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Flavonoids from the Leaves of *Hydrastis* (Hydrastidaceae): the Phytochemical Comparison with *Glaucidium*

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Abstract Three flavonoids were isolated from the leaves of *Hydrastis canadensis*, together with another phenolic compound, chlorogenic acid. They were identified as quercetin 3-*O*-gentiobioside, quercetin 3-*O*-galactoside and quercetin 3-*O*-glucoside by LC-MS, acid hydrolysis, and direct TLC and HPLC comparisons with authentic specimens. The flavonoids in the leaves of *Glaucidium palmatum* have previously been identified as three rare 3-*O*-allosides of quercetin, kaempferol and rhamnocitrin by Iwashina and Ootani (1990). It was proved that the flavonoids of both *H. canadensis* and *G. palmatum* were flavonol 3-*O*-hexosides, suggesting the phytochemical affinity between both genera. However, the glycosidic sugars of *H. canadensis* were the common glucose and galactose, and that of *G. palmatum* was the rare allose, showing intergeneric differences or geographic isolation between the two genera.
Key words: *Hydrastis canadensis*, Hydrastidaceae, Ranunculaceae *sensu lato*, flavonols, quercetin glycosides.

Introduction

The genus *Hydrastis* (Hydrastidaceae, Ranunculaceae *sensu lato*) consists of only one species, *H. canadensis* L., and is endemic to central and northeastern America. The genus *Hydrastis* is commonly treated as Ranunculaceae *sensu lato* (e.g., Cronquist, 1981). However, the genus was recently classified into Hydrastidaceae with another genus *Glaucidium*, which is endemic to Japan and consists of only one species, *G. palmatum* Sieb.& Zucc. (Tobe, 2003). Their close affinity was supported by strong molecular evidence using 18S rDNA, *rbc*L and *atp*B gene sequences (Soltis *et al.*, 2000).

As chemical substances contained in *H. canadensis*, nine isoquinoline alkaloids, i.e., berberine, β-hydrastine, canadine, canadaline, hydrastidine, isohydrastidine, (*S*)-corypalmine, (*S*)-isocorypalmine and (*S*)-tetrahydropalmatine, have been isolated from the rhizomes and roots (Gleye *et al.*, 1974; Messana *et al.*, 1980; Weber *et al.*, 2003). D-Galactose and a ribitol-like substance have also been isolated from the same organs (Iriki and Minamisawa, 1983). Recently, two rare flavonoids, 6,8-di-*C*-methylluteolin 7-methyl ether and 6-*C*-methylluteolin 7-methyl ether, were isolated from commercially available samples of the roots, along with β-sitosterol 3-*O*-β-D-glucoside and some alkaloids described above (Hwang *et al.*, 2003). However, the chemical components in the leaves have not been reported.

In this paper, the isolation and identification of flavonoids in the leaves of *H. canadensis* were described, and the phytochemical relationship with the genus *Glaucidium*, which have already been reported its flavonoid composition by us (Iwashina and Ootani, 1990), were chemotaxonomically discussed.

Materials and Methods

Plant materials

Plant materials were collected in Shannon County near Mountain View, Missouri, along the Jack's Fork River in 1990, and cultivated in Shaw Nature Reserve, Missouri Botanical Garden, USA.

High performance liquid chromatography (HPLC)

Flavonoid composition of the leaves was surveyed by HPLC using Shim-pack CLC-ODS (I.D. 6.0×150 mm, Shimadzu), at flow-rate: 1.0 ml/min, injection: 10 μl, detection: 190–400 nm, and eluent: MeCN/H_2O/H_3PO_4 (22 : 78 : 0.2).

Liquid chromatography-mass spectra (LC-MS)

LC-MS was surveyed with Symmetry C_{18} column (I.D. 2.1×150 mm, Waters), at flow-rate: 0.18 ml/min, eluent: 15% MeCN→45% MeCN (30 min), ESI^+ 3.5 kV, cone voltage 30 V, ESI^- 3.5 kV, cone voltage 30 V, 400°C, ion energy 1.0 V.

Extraction and isolation of phenolic compounds

Dry leaves (8.0 g) were extracted with MeOH. After filtration, the extracts were concentrated to a small volume and applied to prep. PC using solvent systems: 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). Flavonoids were eluted with MeOH and purified by Sephadex LH-20 column chromatography (solvent system: 70% MeOH).

Identification of phenolic compounds

Flavonoids and phenolic acid were identified by UV spectroscopy using shift reagents (Mabry *et al.*, 1970), characterization of acid hydrolysates (aglycones and glycosidic sugars), LC-MS data, and finally direct TLC and HPLC comparisons with authentic specimens. Thin-layer chromatographic, HPLC, LC-MS and UV spectral properties of isolated phenolic compounds were as follows.

Quercetin 3-*O*-gentiobioside (**1**). TLC (cellulose): Rf 0.29 (BAW), 0.41 (BEW), 0.33 (15%HOAc); color UV– dark purple, UV/NH_3– yellow. HPLC: retention time (Rt) 4.23 min. LC-MS: *m/z* 625 $[M-H]^-$ calcd. for $C_{27}H_{30}O_{17}$ (quercetin+2 mol glucose), 463 [M−monoglucosyl−H]$^-$ (quercetin+1 mol glucose) and 301 [M−diglucosyl−H]$^-$ (quercetin). UV: λmax (nm) MeOH 257, 266sh, 358; +NaOMe 274, 328, 410 (inc.); +$AlCl_3$ 273, 433; +$AlCl_3$/HCl 267, 301, 362, 395sh; +NaOAc 273, 326, 396; +NaOAc/H_3BO_3 262, 379.

Mixture of quercetin 3-*O*-galactoside (**2a**) and quercetin 3-*O*-glucoside (**2b**). TLC (cellulose): Rf 0.61 (BAW), 0.71 (BEW), 0.19 (15%HOAc); color UV– dark purple, UV/NH_3– yellow. HPLC: Rt 5.47 (quercetin 3-*O*-galactoside) and 5.61 (quercetin 3-*O*-glucoside).

UV: λmax (nm) MeOH 257, 266sh, 360; +NaOMe 273, 331, 410 (inc.); +$AlCl_3$ 274, 434; +$AlCl_3$/HCl 267, 301, 363, 396sh; +NaOAc 274, 325, 392; +NaOAc/H_3BO_3 262, 380.

Chlorogenic acid (**3**). HPLC: Rt 3.67 min. UV: λmax (nm) MeOH 244sh, 297sh, 328; +NaOMe 263, 310sh, 377 (inc.); +$AlCl_3$ 257sh, 316sh, 360; +$AlCl_3$/HCl 238, 297sh, 326; +NaOAc 296sh, 338, 376sh; +NaOAc/H_3BO_3 256sh, 304sh, 349.

Results

Three flavonoids were detected from the leaves of *Hydrastis canadensis* by HPLC survey (Fig. 1) and isolated by various chromatographic manners. Flavonoid **1** produced quercetin and glucose, which were characterized by direct TLC and HPLC comparisons with authentic specimens, by acid hydrolysis. Liquid chromatography-MS survey of **1** indicated the molecular ion, *m/z* 625 $[M-H]^-$, showing the presence of quercetin and 2 mol glucose. The attachment of sugar to 3-hydroxyl group of quercetin was shown by UV spectroscopy using shift reagents (see Materials and Methods, Mabry *et al.*, 1970). Finally, flavonoid **1** was identified as quercetin 3-

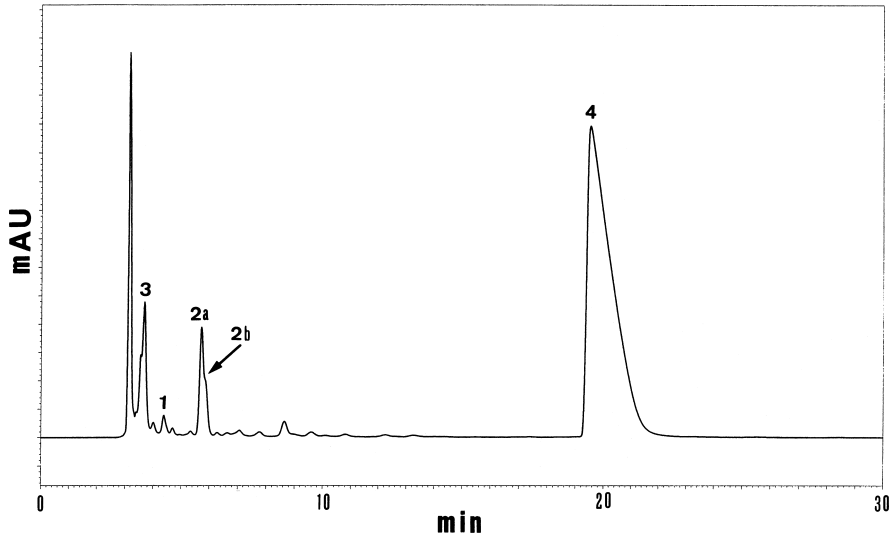

Fig. 1. High performance liquid chromatogram of MeOH extract from the leaves of *Hydrastis canadensis*. **1**=quercetin 3-*O*-gentiobioside, **2a**=quercetin 3-*O*-galactoside, **2b**=quercetin 3-*O*-glucoside, **3**=chlorogenic acid, **4**=isoqinoline alkaloids such as berberin, and other peaks=unknown phenolic acids.

O-glucosyl-(1→6)-glucoside, i.e., quercetin 3-*O*-gentiobioside (Fig. 2), by direct TLC and HPLC comparison with authentic sample.

It was shown by HPLC survey that flavonoid **2** is a mixture of two compounds. Quercetin, glucose and galactose were liberated by acid hydrolysis. UV spectral properties showed that the compound was mixture of quercetin 3-*O*-glycosides. It was shown by direct HPLC comparisons with authentic hyperin and isoquercitrin that flavonoid **2** was the mixture of quercetin 3-*O*-galactoside (**2a**) and quercetin 3-*O*-glucoside (**2b**) (Fig. 2).

A phenolic acid **3** was also isolated from the leaves. It was identified as 3-caffeoylquinic acid, i.e., chlorogenic acid, by UV spectral scopy and direct HPLC comparison with authentic specimen. Moreover, the peak of a major substance appeared on HPLC chromatogram. It was characterized as some yellow isoquinoline alkaloids such as berberin by UV spectral properties.

Discussion

In this survey, three quercetin glycosides, i.e., 3-*O*-galactoside (hyperin), 3-*O*-glucoside (isoquercitrin) and 3-*O*-gentiobioside (=glucosyl-(1→6)-glucoside) were found from the leaves of *Hydrastis canadensis*. Those glycosides are common flavonoids in the plant kingdom. On the other hand, the flavonoids in the leaves of *Glaucidium palmatum* have previously been identified as three rare 3-*O*-allosides of quercetin, kaempferol and rhamnocitrin (Iwashina and Ootani, 1990) (Fig. 2). Thus, it was proved that the flavonoids of both *H. canadensis* and *G. palmatum* were flavonol 3-*O*-hexosides, suggesting the phytochemical affinity between both genera. However, the glycosidic sugars of *H. canadensis* were the common glucose and galactose, and that of *G. palmatum* was the rare allose, showing intergeneric differences or geographic isolation between the two genera. The presence or absence of other chemical components in the roots, e.g., isoquinoline alkaloids (presence in *H. canadensis*) (Gleye *et al.*, 1974; Messana *et al.*, 1980; Weber *et al.*, 2003, Their alkaloids were also found from the leaves in this survey), *C*-methylflavones (presence in *H. canadensis*) (Hwang *et al.*, 2003), and a coumarin, glaupalol (presence in *G. palmatum*) (Irie *et al.*, 1968; Yamamoto *et al.*, 1971), also suggests strong inter-

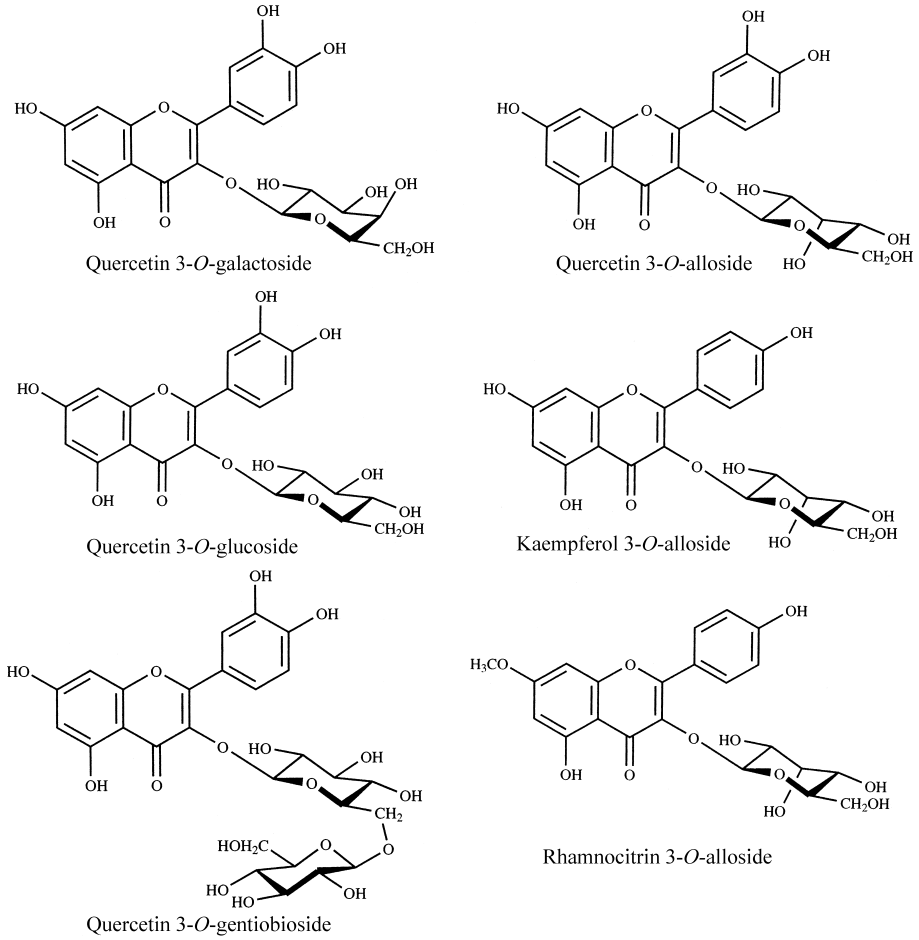

Fig. 2. Chemical structures of the flavonoids isolated from the leaves of *Hydrastis canadensis* (left) and *Glaucidium palmatum* (right).

generic chemical differences between the *Hydrastis* and *Glaucidium*.

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