Natural Product Sciences
6(4) : 199~200 (2000)

A Flavonol Diglucoside from the Leaves of *Brassica juncea*

Jae Sue Choi[1,*], Jung Eun Kim[1], Jae I Kim[2], Hong Sik Cheigh[2], and Takako Yokozawa[3]

[1]Faculty of Food Science and Biotechnology, Pukyong National University, Pusan 608-737, South Korea,
[2]Department of Food and Nutrition, Pusan National University, 609-735, South Korea,
[3]Research Institute for Wakan-Yaku, Toyama Medical and Pharmaceutical University, Sugitani, Toyama 930-0194, Japan

Abstract – A flavonol diglucoside was isolated from the leaves of *Brassica juncea* L. The structure of the compound was elucidated as isorhamnetin 3,7-*di*-*O*-β-D-glucopyranoside (1) on the basis of chemical and spectral evidence.

Key words – *Brassica juncea*; Brassicaceae; flavonol diglucoside; isorhamnetin 3,7-*di*-*O*-β-D-glucopyranoside

Introduction

The *Brassica juncea* L. (Brassiaceae) is a well-known herbaceous plant, biennial or perennial, growing in China, Japan and Korea (Lee, 1985). The leaves are consumed as food spices or a number of folkloric uses, such as a stimulant, diuretic and expectorant in Korea (Farrel, 1985). The leaves have a characteristic and prickly taste, due to the presence of glucosinolates. A previous phytochemical investigation performed on this species resulted in the isolation of glucosinolates [Hill *et al.*, 1987; Han *et al.*, 1987], nucleodites (Kim *et al.*, 2000) and flavonoid (Kang, 1995). The present paper deals with the structure elucidation of a flavonol glycoside **1** on the basis of spectroscopic analysis, including 2D NMR spectroscopic techniques.

Experimental

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

Extraction and isolation – The dry leaves (3.67 kg) were refluxed with MeOH for three hr. (9L×3). The total filtrate was concentrated to dryness *in vacuo* at 40 to render the MeOH extract (400 g), and this extract was suspended in distilled H_2O and partitioned with CH_2Cl_2 (76 g), EtOAc (2.5 g), n-BuOH (31 g) and H_2O (285 g) in sequence. Then BuOH fraction (31 g) was subjected to Si-gel CC. Elution with CH_2Cl_2 with increasing amounts of MeOH (50%, 10-20%, 30%) and then MeOH gave 11 subfractions. The subfraction No. 6 (10.3 g) was further purified by Sephadex LH-20 using MeOH as solvent to give compound 1 (450 mg).

Isorhamnetin 3,7-*di*-O-β-D-glucopyranoside (1). Amorphous yellowish powder; $[a]_D^{20}$ – 6.25° (c0.016, MeOH); UV max (MeOH) 255 (log ε 4.61), 266 (sh, 4.53), 354 (4.51) nm; + NaOMe 248 (sh, 4.49), 265 (4.57), 280 (sh, 4.43), 397 (4.61); + NaOAc 255 (4.60), 267 (sh, 4.54), 356 (4.47) ; + NaOAc + H_3BO_3 255 (4.61), 266 (sh, 4.74), 355 (4.51); + $AlCl_3$ 270 (4.62), 300 (sh, 4.17), 356 (sh, 4.37), 402 (4.45); + $AlCl_3$ + HCl 230 (sh, 4.38), 269 (4.59), 300 (sh, 4.17), 357 (4.36), 401 (4.43); ^{1}H-NMR (DMSO-d_6, 500MHz) δ : 12.61 (1H, s, OH), 9.85 (1H, s, OH), 7.95 (1H, d, J=1.68 Hz, H-2'), 7.53 (1H, dd, J= 1.68 & 8.40 Hz, H-6'), 6.93 (1H, d, J=8.4 Hz, H-5'), 6.81 (1H, d, J=1.80 Hz, H-8), 6.45 (1H, d, J=1.80 Hz, H-6), 5.58 (1H, d, J=7.20 Hz, H-1"), 5.09 (1H, d, J=7.02 Hz, H-1'''), 3.85 (3H, s, OMe); ^{13}C-NMR (DMSO-d_6, 125MHz) δ : 177.63 (C-4), 162.89 (C-7), 160.88 (C-5), 156.91 (C-2), 156.05 (C-9), 149.61 (C-4'), 146.96 (C-3'), 133.30 (C-3), 122.24 (C-6'), 120.99 (C-1'), 115.25 (C-5'), 113.54 (C-2'), 105.71 (C-10), 100.69 (C-1"), 99.77 (C-1'''), 99.41 (C-6), 94.58 (C-8), 77.51 or 77.23 (C-3" or C-3'''), 76.45 (C-5" & C-5'''), 74.37 or 73.11 (C-2" or C-2'''), 69.84 or 69.63 (C-4" or C-4'''), 60.85 or 60.66 (C-6"

*Author for correspondence.

or C-6'''), 55.72 (OMe); HRFABMS *m/z*: see text.

Results and Discussion

Compound 1 was isolated as an amorphous yellowish powder, which gave characteristic flavonol glycoside color reaction i.e. pink with Mg-HCl test and a positive Molisch test. The molecular formula of **1** was determined to be $C_{28}H_{32}O_{17}$ by HRFABMS [$(M+H)^+$: *m/z* 641.1714 for $C_{28}H_{33}O_{17}$, Δ-0.3 mmu]. The IR spectrum showed a broad hydroxyl and α,β-unsaturated carbonyl absorptions at 3,367 and 1,658 cm^{-1} respectively, and a C-O stretching band at 1,056 cm^{-1}, indicating its glycosidic nature. The UV spectrum of **1** exhibited absorption maxima typical of a number of 3-hydroxyl substituted flavonol at 255 nm and 354 nm (Mabry *et al.*, 1970). The bathochromic shift of band I in the presence of $AlCl_3$ and $AlCl_3$+HCl indicated the presence of free 5-hydroxyl group while the absence of a shift with NaOAc indicated that the 7-hydroxyl was substituted. And also a bathochromic shift with NaOMe, with an increase in intensity of band I, indicated the presence of a free 4'-hydroxyl group in **1**. The ^{1}H-NMR spectrum of **1** showed a methoxy singlet at δ3.85, two *meta*-coupled doublets of one proton each at δ6.45 (J=1.80 Hz, H-6) and δ6.81 (J=1.80 Hz, H-8), one *ortho*-coupled doublet of one proton at δ6.93 (J=8.40 Hz, H-5'), a double-doublet of one proton at δ7.53 (J=1.68 and 8.40 Hz, H-6'), a *meta*-coupled doublet of one proton at δ7.95 (J=1.68 Hz, H-2') and a singlet of one proton at δ12.61 (5-OH). These data indicated that **1** was a 3,5,7,3',4'-oxygenated flavonoid derivative. The appearance of the H-2' signal at lower field than the H-6 signal suggested the presence of a 3'-methoxy-4'-hydroxy moiety in the B ring (Mabry *et al.*, 1970). It also showed the proton signals due to the sugar moieties between δ3.40-5.60 including two anomeric proton signals (δ5.09, d, J=7.0Hz; δ5.58, d, J=7.20Hz). The sugars appear to be two mole each of β-D-glucopyranoses according to ^{13}C-NMR spectra. Detailed analysis of the ^{1}H- and ^{13}C-NMR spectra, aided by DEPT, HMQC and HMBC experiments, allowed establishment of the structure of **1**. Carbon-13 signals of the aglycone carbones in **1** were readily assigned by careful analysis of the HMQC and HMBC spectra and by comparisons with the ^{13}C-NMR data for related flavonol glycosides (Agrawal, 1989). The configuration of each glucopyranose moiety was determined to be β not only by the J value of the anomeric proton signal, but also by comparison of the ^{13}C-NMR data with those for corresponding methyl α-D- and β-D-glucopyranosides (Yoshimoto *et al.*, 1980). The glycosidic linkage site of each β-D-glucopyranose was determined to be C-3 and C-7, based on the long-range C-H coupling between H-1' (or H-1'') and C-3 (or C-7) in the HMBC experiment. In addition, a methoxyl group was found to be attached to C-3' according to long-range C-H coupling between OCH_3 and C-3' in the HMBC experiment.

On the basis of these results, the structure of **1** was established as 3,5,7,3',4'-pentahydroxyflavone 3'-methoxy-3,7-*di*-*O*-β-D-glucopyranoside (isorhamnetin 3,7-*di*-*O*-β-D-glucopyranoside). This is the first report of its occurrence in Brassica species to our best knowledge.

Acknowledgements

This research was supported by a grant (PF002201-07) from Plant Diversity Research Center of 21st Frontier Research Program.

References

Agrawal, P. K. Carbon 13-NMR of Flavonoids. Agrawal, P. K. Ed.; Elsevier: Amsterdam, The Netherlands, 1989.

Farrell, K. T. Spices, Condiments, and Seasonings. Van Nostrand Company, p. 150 (1985).

Han, Y. B., Kim, M. R., Han, B. H. and Han, Y. N. Studies on anti-oxidant component of mustard leaf and seed. *Kor. J. Pharmacogn.*, **18**(1), 41-49 (1987).

Hill, C. B., Williams, P. H., Varlson, D. G. and Tookey, H. I. *J. Amer. Soc. Hort. Sci.* **112**, 309-313 (1987).

Kang, S. K. Structural analysis of major antimicrobial substance obtained from leaf mustard (*Brassica juncea*). *J. Korean Soc. Food Nutr.* **24**(5), 702-706 (1995).

Kim, J. I., Choi, J. S., Kim, W. S. and Cheigh, H. S. Studies of Identification and composition of nucleosides from mustard leaf and mustard leaf kimchi. *J. Korean Soc. Food Sci. Nutr.* **29**(5), 796-801 (2000).

Lee, C.B., Illustrated Flora of Korea. P.387. Hyang Moon Sa, Seoul, 1985.

Mabry, T. J. Markham, K. R. and Thomas, M. B. (1970). The Systematic Identification of Flavonoids. Springer, Heidelberg.

Yoshimoto, K., Itatani, Y., Shibata, K. and Tsuda, Y. Syntheses and ^{1}H- and ^{13}C-nuclear magnetic resonance spectra of all positional isomers of methyl mono-*O*-tetradecanoyl-α- and β-D-glucopyranosides. *Chem. Pharm. Bull.* **28**, 208 (1980).

(Accepted November 13, 2000)