Arch Pharm Res Vol 25, No 5, 621-624, 2002

Archives of Pharmacal Research
http://apr.psk.or.kr

A New Kaempferol 7-*O*-Triglucoside from the Leaves of *Brassica juncea* L.

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(Received May 10, 2002)

From the leaves of *Brassica juncea*, a new rare kaempferol 7-*O*-triglucoside isolated and characterized as kaempferol 7-O-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-glucopyranoside (**1**) based on the spectroscopic evidences. This compound was found to be a scavenger of 1,1-diphenyl-2-picrylhydrazyl radical.

Key words : *Brassica juncea*, Flavonoid triglucoside, Radical scavenging

INTRODUCTION

Brassica juncea L., a cormophyte vegetable that belongs to the Cruciferous family, originated from China and has been widely distributed in Korea and Japan. Previously we reported that the BuOH fraction of *B. juncea* exerts *in vitro* radical scavenging activities using spin trapping and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and *in vivo* antioxidant effects on oxidative stress in streptozotocin-induced diabetic rats (Kim *et al.*, 2002). From this BuOH fraction, isorhamnetin 3,7-diglucoside was isolated as one of the active principles (Choi *et al.*, 2000, Yokozawa *et al.*, 2002). In the course of our continuous study for searching an active component from this plant, a new rare kaempferol 7-*O*-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-glucopyranoside (**1**) was isolated along with known kaempferol-3-*O*-(2-*O*-feruloyl-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside)-7-*O*-β-D-glucopyranoside (**2**), kaempferol-3-*O*-β-D-glucopyranosyl-(1 → 2)-*O*-β-D-glucopyranoside-7-*O*-β-D-glucopyranoside (**3**) and 1-*O*-sinapoyl glucopyranoside (**4**). These compounds were individually evaluated for scavenging activity on DPPH radicals.

MATERIALS AND METHODS

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General experimental procedures

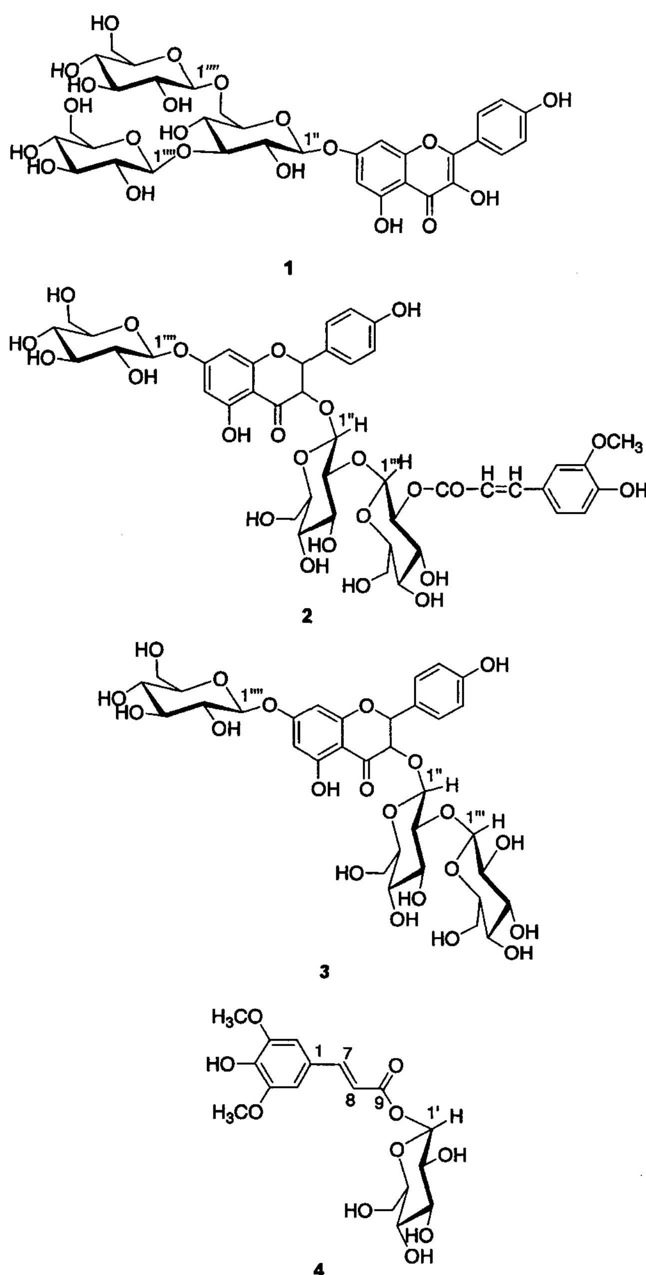
Optical rotation was determined on a Mitamura-Riken polarimeter. UV and IR spectra were recorded on a Varian Carry UV-visible spectrophotometer and a Perkin Elmer FT-IR spectrometer, respectively. LR-FAB mass data were recorded on a JEOL JMS-HX110/110A spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured by Bruker DMX 600 and Varian UNITY-Plus 400 spectrometers. Chemical shifts were referenced to the respective residual solvent peaks (δ_H 2.50 and δ_C 39.5 for DMSO-d_6, δ_H 3.30 and δ_C 49.0 for CD_3OD). COSY, HMQC, and HMBC spectra were recorded on a Bruker DMX 600 using pulsed field gradients. Column chromatography was carried out using Si gel (Merck, 70-230 mesh). TLC was performed on the precoated Merck Kieselgel 60 F_{254} plate (0.25 mm), and 50 % H_2SO_4 was used as spray reagent. L-ascorbic acid and DPPH were purchased from Sigma Chemical Co. Ltd. (St. Louis, MO).

Plant material

The leaves of *Brassica juncea* L. were collected in Yosu, Chonnam Province, Korea, August 1998. A voucher specimen (No. 980802) was deposited in the Herbarium of the Department of Food and Nutrition, Pusan National University.

Extraction and isolation

The powdered dried leaves (3.67 kg) of *B. juncea* were refluxed with MeOH for three hours (9 L × 3). The total filtrate was concentrated to dryness *in vacuo* at 40°C to



Structures of compounds **1** - **4**

render the MeOH extract (400 g), and this extract was suspended in distilled water and partitioned with CH_2Cl_2 (76 g), EtOAc (2.5 g), *n*-BuOH (31 g), and H_2O (286 g) in sequence. Each extract was tested for their DPPH radical scavenging activity. Though EtOAc and BuOH fractions showed similar strong DPPH radical scavenging activities, BuOH fraction showed much higher yield than EtOAc fraction. Therefore, the BuOH fraction (30 g) was applied to a Si gel column which was eluted with CH_2Cl_2-MeOH (gradient) to yield 22 subfractions. Fraction 4 (1.2 g) was further chromatographed on a Si gel column with CH_2Cl_2-MeOH (10:1) to give compound **4** (86.5 mg), and fraction 16 (1.8 g) with EtOAc-MeOH-H_2O (21:4:3) to afford compounds **2** (45 mg) and **3** (35 mg). Fracton 18 (1.2 g) was chromatographed on a Si gel column eluting with EtOAc-MeOH-H_2O (21:4:3) to get compound **1** (45 mg).

Compound 1 (kaempferol 7-*O*-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-glucopyranoside) : Yellow amorphous powder; $C_{33}H_{40}O_{21}$, FAB-MS (negative) *m/z*: 771 $[M-H]^-$, 609 $[M-H-162]^-$, 447 $[M-H-162\times2]^-$; UV λ max (MeOH) 268 (log ε 4.26), 348 (4.16), 371 (4.19), 399 (4.14) nm ; + NaOMe 246 (4.60), 270 (4.59), 302 (4.39), 390 (4.51); + NaOAc 271 (4.23), 348 (4.11), 374 (4.11), 401 (4.04); + NaOAc + H_3BO_3 270 (4.23), 348 (4.13), 372 (4.13) ; + $AlCl_3$ 272 (4.44), 302 (4.27), 348 (4.31), 378 (4.37), 404 (4.35); + $AlCl_3$ + HCl 272 (4.33), 302 (4.14), 348 (4.21), 396 (4.21); ^{1}H-NMR (400 MHz, DMSO-d_6) δ : 4.02 (1H, d, *J*=7.6Hz, H-1'''), 5.07 (1H, d, *J*=7.2 Hz, H-1''), 5.39 (1H, d, *J*=7.2 Hz, H-1''''), 6.44 (1H, *J*= 2.1 Hz, H-6), 6.76 (1H, d, *J*= 2.1 Hz, H-8), 6.89 (2H, d, *J*= 8.9 Hz, H-3', 5'), 8.05 (2H, d, *J*=8.9 Hz, H-2', 6'), 9.84 (1H, s, OH), 10.21 (1H, s, OH), 12.60 (1H, s, 5-OH); ^{13}C-NMR (100 MHz, DMSO-d_6) δ : 177.56 (C-4), 162.85 (C-7), 160.83 (C-5), 160.08 (C-4'), 157.06 (C-9), 156.02 (C-2), 133.5 (C-3), 131.04 (C-2', 6'), 120.70 (C-1'), 115.12 (C-3', 5'), 105.70 (C-10), 103.19 (C-1'''), 99.83 (C-1''), 99.30 (C-6), 100.90 (C-1''''), 94.65 (C-8), 77.14 (C-3''), 76.55 (C-5''/C-5'''), 76.40 (C-5''''), 76.28 (C-3'''), 76.22 (C-3''''), 74.06 (C-2''), 73.33 (C-2'''), 73.09 (C-2''''), 69.73 (C-4''/4'''), 69.55 (C-4''''), 68.01 (C-6''), 60.80 (C-6'''), 60.60 (C-6'''').

Acid Hydrolysis of Compound 1 : On acid hydrolysis with 5%-H_2SO_4 (3 hr), **1** gave glucose (co-TLC, *n*-BuOH-HOAc-H_2O, 4:1:5) and an aglycone **(1a),** identified as kaempferol.

Compound 1a (kaempferol) : ^{1}H-NMR (400 MHz, DMSO-d_6) δ : 6.19 (1H, *J*= 2.0 Hz, H-6), 6.44 (1H, d, *J*= 2.0 Hz, H-8), 6.92 (2H, d, *J*= 8.0 Hz, H-3', 5'), 8.06 (2H, d, *J*=8.0, H-2', 6'), 12.47 (1H, brs, 5-OH); ^{13}C-NMR (100.0 MHz, DMSO-d_6) δ : 175.90 (C-4), 163.88 (C-7), 160.70 (C-5), 159.18 (C-4'), 156.16 (C-9), 146.80 (C-2), 135.65 (C-3), 129.50 (C-2', 6'), 121.66 (C-1'), 115.43 (C-3', 5'), 103.04 (C-10), 98.19 (C-6), 93.47 (C-8)

Compound 2 (kaempferol-3-*O*-(2-*O*-feruloyl-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside)-7-*O*-glucopyranoside) : FAB-MS (negative) *m/z* : 947.0 $[M-H]^-$, 785 $[M-H-162]^-$, 771 $[M-H-feruloyl]^-$, 609 $[M-H\ feruloylglucosyl]^-$, 447 $[M\ H\ feruloylsophorosyl]^-$; UV : λ max (MeOH) 244 (log ε 4.30), 268 (4.29), 297 (4.19), 330 (4.33), 354 (4.18) nm ; + NaOMe 245 (4.39), 271 (4.34), 348 (4.39), 380 (4.55) ; + NaOAc 221 (4.28), 244 (4.10), 268 (4.09), 332 (4.14) ; + NaOAc + H_3BO_3 243 (4.15), 268 (4.16), 333 (4.19), 352 (4.08) ; + $AlCl_3$ 234 (4.06), 270 (4.03), 302

(3.95), 336 (4.06), 354 (3.95); + $AlCl_3$ + HCl 234 (4.04), 277 (4.04), 299 (3.97), 335 (4.07), 399 (3.69) ; ^{1}H-NMR (600 MHz, DMSO-d_6) : KP-moiety δ 6.42 (1H, d, *J*=2.0 Hz, H-6), 6.72 (1H, d, *J*=2.0 Hz, H-8), 8.00 (2H, d, *J*=8.9 Hz, H-2' and H-6'), 6.92 (2H, d, *J*=8.9 Hz, H-3' and H-5') ; Feruloyl moiety δ 7.16 (1H, brd, *J*=2.0 Hz, H-2), 6.73 (1H, d, *J*=8.0 Hz, H-5), 6.96 (1H, brd, *J*=8.0 Hz and 2.0 Hz, H-6), 6.38 (1H, d, *J*=15.9 Hz, H-8), 7.46 (1H, d, *J*=15.9 Hz, H-9), 3.77 (1H, s, OCH_3) ; 3-*O*-sugar moiety δ 5.73 (1H, d, *J*=7.6 Hz, H-1''), 3.53 (1H, t, *J*=7.6 Hz, H-2''), 3.05 (1H, d, *J*=5.0 Hz, H-5''), 5.09 (1H, d, *J*=8.0 Hz, H-1'''), 4.69 (1H, dd, *J*=9.0 Hz and 8.6 Hz, H- 2''') ; 7-*O*-sugar moiety δ 5.07 (1H, d, *J*=7.6 Hz, H-1'''') ; ^{13}C-NMR (150 MHz, DMSO-d_6) : KP-moiety δ 155.87 (C-2), 133.13 (C-3), 177.50 (C-4), 160.81 (C-5), 99.28 (C-6), 162.71 (C-7), 94.36 (C-8), 156.24 (C-9), 105.66 (C-10), 120.83 (C-1'), 130.93 (C-2' and C-6'), 115.30 (C-3' and C-5'), 160.11 (C-4') ; Feruloyl moiety δ 125.69 (C-1), 111.03 (C-2), 147.80 (C-3), 149.07 (C-4), 115.44 (C-5), 122.56 (C-6), 165.80 (C-7), 115.10 (C-8), 144.49 (C-9), 55.60 (OCH_3) ; 3-*O*-sugar moiety δ 97.17 (C-1''), 79.27 (C-2''), 76.92 (C-3''), 70.23 (C-4''), 77.29 (C-5''), 60.94 (C-6''), 99.28 (C-1'''), 73.69 (C-2'''), 74.50 (C-3'''), 70.23 (C-4'''), 76.45 (C-5'''), 60.60 (C-6''') ; 7-*O*-sugar moiety δ 99.81 (C-1''''), 73.13 (C-2''''), 76.35 (C-3''''), 69.63 (C-4''''), 77.17 (C-5''''), 60.63 (C-6'''')

Compound 3 (kaempferol-3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside-7-*O*-β-D-glucopyranoside) : FAB-MS (negative) *m/z* : 771 $[M-H]^-$, 609 $[M-H-162]^-$, 447 $[M-H-162 \times 2]^-$; UV : λ max (MeOH) 259 (log ε 4.40), 341 (4.22) nm ; + NaOMe 267 (4.46), 342 (4.22), 388 (4.30) ; + NaOAc 258 (4.46), 344 (4.26) ; + NaOAc + H_3BO_3 257 (4.44), 345 (4.25) ; + $AlCl_3$ 266 (4.37), 293 (4.11), 343 (4.13), 391 (4.03); + $AlCl_3$ + HCl 266 (4.30), 292 (4.04), 334 (4.06), 390 (3.92) ; ^{1}H NMR (400 MHz, DMSO-d_6) δ : 6.43 (1H, d, *J*=1.8 Hz, H-6), 6.79 (1H, d, *J*=1.8 Hz, H-8), 8.07 (2H, d, *J*=8.9 Hz, H-2' and H-6'), 6.93 (2H, d, *J*=8.9 Hz, H-3' and H-5') ; 3-*O*-sugar moiety δ 5.70 (1H, d, *J*=7.0 Hz, H-1''), 4.61 (1H, d, *J*=7.8 Hz, H-1''') ; 7-*O*-sugar moiety δ 5.08 (1H, d, *J*=7.6 Hz, H-1'''') ; ^{13}C NMR (100 MHz, DMSO-d_6) δ : 155.9 (C-2), 133.1 (C-3), 177.6 (C-4), 160.9 (C-5), 99.2 (C-6), 162.7 (C-7), 94.4 (C-8), 156.2 (C-9), 105.6 (C-10), 120.6 (C-1'), 131.0 (C-2' and C-6'), 115.3 (C-3' and C-5'), 160.3 (C-4') ; 3-*O*-sugar moiety δ 99.7 (C-1''), 82.5 (C-2''), 76.4 (C-3''), 69.6 (C-4''), 77.2 (C-5''), 60.5 (C-6''), 104.2 (C-1'''), 74.4 (C-2'''), 76.5 (C-3'''), 69.6 (C-4'''), 77.0 (C-5'''), 60.6 (C-6''') ; 7-*O*-sugar moiety δ 97.8 (C-1''''), 73.5 (C-2''''), 76.6 (C-3''''), 69.6 (C-4''''), 77.6 (C-5''''), 60.8 (C-6'''')

Compound 4 (1-*O*-sinapoyl glucopyranoside) : Amorphous white powder; $[\alpha]_D^{20}$ -6.25° (c0.016, MeOH); FAB-MS (positive) *m/z* : 409 $[M + Na]^+$; IR (KBr) : 3449, 1701, 1025~1110 cm^{-1} ; UV : λ max (MeOH) 226 (log ε 4.28), 240 (4.29), 331 (4.39) nm ; + NaOMe 213 (5.13), 260 (4.53), 385 (4.61) ; ^{1}H NMR (400 MHz, DMSO-d_6) δ : 7.05 (2H, s, H-2 and H-6), 7.64 (1H, d, *J*=15.9 Hz, H-7), 6.55 (1H, d, *J*=15.9 Hz, H-8), 3.80 (6H, s, OCH_3), 5.46 (1H, d, *J*=8.0 Hz, H-1') ; ^{13}C NMR (100 MHz, DMSO-d_6) δ : 124.23 (C-1), 106.40 (C-2 and C-6), 148.02 (C-3 and C-5), 146.02 (C-4), 138.56 (C-7), 114.32 (C-8), 165.34 (C-9), 56.08 (CH_3), 94.19 (C-1'), 72.53 (C-2'), 77.81 (C-3'), 69.53 (C-4'), 76.51 (C-5'), 60.59 (C-6')

DPPH radical scavenging effect

The DPPH radical scavenging effect was determined as described by Blois (1958). Four milliliters of MeOH solution of varying sample concentrations (1.5-45 μM) was added to 1.0 mL DPPH methanol solution (1.5×10^{-1} M). After mixing gently and leaving for 30 min at room temperature, the optical density was measured at 520 nm using a spectrophotometer. The antioxidant activity of each sample was expressed in terms of IC_{50} (μg/mL or μM required to inhibit DPPH radical formation by 50%) and calculated from the log-dose inhibition curve.

RESULTS AND DISCUSSION

Column chromatography of the BuOH-soluble part of the methanol extract of the leaves of *B. juncea* yielded three flavonoids, compounds **1-3** and a phenolic glucoside (**4**) in the order of decreasing polarity. The structure of the known compounds **2-4** was identified by comparisons with published spectral data as kaempferol-3-*O*-(2-*O*-feruloyl-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside)-7-*O*-β-D-glucopyranoside (**2**), kaempferol-3-*O*-β-D-glucopyranosyl-(1 → 2)-*O*-β-D-glucopyranoside-7-*O*-β-D-glucopyranoside (**3**) and 1-*O*-sinapoyl glucopyranoside (**4**) (Yoshida *et al.*, 1987; Nielsen *et al.*, 1993; Dietz & Winterhalter, 1996).

Compound **1** was obtained as an amorphous yellowish powder, which gave characteristic flavonol glycoside color reaction i.e. pink with Mg-HCl test and a positive Molisch test. Negative ion FAB mass spectrum [*m/z* 771 : $(M-H)^-$] suggested that **1** was a kaempferol trioside. Acid hydrolysis of compound **1** yielded kaempferol and D-glucose. The ^{1}H NMR spectrum showed that all glucose units were β-linked to other glucose units or to the aglycone since the coupling constants between the anomeric protons and H-2 were always about 7.5 Hz. The UV spectrum of **1** exhibited absorption maxima typical of a number of 3-hydroxyl flavonol at 268 and 371 nm (Mabry *et al.*, 1970). A bathochromic shift of 19 nm in band I with NaOMe, and 25 nm in band I with $AlCl_3$ + HCl suggested free hydroxyl groups at C-4' and C-5 positions, respectively. While the absence of a bathochromic shift in Band II after treatment with NaOAc indicated that glucose was attached to the flavonol aglycone at C-7.

Detailed analysis of the ^{1}H- and ^{13}C-NMR spectra, aided by DEPT, COSY, HMQC, and HMBC experiments, allowed establishment of the structure of **1**. Carbon-13 signals of the aglycone carbons in **1** were readily assigned by careful analysis of the HMQC and HMBC spectra and by comparisons with ^{13}C-NMR data for related kaempferol glycosides (Agrawal, 1989). In the HMBC spectrum of **1**, the signal of glucose anomeric proton at δ 5.07 was correlated with that of kaempferol C-7 at δ 162.85; the signals of other glucose anomeric protons at δ 4.02 (glucose-a) and δ 5.39 (glucose-b) were correlated with that of the inner glucose C-3 at δ 77.14 and C-6 at δ 68.01, respectively, suggesting that the inner glucose was connected with kaempferol at C-7, the glucose-a connected with inner glucose at C-3 and glucose-b connected with the inner glucose at C-6.

On the basis of these results, the structure of **1** was established as kaempferol 7-*O*-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside. This compound has not previously been found in plants.

Compounds **1-4** showed scavenging activities of DPPH radical, with IC_{50} values of 8.4, 24.7, 318.7 and 8.6 μg/ml, respectively. These radical scavenging activities were comparable to that of L-ascorbic acid (IC_{50} = 1.9 μg/ml).

ACKNOWLEDGEMENTS

The high resolution NMR (600 MHz) and FABMS spectra were provided by the Korea Basic Science Institute. This research was supported by a grant (PF002201-07 and -08) from Plant Diversity Research Center of 21st Century Frontier Research Program funded by Ministry of Science and Technology of Korean government.

REFERENCES

Agrawal, P. K., *Carbon 13-NMR of flavonoids.* Amsterdam, The Netherlands, pp. 283-364 (1989).

Blois, M. S., Antioxidant determinations by the use of a stable free radical. *Nature*, 181, 1990-1200 (1958).

Choi, J. S., Kim, J. E., Kim, J. I., Cheigh, H. S. and Yokozawa, T., A flavonol diglucoside from the leaves of *Brassica juncea. Nat. Prod. Sci.,* 6, 199-200 (2000).

Dietz, H. and Winterhalter, P., Phytotoxic constituents from *Bunias orientalis* leaves. *Phytochemistry,* 42, 1005-1010 (1996).

Kim, H. Y., Yokozawa, T., Cho, E. J., Cheigh, H. S., Choi, J. S. and Chung, H. Y., *In vitro* and *in vivo* antioxidants effects of mustard leaf (*Brassica juncea*). *Phytotherapy Research,* accepted (2002).

Mabry, T. J., Markham, K. R. and Thomass, M. B., *The systematic identification of flavonoids.* Springer, Heidelberg, pp. 41-61 (1970).

Nielsen, J. K., Olsen, C. E. and Petersen, M. K., Acylated flavonol glycoside from cabbge leaves. *Phytochemistry,* 34 (2), 539-544 (1993).

Yokozawa, T., Kim, H. Y., Cho, E. J., Choi, J. S. and Chung, H. Y. : Antioxidant effects of isorhamnetin 3,7-di-*O*-β-D-glucopyranoside isolated from mustard leaf (*Brassica juncea*) in rats with streptozotocin-induced diabetes. *J. Agricultural and Food Chemistry,* 50, 5490-5495 (2002).

Yoshida, T., Saito, T. and Kadoya, S., New acylated flavonol glycoside in *Allium tuberosum* Rottler. *Chem. Pham. Bull.,* 35, 97-107 (1987).