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A FLAVONE DIGLYCOSIDE FROM *CIRSIUM JAPONICUM* VAR. *USSURIENSE*

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Abstract—Chromatographic separation of the butanol-soluble part of the methanol extract of whole plants of *Cirsium japonicum* var. *ussuriense* resulted in the isolation of a new flavonoid, hispidulin 7-neohesperidoside, together with the known cirsimaritin 4′-glucoside and acacetin 7-rutinoside.

INTRODUCTION

Cirsium japonicum DC. var. *ussuriense* (Regel) Kitam. is a herbaceous perennial native to Korea, whole plants of which have been used in a folklore medicine for its antiflammatory properties. Since its chemistry has not yet been investigated, we have examined the whole plants and here report the isolation of a new compound, hispidulin 7-neohesperidoside (**1**). In addition, cirsimaritin 4′-glucoside and acacetin 7-rutinoside are also reported for the first time from this plant.

RESULTS AND DISCUSSION

Column chromatography of the butanol-soluble part of the whole plant methanol extract of *C. japonicum* var. *ussuriense* followed by crystallization yielded a pale yellow powder of **1**, mp 192–196°, which gave characteristic flavone glycoside colour reactions i.e. pink with Zn + HCl and Mg + HCl, and a positive Molisch test. The IR spectrum of **1** showed a broad hydroxyl and α,β-unsaturated carbonyl absorptions at 3380 and 1659 cm^{-1} respectively, and a C–O stretching band at 1073 cm^{-1}, indicating its glycosidic nature. The UV spectrum of **1** exhibited absorption maxima typical of a flavone at 276 nm and 331 nm [1]. The bathochromic shift of band I in the presence of $AlCl_3$ and $AlCl_3$ + HCl indicated the presence of free 5-hydroxyl group. Also a bathochromic shift with NaOMe, without a decrease in intensity, showed the presence of a free 4′-hydroxyl group [1] while the absence of a shift with NaOMe indicated that the 7-hydroxyl was substituted. The ^{1}H NMR spectrum of **1** showed two singlets at δ6.83 and 7.02 assigned to the proton at C-3 and C-8 and two *ortho*-coupled doublets at 6.93 and 7.93, which indicated that the flavone moiety was oxygenated at C-5, C-6, C-7 and C-4′. In addition, a singlet at 3.83 indicated the presence of one methoxyl group. Two anomeric proton signals at 4.65 and 5.33 indicated that 2 mol of sugars were linked, one of which was assumed to be rhamnose by the diagnostic methyl signal at 1.14.

Acid hydrolysis of **1** yielded an aglycone, mp 301–304°, D-glucose and L-rhamnose. The NaOAc spectrum of the aglycone showed a bathochromic shift of band II, unlike that of **1**, where no shift occurred indicating the presence of free 7-hydroxyl group [1]. Therefore, the sugars must be attached to the 7-hydroxyl group, and the methoxyl group located at C-6. Therefore, the aglycone of **1** was identified as hispidulin which was confirmed by direct comparison with an authentic sample (UV, TLC and mmp). The ^{1}H NMR data of **1** supported that the interglycosidic linkage of disaccharide must be neohesperidoside because the anomeric proton of the rhamnose appears between 4.9 and 5.0 ppm, which is typical of 7- and 3-*O*-neohesperidosides (the corresponding rutinosides show values between 4.2 and 4.4 ppm) and the signal for the methyl residue appears between 1.1 and 1.3 ppm (compared well with a value between 0.9 and 1.0 ppm for rutinosides) [1]. The ^{13}C NMR data of **1** also supported the attachment of neohesperidosyl moiety to the 7-position of hispidulin (see Experimental). The configuration of D-glucose and L-rhammnose was determined as β- and α-linkages, respectively. From the above evidence, **1** was identified as hispidulin 7-*O*-neohesperidoside. This is the first report of its occurrence in nature.

The structures of two other known flavone glycosides isolated in the present study were determined to be

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acacetin 7-*O*-rutinoside and cirsimaritin 4′-*O*-glucoside from their spectral data (see Experimental).

EXPERIMENTAL

Plant material. Fresh whole plants of *Cirsium japonicum* var. *ussuriense* were collected near Jinju in Spring, 1991. A voucher specimen is deposited in the Herbarium of the Botanical Gardens in Suncheon National University.

Isolation of flavonoids. Dried whole plants (1.0 kg) were extracted with MeOH and concd to a dark residue, which was partitioned between $CHCl_3$ and H_2O. The aq. layer was extracted with BuOH. The BuOH extract (20 g) was then subjected to silica gel CC, using $CHCl_3$–MeOH–H_2O (25:8:5, lower phase) as eluent, to yield **1** (200 mg), mp 192–196°; acacetin 7-rutinoside, mp 255–257° [ref. [2], mp 248–250°]; cirsimaritin 4′-glucoside, mp 269–273° [ref. [3], mp 278–280°].

Hispidulin 7-neohesperidoside (5,7,4′-*trihydroxy*-6-*methoxylflavone 7-neohesperidoside*) (**1**). UV λ_{max}^{MeOH} nm (log ε): 276 (4.43), 331 (4.51); with NaOMe 273 (4.42), 389(4.63); + NaOAc 277 (4.56), 337 (4.63); with NaOAc + H_3BO_3 277 (4.66), 337 (4.69); + $AlCl_3$ 286 (4.52), 302 (4.55), 363 (4.66); + $AlCl_3$ + HCl 286 (4.46), 302 (4.47), 353 (4.52). ^{1}H NMR (DMSO-d_6, TMS): δ7.93 (2H, *d*, *J* = 8.75 Hz, H-2′ and H-6′), 7.02 (1H, *s*, H-8), 6.93 (2H, *d*, *J* = 8.75 Hz, H-3′ and H-5′), 6.83 (1H, *s*, H-3), 5.33 (1H, *d*, *J* = 7.25 Mz, anomer H), 4.65 (1H, *br s*, anomer H), 3.83 (3H, *s*, –OMe), 1.14 (3H, *d*, *J* = 6.3 Hz, –Me of rhamnose). ^{13}C NMR (DMSO-d_6, TMS): δ164.4 (C-2), 102.7 (C-3), 182.3 (C-4), 152.1 (C-5), 132.8 (C-6), 155.8 (C-7), 94.5 (C-8), 152.5 (C-9), 105.8 (C-10), 121.1 (C-1′), 128.6 (C-2′), 116.1 (C-3′), 161.5 (C-4′), 116.1 (C-5′), 128.6 (C-6′), 98.0 (G-1), 75.9 (G-2), 77.7 (G-3), 69.8 (G-4), 77.3 (G-5), 60.6 (G-6), 100.0 (R-1), 70.4 (R-2), 70.6 (R-3), 72.0 (R-4), 68.6 (R-5), 18.2 (R-6), 60.3 (OMe).

Acacetin 7-rutinoside. IR ν_{max}^{KBr} cm^{-1}: 3422 (OH), 1664 (α,β-unsaturated C=O), 1612, 1487 (aromatic C=C), 1117, 1082 (glycosidic C–O): UV λ_{max}^{MeOH} nm (log ε): 269 (4.21), 324 (4.29); + NaOMe 292 (4.34), 368 (4.30); + NaOAc 270 (4.21), 325 (4.33); + NaOAc + H_3BO_3 271 (4.21), 324 (4.35); + $AlCl_3$ 275 (4.20), 305 (4.18), 345 (4.38), 386 (4.32); + $AlCl_3$ + HCl 279 (4.22), 304 (4.20), 345 (4.39), 386 (4.27). ^{1}H NMR (DMSO-d_6, 200 MHz, TMS): δ12.9 (1H, *s*, 5-OH), 8.03 (2H, *d*, *J* = 8.8 Hz, H-2′ and H-6′), 7.13 (2H, *d*, *J* = 8 Hz, H-3′ and H-5′), 6.92 (1H, *s*, H-3), 6.78 (1H, *d*, *J* = 1.9 Hz, H-8), 6.44 (1H, *d*, *J* = 1.9 Hz, H-6), 5.12 (1H, *d*, *J* = 6.0 Hz, anomeric H), 4.43 (1H, *br s*, anomeric H), 3.85 (3H, *s*, OMe-4′), 1.02 (3H, *d*, *J* = 5.9 Hz, Me of rhamnose). ^{13}C NMR (DMSO-d_6, TMS): δ163.9 (C-2), 103.8 (C-3), 182.1 (C-4), 161.2 (C-5), 100.5 (C-6),[a] 157.0 (C-7), 94.8 (C-8), 163.0 (C-9), 105.5 (C-10), 122.7 (C-1′), 128.4 (C-2′ and C-6′), 114.7 (C-3′ and C-5′), 162.4 (C-4′), 100.0 (G-1),[a] 73.1 (G-2), 76.3 (G-3), 69.8 (G-4), 75.9 (G-5), 66.1 (G-6), 100.4 (R-1),[a] 70.8 (R-2),[b] 70.4 (R-3),[b] 72.1 (R-4), 68.3 (R-5), 17.8 (R-6), 55.6 (OMe). [a,b]Assignments interchangeable.

Cirsimaritin 4′-glucoside. IR ν_{max}^{KBr} cm^{-1}: 3322 (OH), 1659 (α,β-unsaturated C=O), 1608, 1498 (aromatic C=C), 1125, 1067 (glycosidic C–O); UV λ_{max}^{MeOH} nm (log ε) 276 (4.30), 326 (4.32); + NaOMe 292 (4.0), 368 (4.34); + NaOAc 278 (4.27), 326 (4.44); + NaOAc + H_3BO_3 278 (4.29), 325 (4.47); + $AlCl_3$ 283 (4.48), 290 (sh, 4.49), 346 (4.52); + $AlCl_3$ + HCl 2.78 (4.48), 292 (sh, 4.50), 338 (4.48). ^{1}H NMR (DMSO-d_6, TMS) δ: 12.85 (1H, *s*, 5-OH), 8.07 (2H, *d*, *J* = 8.9 Hz, H-2′ and H-6′), 7.19 (2H, *d*, *J* = 8.9 Hz, H-3′ and H-5′), 6.98 (1H, *s*, H-3), 6.69 (1H, *s*, H-8), 5.38 (1H, *d*, *J* = 5.2 Hz, anomeric H), 3.92 (3H, *s*, 6-OMe), 3.73 (3H, *s*, 4′-OMe); ^{13}C NMR (DMSO-d_6, TMS); δ163.4 (C-2), 103.6 (C-3), 182.3 (C-4), 152.0 (C-5), 131.9 (C-6), 158.7 (C-7), 91.7 (C-8), 152.7 (C-9), 105.2 (C-10), 123.8 (C-1′), 128.2 (C-2′ and C-6′), 116.6 (C-3′ and C-5′), 160.3 (C-4′), 99.8 (G-1), 73.2 (G-2), 76.5 (G-3), 69.6 (G-4), 77.2 (G-5), 60.6 (G-6), 60.0 (OMe), 56.5 (OMe).

Acid hydrolysis of **1**. A soln of **1** (50 mg) in 5% H_2SO_4 (10 ml) was refluxed for 4 hr. The solid, after cooling and separating, was crystallized from MeOH to give yellow needles (18 mg) of an aglycone, mp 301–304° [ref. [4], mp 304–305°]; UV λ_{max}^{MeOH} nm: 272, 332; + NaOMe 276, 328, 394; + NaOAc 274, 302sh, 332sh, 384; + NaOAc + H_3BO_3 276, 328, 340, 396; + $AlCl_3$ 264, 284, 300, 346, 353, 386; + $AlCl_3$ + HCl 278, 304, 346, 386. The aq. layer was concd in vacuo. D-Glucose and L-rhamnose were identified by TLC on cellulose in pyridine–EtOAc–HOAc–H_2O (5:5:1:3) R_f 0.4 and 0.6, respectively.

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