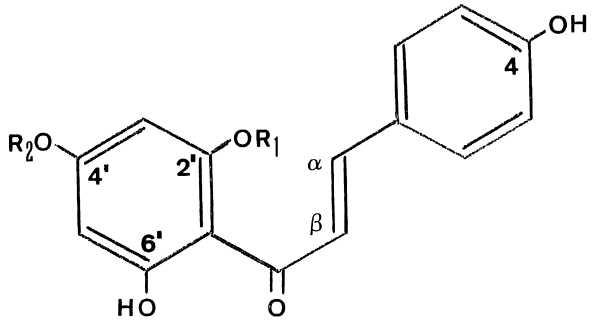
Table 1
^{1}H NMR spectral data of flavonoids **1** and **2** in pyridine-d_5 (500 MHz)

Chalcones	1	2
H-2,6	7.96 (*d*, *J*=8.6 Hz)	7.96 (*d*, *J*=8.6 Hz)
H-3,5	7.06 (*d*, *J*=8.6 Hz)	7.07 (*d*, *J*=8.6 Hz)
H-3′	7.19 (*d*, *J*=2.1 Hz)	7.12 (*d*, *J*=2.1 Hz)
H-5′	6.66 (*d*, *J*=2.1 Hz)	6.72 (*d*, *J*=2.1 Hz)
H-α	8.66(*d*, *J*=15.3 Hz)	8.66 (*d*, *J*=15.6 Hz)
H-β	8.17(*d*, *J*=15.3 Hz)	8.19 (*d*, *J*=15.6 Hz)
6′-OH	14.46 (*s*)	14.46 (*s*)
Glucosyl		
2′-H-1	5.93 (*d*, *J*=7.6 Hz)	5.88 (*d*, *J*=7.6 Hz)
4′-H-1	5.90 (*d*, *J*=7.9 Hz)	6.00 (*d*, *J*=7.6 Hz)
4″-H-1		5.01 (*d*, *J*=7.6 Hz)

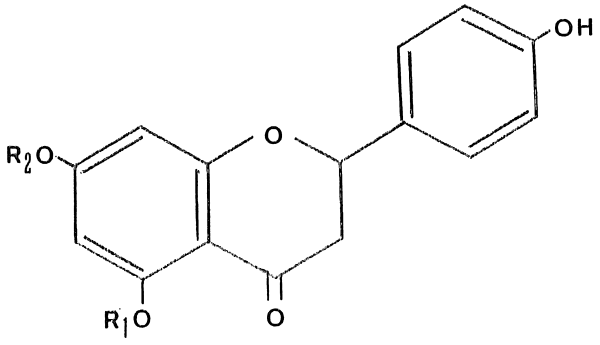
the sugar by hot NaOAc treatment. The UV absorption maximum of the flavanone glycoside appeared at 390 nm with an increase in peak intensity on addition of NaOMe due to isomerization of the flavanone to a chalcone. This showed that the flavanone has a free 4′-hydroxyl and a substituted 5-hydroxyl group (Mabry et al., 1970). Moreover, since no wavelength shift was observed with NaOAc addition, the presence of a substituted 7-hydroxyl group was also confirmed. From these results, flavanone glycoside **3** was identified as naringenin 5,7-di-*O*-glucoside. Assignment of the ^{13}C NMR spectrum was accomplished based on comparison with the data of 2′,4′-dihydroxy-4,6′-dimethoxychalcone (Thuy et al., 1998), 4,2′-dihydroxy-4′,6′-dimethoxychalcone 4-*O*-β-D-glucopyranoside and 4-*O*-apiofuranosyl-(1 → 2)-β-D-glucopyranoside (Fukunaga et al., 1987), chalcononaringenin 2′-*O*-glucoside (Yamaguchi et al., unpublished data), and analysis of the ^{1}H-^{13}C COSY spectrum. Thus, **1** was identified as chalcononaringenin 2′,4′-di-*O*-β-D-glucopyranoside.



1 $R_1 = R_2$ = glucosyl

2 R_1 = glucosyl, R_2 = gentiobiosyl

Flavonoid **2** was also isolated as a yellow powder. The UV spectrum of **2** showed an almost identical pattern to that of **1**. Naringenin and glucose were also liberated by acid hydrolysis. The conversion of the chalcone glycoside **2** to the corresponding flavanone glycoside **4** by hot NaOAc treatment (Shimokoriyama, 1957) afforded naringenin 5,7-*O*-glycoside which was characterized by UV spectral data (Mabry et al., 1970). FAB mass spectra of **2** indicated a molecular ion peak at *m*/*z* 759 $[M+H]^+$ suggesting it to be a chalcononaringenin triglucoside. The ^{1}H NMR spectral data (Table 1) were also almost identical to those of **1**, except for the presence of an additional glucosyl anomeric proton (δ5.01, *d*, *J*=7.6 Hz). Two other glucosyl anomeric protons, δ5.88 and 6.00, were shown to be due to 2′- and 4′-glucosyls by comparisons with ^{1}H NMR spectral data of authentic chalcononaringenin 2′-*O*-glucoside (δ5.84) (Yamaguchi et al., unpublished data).



3 $R_1 = R_2$ = glucosyl

4 R_1 = glucosyl, R_2 = gentiobiosyl

It was shown by comparison of the ^{13}C NMR spectral data of **2** with those of **1** and authentic chalcononaringenin 2′-*O*-glucoside that the third glucosyl group is attached to the 6-position of the 4′-glucosyl group of **1**, i.e. no shift of the 2′-glucosyl C-1 (δ102.1, 102.0 and 102.0) was observed in **2**, **1** and authentic chalcononaringenin 2′-*O*-glucoside (Yamaguchi et al., unpublished data), and the significant shift of the 4′-glucosyl C-1 and C-5 (δ100.8 and 77.4) of **2** as compared to those of **1** (δ101.1 and 79.0) (Table 2) as shown in triterpene glycosides etc. (Usui et al., 1973; Kasai et al., 1977; Tori et al., 1977; Kitajima et al., 1998). Thus, chalcone **2** was identified as chalcononaringenin 2′-*O*-β-D-glucopyranoside-4′-*O*-β-gentiobioside. This is the first report of naturally occurring di- and tri-glucosides of chalcononaringenin.

Seven known flavonol glycosides, quercetin 3-*O*-galactoside, quercetin 3-*O*-robinobioside, quercetin 3-*O*-β-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside, kaempferol 3-*O*-galactoside, kaempferol 3-*O*-glucoside, kaempferol 3-*O*-galactoside-7-*O*-rhamnoside and isorhamnetin 3-*O*-rhamnosylgalactoside were also isolated and identified by UV, FAB mass, ^{1}H and ^{13}C NMR spectra, characterization of acid hydrolysates, and PC and HPLC comparisons with authentic specimens.

Table 2
^{13}C NMR spectral data of chalcones **1** and **2** in pyridine-d_5 (125 MHz)

Chalcones	**1**	**2**
Aglycones		
C-α	125.2	125.3
C-β	144.4	144.3
C-β′	193.9	193.9
C-1′	108.4	108.4
C-2′	164.2	164.2
C-3′	98.9	98.8
C-4′	166.7	166.9
C-5′	95.7	95.8
C-6′	161.6	161.5
C-1	127.3	127.4
C-2,6	131.7	131.7
C-3,5	116.9	116.9
C-4	161.0	161.0
Glucose	2′-gl 4′-gl	2′-gl 4′-gl
C-1	102.0, 101.1	102.1, 100.8
C-2	75.1[a], 74.8[a]	75.1[d], 74.9[d]
C-3	79.0, 78.5	78.7[e], 78.5[e]
C-4	71.6[b], 71.7[b]	71.6[f], 71.4[f]
C-5	79.1, 79.0	79.2[g], 77.4
C-6	62.5[c], 62.7[c]	62.5[h], 69.9
C-1′		105.4
C-2′		74.8[d]
C-3′		78.5[e]
C-4′		71.1[f]
C-5′		78.6[g]
C-6′		62.5[h]

[a–h] Assignments may be interchaged.

Kaempferol 3,7-di-*O*-glycoside, quercetin 3-methyl ether 7-*O*-glycoside and quercetin 3,7-di-*O*-glycoside have been isolated from the chloroplasts of *A. canadense* (Saunders and McClure, 1976). From our results, these diglycosides were found to be kaempferol and quercetin 3-*O*-galactoside-7-*O*-rhamnosides. Chalcones have not been reported hitherto from the Aristolochiaceae.

3. Experimental

3.1. General

^1H and ^{13}C NMR spectra (500 and 125 MHz) were recorded in pyridine-d_5 using a JEOL A-500 spectrometer with TMS as an internal standard. ^{13}C-^1H COSY was obtained with the usual pulse sequence and data processing was performed with standard JEOL software. FAB mass spectra were recorded with a JEOL HX-110 spectrometer using glycerol as a matrix.

3.2. Plant material

A. canadense L. was cultivated in the Botanical Garden, Faculty of Agriculture, Hokkaido University, Sapporo, Hokkaido, Japan.

3.3. Extraction and isolation

Fresh leaves (325 g) of *A. canadense* were extracted with MeOH. After concentration, the aqueous residue was washed with petroleum ether, then extracted with EtOAc. The EtOAc solution containing kaempferol 3-*O*-galactoside, kaempferol 3-*O*-glucoside and quercetin 3-*O*-galactoside, and aqueous residue containing other flavonoids were applied to preparative paper chromatography (PPC) using BAW (*n*-BuOH/HOAc/H_2O = 4:1:5, upper phase), 15% HOAc and then BEW (*n*-BuOH/EtOH/H_2O = 4:1:2.2) as solvent systems. After purification of the isolated flavonoids by Sephadex LH-20 column chromatography (solvent system: eluting with 70% MeOH), chalcones **1** and **2** were obtained as yellow powders (yield: ca. 20 mg and 40 mg, respectively).

3.4. Chalcononaringenin 2′,4′-di-*O*-β-D-glucoside (*1*)

PC: R_f 0.35 (BAW), 0.42 (BEW), 0.39 (15%HOAc), 0.23 (5%HOAc); UV— dark green, UV/NH_3— bright orange; UV λ_{max} nm: MeOH 243sh, 368; +NaOMe 243, 393; +$AlCl_3$ 250, 326, 422; +$AlCl_3$/HCl 245sh, 399; +NaOAc 307sh, 375, 437sh; +NaOAc/H_3BO_3 306sh, 371, 450sh; Positive FAB MS: *m/z* 597.1843 $[M+H]^+$ (base, calcd. for $C_{27}H_{33}O_{15}$, 597.1819); ^1H and ^{13}C NMR spectral data: see Tables 1 and 2.

3.5. Chalcononaringenin 2′-*O*-β-D-glucoside-4′-*O*-β-gentiobioside (*2*)

PC: R_f 0.24 (BAW), 0.31 (BEW), 0.52 (15% HOAc), 0.41 (5% HOAc); UV— dark green, UV/NH_3— bright orange; UV λ_{max} nm: MeOH 243sh, 368; +NaOMe 243, 394; +$AlCl_3$ 254sh, 326, 425; +$AlCl_3$/HCl 250sh, 400; +NaOAc 308sh, 379, 434sh; +NaOAc/H_3BO_3 309sh, 372, 450sh; Positive FAB MS: *m/z* 759.2357 $[M+H]^+$ (base, calcd. for $C_{33}H_{43}O_{20}$, 759.2345); ^1H and ^{13}C NMR spectral data: see Tables 1 and 2.

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