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PHYTOCHEMISTRY

Hispidulin and nepetin 4′-glucosides from *Cirsium oligophyllum*

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Abstract

Two flavone glycosides, hispidulin 4′-*O*-β-D-glucopyranoside and nepetin 4′-*O*-β-D-glucopyranoside, were isolated from the leaves of *Cirsium oligophyllum* and identified on the basis of their ^{1}H and ^{13}C NMR, FAB-MS, UV spectra and characterization of their hydrolysates; hispidulin 7,4′-glycoside, vicenin-2, hispidulin and nepetin were also isolated.

Keywords: *Cirsium oligophyllum*; Compositae; Flavone glycosides; Hispidulin 4′-glucoside; Nepetin 4′-glucoside; ^{1}H and ^{13}C NMR spectroscopy; FAB-MS

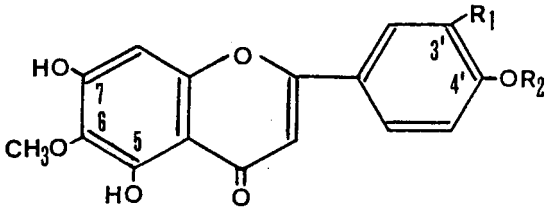
1. Introduction

We have previously isolated and identified the flavonoids, pectolinarigenin 7-rutinoside (pectolinarin), acacetin 7-rutinoside (linarin), apigenin 5-glucoside and luteolin 5-glucoside from *Cirsium* species during chemotaxonomical studies on this genus (Iwashina, Ito, & Ootani, 1988; 1989; Iwashina, Kadota, Ueno, & Ootani, 1995; Iwashina & Ootani, 1998).

In the present paper, we describe the isolation and identification of two hitherto unknown glycosides of known flavone methyl ethers, i.e. hispidulin 4′-glucoside (**1**) and nepetin 4′-glucoside (**2**), together with hispidulin 7,4′-glycoside (**3**), the previously described vicenin-2 (**4**), hispidulin (**5**) and nepetin (**6**), from the leaves of *C. oligophyllum*, a species endemic to Japan.

2. Results and discussion

Six flavonoids were isolated from the leaves of *C. oligophyllum*. Acid hydrolysis of flavonoid **1** gave glucose and hispidulin (**5**) (5,7,4′-trihydroxy-6-methoxyflavone), which was identified by UV (Mabry, Markham, & Thomas, 1970), FAB-mass and ^{1}H NMR spectra (Seth, Dandey, & Dasgupta, 1982; Markham & Geiger, 1994); hispidulin was also isolated in small amount as the aglycone from the crude extracts. The UV spectra of glycoside **1** suggested the presence of



1 $R_1 = H$, R_2 = glucosyl
2 $R_1 = OH$, R_2 = glucosyl
5 $R_1 = R_2 = H$
6 $R_1 = OH$, $R_2 = H$

free 5-hydroxyl and substituted 4′-hydroxyl groups via treatment with +NaOMe and $+AlCl_3$ (Mabry et al., 1970), whereas the presence of 7-hydroxyl and 6-meth-

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oxyl groups was indicated by comparison of C-6 (δ 131.5) and C-8 (δ 94.5) ^{13}C NMR signals of **1** with those (δ 131.3 and 94.2) of 5,7-dihydroxy-6,4′-dimethoxyflavone (Horie et al., 1998). Inspection of the ^{1}H NMR spectrum revealed a resonance corresponding to H-3′,5′ (δ 7.40), which was at much lower field than that of the aglycone (δ7.04), suggesting attachment of the glucose to the 4′-hydroxyl group (Veit, Geiger, Czygan, & Markham, 1990; Markham & Geiger, 1994). Since the coupling constant of the anomeric proton (δ 5.25) of **1** indicated $J=7.3$ Hz, glucose is linked via a β-linkage to the aglycone. In addition, the FAB-mass spectrum of **1** gave a molecular ion [M+H] at m/z 463, in agreement with the attachment of 1 mol glucose to hispidulin. Thus, **1** was identified as hispidulin 4′-*O*-β-D-glucopyranoside.

Acid hydrolysis of **2** liberated glucose and nepetin (**6**) (5,7,3′,4′-tetrahydroxy-6-methoxyflavone), which was identified by the UV, FAB-mass and ^{1}H NMR spectra (Seth et al., 1982); the paper-chromatographic (PC) (R_fs and color reactions) and UV spectral properties of the isolated nepetin (**6**), which was also isolated in a small amount from the crude extracts, was identical to that of an authentic standard.

The UV spectra of glycoside **2** suggested the presence of 5- and 3′-hydroxyl as well a substituted 4′-hydroxyl groups via treatment with +NaOMe and +$AlCl_3$ (Mabry et al., 1970), whereas the presence of 7-hydroxyl and 6-methoxyl groups of **2** was indicated by comparison of ^{13}C NMR signals C-6 (δ 131.5) and C-8 (δ 94.4) as for **1** (Horie et al., 1998). The FAB-mass spectrum suggested a molecular ion [M+H] at m/z 479, in agreement with attachment of 1 mol glucose to nepetin (**6**). Moreover, in the ^{1}H NMR spectrum, the presumed H-5′ (δ 7.46) proton appeared at a lower field than that of the aglycone (δ7.01), suggesting attachment of the glucose moiety to the 4′-hydroxyl group (Iwashina, Kamenosono, & Yabuya, 1984). Since the coupling constant of the anomeric proton (δ 5.32) of **2** indicated $J=8.9$ Hz, glucose is linked via a β-linkage to the aglycone. Thus, **2** was identified as nepetin 4′-*O*-β-D-glucopyranoside.

Compound **3** was characterized as hispidulin 7,4′-diglycoside by UV and PC properties (R_fs and color reactions) and acid hydrolysis. Flavonoid **4** was identified as vicenin-2, by UV spectra, hot acid treatment and direct PC comparison with that of an authentic sample (Iwashina, Kamenosono, & Yabuya, 1996).

In a chemotaxonomical survey of *Cirsium* species, some methylated flavone glycosides have been reported (Morita & Shimizu, 1963; Wallace & Bohm, 1971; Morita, Shimizu, & Arisawa, 1973; Lin, Arisawa, Shimizu, & Morita, 1978; Park, Lee, & Choi, 1995). A major methylated flavone which is found in many *Cirsium* species is pectolinarigenin 7-rutinoside, and is frequently accompanied with acacetin 7-rutinoside (Nakaoki & Morita, 1959, 1960; Wagner, Hörhammer, & Kirchner, 1960; Morita, Fukuta, & Shimizu, 1964; Morita et al., 1973; Gardner, 1973; 1974). Other methylated flavone glycosides, cirsimaritin (5,4′-dihydroxy-6,7-dimethoxyflavone) 4′-glucoside and 4′-rutinoside (Morita & Shimizu, 1963; Wallace & Bohm, 1971), cirsilineol (5,4′-dihydroxy-6,7,3′-trimethoxyflavone) and cirsiliol (5,3′,4′-trihydroxy-6,7-dimethoxyflavone) 4′-glucosides (Morita et al., 1973), cirsitakaogenin (5,7-dihydroxy-8,4′-dimethoxyflavone) 7-glucoside (Lin et al., 1978), and hispidulin 7-neohesperidoside (Park et al., 1995) have been reported.

Hispidulin 4′-glucoside and nepetin 4′-glucoside were found for the first time.

3. Experimental

3.1. General

UV spectra were measured according to Mabry et al. (1970). The NMR spectra were performed in DMSO-d_6 at 270 MHz (^{1}H NMR) and 67 MHz (^{13}C NMR). FAB-MS were measured using nitrobenzyl alcohol (NBA).

3.2. Plant materials

C. oligophyllum (Franch. & Savat.) Matsum. was collected in Makioka, Yamanashi Prefecture, Japan. Voucher specimens were deposited in the National Science Museum, Tokyo (TNS) in Tsukuba, Japan.

3.3. Extraction and isolation

Fresh leaves (ca. 250 g) of *C. oligophyllum* were extracted with MeOH. The extracts were filtered and concentrated to a small volume. Constituents **3**–**6** were isolated by preparative paper chromatography (PPC) using the solvent system; BAW (*n*-BuOH/HOAc/H_2O=4:1:5, upper phase) and 15% HOAc, respectively, and were further purified by Sephadex LH-20 CC (eluent: 70% MeOH).

A mixture of **1** and **2** could not be separated by PPC, and this was applied to Polyamide CC eluted with 70% MeOH. After further purification by Sephadex LH-20 CC (eluent: 70% MeOH), **1** and **2** were individually obtained as pale yellow powders, ca. 20 and 50 mg, respectively.

3.4. Hispidulin 4′-glucoside (*1*)

PC: R_f 0.65 (BAW), 0.72 (BEW: *n*-BuOH/EtOH/H_2O=4:1:2.2), 0.44 (15% HOAc), 0.15 (5% HOAc); UV and UV/NH_3 — dark purple. UV λ_{max} nm:

MeOH 275, 327; +NaOMe 275, 367 (dec.); +$AlCl_3$ 294, 298sh, 352, 386sh; +$AlCl_3$/HCl 294, 297sh, 346, 387sh; +NaOAc 275, 369; +NaOAc/H_3BO_3 277, 330. ^{1}H NMR (270 MHz, DMSO-d_6): δ 13.22 (1H, s, 5-OH), 8.25 (2H, d, J=8.9 Hz, H-2′ and H-6′), 7.40 (2H, d, J=8.9Hz, H-3′ and H-5′), 7.11 (1H, s, H-8), 6.84 (1H, s, H-3), 5.25 (1H, d, J=7.3 Hz, glucosyl anomeric H), 3.97 (3H, s, 6-OMe). ^{13}C NMR (67 MHz, DMSO-d_6): (hispidulin nucleus) δ 163.2 (C-2), 103.4 (C-3), 182.2 (C-4), 152.6 (C-5), 131.5 (C-6), 157.7 (C-7), 94.5 (C-8), 152.8 (C-9), 104.2 (C-10), 124.1 (C-1′), 128.2 (C-2′ and C-6′), 116.7 (C-3′ and C-5′), 160.4 (C-4′), 60.0 (6-OMe); (glucosyl carbons) δ 100.0 (C-1″), 73.3 (C-2″), 77.2 (C-3″), 69.7 (C-4″), 76.6 (C-5″), 60.7 (C-6″). FAB-MS (NBA): $[M+H]^+$ at m/z 463 (hispidulin monoglucoside) and $[M\text{-glucosyl}+H]^+$ at m/z 301(hispidulin).

3.5. Nepetin 4′-glucoside (**2**)

PC: R_f 0.60 (BAW), 0.68 (BEW), 0.33 (15% HOAc), 0.08 (5% HOAc); UV and UV/NH_3 — dark purple. UV λ_{max} nm: MeOH 275, 335; +NaOMe 267, 377 (dec.); +$AlCl_3$ 260, 288, 363, 395sh; +$AlCl_3$/HCl 256, 290, 354, 390sh; +NaOAc 272, 375; +NaOAc/H_3BO_3 276, 337. ^{1}H NMR (270 MHz, DMSO-d_6): δ 13.22 (1H, s, 5-OH), 7.73 (2H, d, J=8.6 Hz, H-2′ and H-6′), 7.46 (1H, d, J=8.3 Hz, H-5′), 7.04 (1H, s, H-8), 6.83 (1H, s, H-3), 5.32 (1H, d, J=8.9 Hz, glucosyl anomeric H), 3.97 (3H, s, 6-OMe). ^{13}C NMR (67 MHz, DMSO-d_6): (nepetin nucleus) δ 163.3 (C-2), 103.6 (C-3), 182.2 (C-4), 152.8 (C-5), 131.5 (C-6), 157.5 (C-7), 94.4 (C-8), 152.5 (C-9), 104.2 (C-10), 124.8 (C-1′), 113.7 (C-2′), 147.0 (C-3′), 148.6 (C-4′), 116.1 (C-5′), 118.6 (C-6′), 60.0(6-OMe); (glucosyl carbons) δ 101.3 (C-1″), 73.3 (C-2″), 75.9 (C-3″), 69.9 (C-4″), 77.4 (C-5″), 60.8 (C-6″). FAB-MS (NBA): $[M+H]^+$ at m/z 479 (nepetin monoglucoside) and $[M\text{-glucosyl}+H]^+$ at m/z 317 (nepetin).

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