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FLAVONOL GLYCOSIDES FROM THE FLOWERS OF *CUCURBITA PEPO*

HIDEJI ITOKAWA, YOSHIAKI OSHIDA, AKIRA IKUTA, HIDEO INATOMI* and SHIRO IKEGAMI†

Tokyo College of Pharmacy, 1432-1, Horinouchi, Hachioji, Tokyo, Japan; * Faculty of Agriculture, Meiji University, 5158 Ikuta, Tamaku, Kawasaki, Kanagawa, Japan; † Faculty of Pharmaceutical Science, Teikyo University, Sagamiko, Kanagawa, Japan

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Key Word Index—*Cucurbita pepo*; Cucurbitaceae; male flowers; FDMS; CIMS; flavonol glycosides; rhamnazin 3-rutinoside; isorhamnetin 3-rutinoside-4′-rhamnoside.

Abstract—Two new flavonol glycosides were isolated from the male flowers of *Cucurbita pepo* and their structures determined by UV, NMR and FDMS as rhamnazin 3-rutinoside and isorhamnetin 3-rutinoside-4′-rhamnoside. Isorhamnetin 3-rutinoside was also isolated.

INTRODUCTION

Cucurbita pepo (pumpkin) has been the subject of several chemical investigations [1–3], but so far no studies on floral constituents have been published. We now report the isolation and identification of two new flavonol glycosides and a known glycoside from the male flowers of this plant.

RESULTS AND DISCUSSION

The butanol-soluble fraction of the methanolic extract of the male flowers after separation by column chromatography gave the three compounds **1**, **2** and **3**. These constituents were easily separated as yellow spots on TLC in *n*-BuOH–HOAc–H_2O, appeared dark in UV (365 nm) and turned yellow-orange when sprayed with 10% H_2SO_4 and heated.

1 crystallized from MeOH and afforded a pale yellow powder, which gave characteristic flavonoid colour reactions. The IR spectrum showed at ν_{max}^{KBr} 3650–3000 cm^{-1} (OH), 1650 cm^{-1} (α,β-unsaturated carbonyl) and 1090–1030 cm^{-1} (C–O stretching bond). The UV spectrum gave maxima at 253 and 353 nm, and confirmation of the substitution of the 3-hydroxyl group was shown by the hypsochromic shift of band I compared with that of its aglycone ($\Delta\lambda_{max}$ 17 nm) [4]. Also the ^1H NMR spectrum of **1** in CD_3OD exhibited a doublet at δ 1.10 (J = 5.9 Hz), which is a distinguishing feature for rhamnosyl Me and two singlets at 3.77 and 3.89 ppm for two OMe groups. Two doublet peaks with *meta*-coupling in ring A appeared at 6.61 and 6.30 ppm, and also three signals at 6.84 (1 H, *d*, J = 9 Hz), 7.56 (1 H, *dd*, J = 9, 2.4 Hz) and 7.90 (1 H, *d*, J = 2.4 Hz) were assigned for C-5′, C-6′ and C-2′ of ring B, respectively.

The rutinosyl was suggested by the ^{13}C NMR spectrum and was confirmed by comparison with the ^{13}C NMR of rutin [5]. Furthermore, anomeric carbon signals of the glycoside at δ 101.0 ppm suggested that the sugar has the β-configuration.

The EIMS (electron impact mass spectrum) showed a base peak at m/z 330 $[Aglyc]^+$ but the nature of the glycosidic units could not be obtained. However, the CIMS (chemical ionization mass spectrum) showed its base peak as a protonated aglycone at m/z 331 $[Aglyc + H]^+$ and fragment peaks of sugar at m/z 326 $[Glu - Rha]^+$, 180 $[Glu]^+$ and 164 $[Rha]^+$, but neither $[M]^+$ nor $[QM]^+$ were observed.

In contrast to the EIMS and CIMS spectra, the FDMS showed a strong protonated molecular ion at m/z 639 and was structurally more informative, and also an $[M + Na]^+$ was present [6]. The octa-acetate of **1** showed two aromatic acetyl signals at 2.32 and 2.40 ppm, and six sugar acetyl signals at 1.94, 1.97, 2.01, 2.03, 2.07 and 2.09 ppm, in the ^1H NMR (in $CDCl_3$). **1** also yielded an aglycone (**4**), D-glucose and L-rhamnose on acid hydrolysis.

4 (M^+, m/z 330, for $C_{17}H_{14}O_7$ by high MS) showed UV maxima at 253 and 370 nm. Free hydroxyls at 4′ and 5 were indicated by bathochromic shifts with NaOMe ($\Delta\lambda_{max}$ 60 nm) and $AlCl_3$ ($\Delta\lambda_{max}$ 53 nm), and substitution at the 7- and 3′-hydroxyls was confirmed by the absence of shifts with NaOAc and NaOAc–H_3BO_3. From the above data, **4** was identified as rhamnazin and this was confirmed by comparison with authentic physical data [7]. The sugars of **1** were identified by comparison with authentic samples using TLC and GLC (TMS–Et_2O). Therefore, **1** is identified as rhamnazin 3-*O*-rutinoside, which is the first report of this glycoside in nature.

Compound **2** was identified by standard procedures: UV, IR, ^1H NMR, ^{13}C NMR, hydrolysis to aglycone and sugar and by comparison with an authentic marker as isorhamnetin 3-*O*-rutinoside.

Compound **3** gave colour reactions and spectral data indicative of a flavonol glycoside. The IR spectrum was similar to those of **1** and **2** and the UV spectrum showed maxima at 250 and 350 nm, and also suggested sugar substitution at the 3-hydroxyl as in **1** and **2**.

The UV data of the aglycone indicated that it is a 5,7-dihydroxyflavonol with substitution at the 3′- and/or 4′-positions. The mass spectral data of **3** were very similar to those of **2**, especially at m/z 316 $[Aglyc]^+$, in the EIMS fragment but the FDMS showed a $[M + H]^+$ ion at m/z 771 and the ^1H NMR spectrum exhibited two doublets at δ 0.98 (J = 5.9 Hz) and δ 1.06 ppm (J = 5.9 Hz) which

were two rhamnosyl Me. The two signals due to the MeCO attached to the aromatic ring appeared as singlets at 2.32 (3H) and 2.47 (3H) ppm in the acetate of **3**. Furthermore, partial acid hydrolysis of **3** yielded isorhamnetin 3-*O*-rutinoside **2**. These facts suggested that **3** was substituted with rhamnose at the 4'-hydroxyl of **2**. Acid hydrolysis of **3** gave isorhamnetin, D-glucose and L-rhamnose, respectively. The ratio of the two sugars was 2:1 (rhamnose:glucose) by GLC. From these data, **3** was identified as isorhamnetin 3-*O*-rutinoside-4'-*O*-rhamnoside and this is the first isolation from a natural source.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a JEOL JMN-PS-100, ^{13}C NMR spectra on a JEOL FX-100, optical rotation on a JASCO DIP-4, EIMS were obtained on a Hitachi RMU-7L, CIMS on a Shimadzu GCMS-6020 with NH_3 gas, FDMS on a JEOL JMS-O1SG II (emitter current 21–24 mA). GLC on a SE-30 column, temp. program (130°, 2°/min), velocity of carrier gas N_2 20 ml/min. TLC on Si gel ($60F_{254}$, Merck) in (1) *n*-BuOH–HOAc–H_2O (5:1:1), (3:1:1), (2) $CHCl_3$–MeOH–NH_4OH (10:10:0.1), (3) $CHCl_3$–MeOH (9:1), (4) pyridine–EtOAc–HOAc–H_2O (5:1:1:3). The MeOH extract obtained from the male flowers of *Cucurbita pepo* was dissolved in H_2O and successively extracted with *n*-hexane, EtOAc and *n*-BuOH. The *n*-BuOH extract was separated by column chromatography with Amberlite XAD-2, eluting with H_2O and MeOH. The MeOH-soluble fraction was purified by repeated column chromatography on Sephadex LH-20 (MeOH) and droplet counter-current chromatography ($CHCl_3$–MeOH–H_2O, 7:13:8 upper). The acetates of each compound (Ac_2O–pyridine at 60°) were purified using prep. TLC with solvent 3. The three flavonol glycosides gave a brownish green colour with $FeCl_3$, yellow with NaOH and yellowish orange with Mg–HCl.

Rhamnazin 3-*O*-*rutinoside* (**1**). Mp 157–158° (MeOH), $[\alpha]_D^{35}$ −3.4° (*c* = 0.12, MeOH). UV λ_{max} nm: MeOH, 253, 262 (sh), 308 (sh), 353; NaOMe, 265, 406; $AlCl_3$, 266, 300, 359, 400 (sh); $AlCl_3$/HCl, 266, 299, 360, 398; NaOAc, 260, 414; NaOAc/H_3BO_3, 253, 263 (sh), 308 (sh), 357. IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 1650 (C–O), 1090–1030 (C–O). ^{1}H NMR (δ, ppm, CD_3OD): 1.10 (*d*, *J* = 5.9 Hz, 3H of Me-Rha); 3.4–5.2 (sugar); 3.77 (*s*, 3H of OMe); 3.89 (*s*, 3H of OMe); 6.16 (*d*, *J* = 2.4 Hz, 1H at C-6); 6.30 (*d*, *J* = 2.4 Hz, 1H at C-8); 6.84 (*d*, *J* = 9 Hz, 1H at C'-5); 7.56 (*dd*, *J* = 9 and 2.4 Hz, 1H at C'-6); 7.90 (*d*, *J* = 2.4 Hz, 1H at C'-2). MS *m/z* (%): 330 (M^+ − sugar, 100), 315 (6.3), 301 (5.6), 287 (6.7), 167 (5.0), 165 (4.0), 151 (5.6), 149 (11.9). ^{13}C NMR (δ, ppm, DMSO-d_6): 177.5 (*s*), 165.2 (*s*), 160.9 (*s*), 156.9 (*s*), 156.5 (*s*), 149.7 (*s*), 147.0 (*s*), 133.4 (*s*), 122.5 (*d*), 121.0 (*s*), 115.4 (*d*), 113.4 (*d*), 105.1 (*s*), 101.0 (*d*), 98.0 (*d*), 92.5 (*d*), 76.6 (*d*), 76.1 (*d*), 74.4 (*d*), 71.9 (*d*), 70.7 (*d*), 70.5 (*d*), 68.4 (*d*), 67.1 (*t*), 56.2 (*q*), 55.8 (*q*), 17.9 (*q*).

Acid hydrolysis of **1**. **1**, 34 mg in 2 ml MeOH on hydrolysis with 5% HCl (3 ml) afforded the aglycone **4**, mp 215–218° (MW calc. for $C_{17}H_{14}O_7$ (MS): 330.0739; found 330.0753). UV λ_{max} nm: MeOH, 253, 262 (sh), 370; NaOMe, 247, 265, 430; $AlCl_3$, 264, 298 (sh), 370 (sh), 423; $AlCl_3$/HCl, 262, 295 (sh), 360, 423; NaOAc, 253, 281, 407 (sh); NaOAc/H_3BO_3, 253, 262 (sh), 370. IR ν_{max}^{KBr} cm^{-1}: 3460, 3270, 1660, 1615, 1590. ^{1}H NMR (δ, ppm, DMSO-d_6): 3.84 (*s*, 6H); 6.35 (*d*, *J* = 2 Hz, 1H); 6.76 (*d*, *J* = 2 Hz, 1H); 6.95 (*d*, *J* = 9 Hz, 1H), 7.73 (*dd*, 9 and 2 Hz, 1H); 7.76 (*d*, *J* = 2 Hz, 1H). MS *m/z* (%): 330 (M^+, 100), 315 (3.8), 301 (5), 287 (6.3), 259 (2.5), 165 (4.4), 151 (9.5), 149 (13.9). The H_2O–MeOH soln layer was neutralized with Dowex 2 × 8 and evapd. Sugars were identified by TLC (solvents 1 and 4) and GLC (TMS ether) as L-rhamnose and D-glucose.

Isorhamnetin 3-O-*rutinoside*-4'-O-*rhamnoside* (**3**). Mp 190.5–192° (MeOH). $[\alpha]_D^{35}$ 64.4° (*c* = 0.3, MeOH). UV λ_{max} nm: MeOH, 250, 264 (sh), 310 (sh), 350; NaOMe, 267, 325, 404; $AlCl_3$, 266, 302, 377; $AlCl_3$/HCl, 266, 300 (sh), 358, 399; NaOAc, 271, 318, 382; NaOAc/H_3BO_3, 252, 264 (sh), 354. IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 1650 (C–O), 1100–1000 (C–O). ^{1}H NMR (δ, ppm, CD_3OD): 0.93 (*d*, *J* = 5.9 Hz, 3H of Me-Rha); 1.06 (*d*, *J* = 5.9 Hz, 3H of Me-Rha); 3.4–5.2 (sugar); 3.96 (*s*, 3H of OMe); 6.17 (*d*, *J* = 2.4 Hz, 1H at C-6); 6.37 (*d*, *J* = 2.4 Hz, 1H at C-8); 6.88 (*d*, *J* = 8.8 Hz, 1H at C'-5); 7.56 (*dd*, *J* = 8.8 and 2.4 Hz, 1H at C'-6); 7.90 (*d*, *J* = 2.4 Hz, C'-2). MS *m/z* (%): 316 (M^+ − sugar, 100), 301 (7), 287 (10), 245 (8). ^{13}C NMR (δ, ppm, DMSO-d_6): 177.0 (*s*), 163.9 (*s*), 161.0 (*s*), 156.2 (*s*), 149.1 (*s*), 146.6 (*s*), 132.3 (*s*), 121.9 (*d*), 120.9 (*s*), 115.0 (*d*), 114.8 (*d*), 103.9 (*s*), 100.8 (*d*), 98.5 (*d*), 93.6 (*d*), 71.6 (*d*), 70.4 (*d*), 68.2 (*d*), 55.5 (*q*), 17.6 (*q*), 17.0 (*q*).

Acid hydrolysis of **3**. **3** (31 mg) in 2 ml MeOH on hydrolysis with 5% HCl (3 ml) afforded the aglycone **6**, mp 306–308°. UV λ_{max} nm: MeOH, 255, 264 (sh), 370; NaOMe, 277, 323, 425; $AlCl_3$, 265, 300 (sh), 365 (sh), 428; $AlCl_3$/HCl, 264, 300 (sh), 358, 427; NaOAc, 273, 317, 392; NaOAc/H_3BO_3, 253, 264 (sh), 370. IR ν_{max}^{KBr} cm^{-1}: 3400–3100, 1650, 1595, 1080–1040. ^{1}H NMR (δ, ppm, DMSO-d_6): 3.86 (*s*, 3H); 6.22 (*d*, *J* = 2 Hz, 1H); 6.50 (*d*, *J* = 2 Hz, 1H); 6.94 (*d*, *J* = 8.8 Hz, 1H); 7.72 (*dd*, *J* = 8.8 and 2 Hz, 1H); 7.77 (*d*, *J* = 2 Hz, 1H). MS *m/z* (%): 316 (M^+, 100), 301 (4), 287 (7), 286 (8), 273 (2), 243 (4), 217 (2). Sugars were identified as above (Rha: Glc = 2:1).

Partial hydrolysis of **3**. A soln of 200 mg of **3** in 0.1 N HCl was heated under reflux for 1 hr. After cooling, the soln was neutralized with Dowex 2 × 8 and purified by column chromatography with Sephadex LH-20 (MeOH) to give 16 mg of **7**, which was identified as isorhamnetin 3-*O*-rutinoside **2** by mp, UV and TLC.

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