Flavonoid Glycosides of *Barbarea vulgaris* L. (Brassicaceae)

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Seven flavonoid derivatives were for the first time isolated from aerial parts of an alimentary and medicinal plant of the Brassicaceae family, *Barbarea vulgaris* L. The products were characterized on the basis of spectroscopic NMR (^{1}H, ^{13}C, COSY, HMQC, HMBC) and FAB-MS data. The occurrence of flavonoids in this plant is interesting for their important nutritional properties and for chemotaxonomical pourposes.

Keywords: *Barbarea vulgaris L.; Brassicaceae; flavonol glycosides; food source; ^{1}H and ^{13}C NMR; $^{1}H-^{1}H$ 2D COSY correlated H; $^{13}C-^{1}H$ 2D, HMQC correlated C; $^{13}C-^{1}H$ 2D; HMBC, correlated C; FAB-MS*

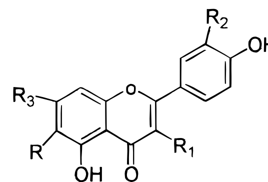
INTRODUCTION

Knowledge of the effects of plant flavonoids on mammalian biology is steadily increasing. There is a considerable amount of epidemiological evidence revealing an association between people who have a diet rich in fresh fruit and vegetables and a decrease in the risk of cardiovascular diseases and certain forms of cancer (Salah et al., 1995). It is generally assumed that the active dietary constituents contributing to these protective effects are the antioxidant nutrients. These, like other components present in trace amounts only in particular food plants, such as carotenoids and glucosinolates, because of their important nutritional properties, may be essential to human health. On average, the daily Western diet contains ∼1 g of mixed flavonoids (Kuhnau, 1976), a quantity that could provide pharmacologically significant concentrations in body fluids and tissues (assuming good absorption from the gastrointestinal tract). The biological activities of flavonoids include action against allergies, inflammation, free radicals, hepatotoxins, platelet aggregation, microbes, ulcers, viruses, and tumors (Dragsted, 1993); block of the angiotensin-converting enzyme (ACE) that raises blood pressure (Middleton and Kandaswami, 1996); and protection of the vascular system and strengthening of the tiny capillaries that carry oxygen and essential nutrients to all cells (Middleton and Kandaswami, 1996). Additionally, flavonoids block the enzymes that produce estrogen, thus reducing the risk of estrogen-induced cancers (Northrup, 1994). They also appear to retard the development of cataracts in individuals with inborn errors in sugar metabolism such as diabetes (Murray, 1994).

One of the objectives of our research group is to improve the knowledge of plants that are used in our countries for medicinal or alimentary purposes through phytochemical and pharmacological studies and prove the existence of metabolites that give a biological response (D'Agostino, 1998; Dini et al., 1995; Senatore, 1996). In this paper we report on the flavonoid glycosides isolated from *Barbarea vulgaris* L. (Brassicaceae).

B. vulgaris is a wild herbaceous plant, biennial or perennial, with an erect and angular stem, shortly branched, growing along trails and roads up to 700 m in elevation throughout the mountains. The plant is also cultivated. Its yellow flowers consist of four petals 12−16 mm across, developing from greenish berry-like buds bunched atop numerous stems. The plant blooms from March to September, and the fruits are small pods (2−3 cm long). The leaves and the young sprouts have a characteristic and prickly taste, due to the presence of some glycosides that for hydrolysis produce allyl and phenyl isosulfocyanates. The leaves and the buds are consumed after cooking. In popular medicine *B. vulgaris* has the same uses as *Nasturtium officinale* R. Brown (Brassicaceae); in fact, is used as a stimulant, diuretic, and expectorant. Also, to the juice obtained from the leaves is attributed the power to denicotinize the leaves of tobacco dipped in it for some time.

Seven flavonoid derivatives (**1**−**7**) described for the



Compound	R	R_1	R_2	R_3
1	H	O-rutinose	OCH_3	OH
2	H	O-glucose	OH	O-rhamnose
3	H	O-glucose	H	OH
4	glucose	H	OCH_3	OH
5	glucose	H	OH	OH
6	H	OH	OCH_3	O-rhamnose
7	H	O-glucose	OCH_3	OH

first time in a plant of the Brassicaceae family were characterized. They can be thought of as markers of chemotaxonomic utility for the plants of this family. These derivatives not were previously characterized by

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10.1021/jf990625k CCC: $19.00 © 2000 American Chemical Society
Published on Web 06/03/2000

Table 1. NMR Data for Compound 1 (in CD_3OD)

	^{1}H	^{13}C	COSY	HMQC
2		158.9		
3		135.5		
4		179.3		
5		163.0		
6	6.24 d 1.8 Hz	100.0	H8 (6.44)	100.0
7		166.1		
8	6.44 d 1.8 Hz	95.0	H6 (6.24)	95.0
9		158.4		
10		105.7		
1′		123.0		
2′	7.97 d 1.85 Hz	114.5	H6′ (7.65)	114.5
3′		150.8		
4′		148.3		
5′	6.94 d 8.32 Hz	116.1		116.1
6′	7.65 dd 1.5, 8.3 Hz	124.0	H2′ (7.97) H5′ (6.94)	124.0
rutinose				
1″	5.26 d 7.4 Hz	102.5		
2″		75.9		
3″		78.1		
4″		72.1		
5″		77.3		
6″		68.5		
1‴	4.5 d 1.5 Hz	100.0		
2‴		71.5		
3‴		72.0		
4‴		73.8		
5‴		69.8		
6‴	1.13 d 6.2 Hz	17.8		
OCH_3	3.97	56.8		

Table 2. NMR Data for Compound 2 (in CD_3OD)

	^{1}H	^{13}C	COSY	HMQC	HMBC
2		157.9			
3		135.8			
4		179.6			
5		163.6			
6	6.47 d 2 Hz	99.8	H8 (6.47)	99.8	C5 (163.6) C8 (95.5) C10 (107.3)
7		162.8			
8	6.75 d 2 Hz	95.5	H6 (6.75)	95.5	C9 (159.5) C10 (107.3)
9		159.5			
10		107.3			
1′	5.34 d 8 Hz	122.9			
2′	7.75 d 2 Hz	117.5	H6′ (6.89)	117.5	C1′ (122.9) C4′ (145.9)
3′		149.9			
4′		145.9			
5′	7.63 d 8.3	123.4	H6′ (6.89)	123.4	C3′ (149.9) C6′ (116.07)
6′	6.89 dd 8.3, 3.2 Hz	116.07	H2′ (7.75) H5′ (7.63)	116.07	C4′ (145.9) C5′ (123.4)
rhamnose					
1″	5.59	103.0			C7 (162.8)
2″		75.7			
3″		78.0			
4″		72.0			
5″		78.0			
6″	1.12 d 6 Hz				
glucose					
1‴	5.59 d (8 Hz)	100.6			
2‴		73.5			
3‴		78.4			
4‴		71.2			
5‴		78.0			
6‴		62.5			

2D NMR. Compound **3** shows an unusual 3-*O*-*β*-D-glucofuranoside structure.

MATERIALS AND METHODS

Material. The Plant material was collected near Potenza (Basilicata region, southern Italy) and identified by F. Senatore. A voucher specimen (DSN/97/23) is stored in the Herbarium of the Department of Chemistry of Natural Substances, University of Naples.

Apparatus. HPLC separations were performed on a Hewlett-Packard HP 1050 series pumping system with a Varian RI-4 refractive index. A detector equipped with a Whatman Partisil M9 10/50 10 ODS-2 column was used.

GC-MS was perofrmed by using a Hewlett-Packard 5890 apparatus, a gas chromatograph fitted with an HP 5970B mass detector, and an HP 59970 MS Chemstation, equipped with an HP-5 column (25 m × 0.2 mm i.d.; 0.33 μm film). GC-MS conditions were as follows: oven temperature, 160 °C; injector temperature, 250 °C; and transfer line temperature, 290 °C. The MS was operated in EI mode with an ionization voltage of 70 eV and an electron multiplier energy of 2000 V.

The NMR spectra were obtained in CD_3OD by means of a Bruker AMX-500 spectrometer, using the solvent shift as reference (δ 3.34 for ^{1}H and δ 49.0 for ^{13}C). The distortionless enhancement by polarization transfer (DEPT) experiments were performed using a transfer pulse of 135° to obtain positive signals for CH and CH_3 and negative ones for CH_2. Polarization transfer delays were adjusted to an average CH coupling of 135 Hz. Two-dimensional homonuclear proton chemical shift correlation (COSY) experiments were measured by employing the conventional pulse sequence. The COSY spectrum was obtained using a data set ($t_1 \times t_2$) of 1024 × 1024 points for a spectral width of 1165 Hz (relaxation delay of 1 s). The data matrix was processed using an unshifted sine bell window function, followed by transformation to give a magnitude spectrum with symmetrization (digital resolution in both F_2 and F_1 dimensions 1.13 Hz for point). The ^{1}H detected heteronuclear multiple quantum coherence (HMQC) experiments were performed according to the method of Martin and Crouch (1991), using an initial BIRD pulse to suppress ^{1}H resonances not coupled to ^{13}C and GARP sequence for ^{13}C decoupling during data acquisition. The spectral width in the ^{1}H dimension was 2994.05 Hz; 256 experiments of 240 scans each (relaxation delay of 1.5 s, delay after BIRD pulse of 0.4 s, fixed delay t_1 of 3.3 ms) were acquired in 1K points. A sine square function was applied in the t_2 dimension (TM, 0.03 Hz TM2, and 0.6 Hz) before Fourier transformation (digital resolution in F_2 dimension of 2.994 Hz/point). ^{1}H detected heteronuclear multiple bound correlation (HMBC) spectroscopy was performed according to the method of Martin and Crouch (1991).

The FAB-MS spectra, in negative ion mode, were obtained by dissolving the samples in a glycerol−thioglycerol matrix and placing them on a copper probe tip prior to bombardment with Ar atoms of energy 2−6 kV in a Kratos MS 902 spectrometer equipped with a Kratos FAB source.

Extraction and Isolation. Aerial parts of *B. vulgaris* (317.2 g) were defatted with petroleum ether and $CHCl_3$ and then extracted with MeOH. The extract (40 g) was chromatographed on a Sephadex LH-20 column with MeOH as eluent. Fractions (20 mL) were collected and checked by TLC [silica gel plates in *n*-BuOH/HOAc/H_2O (60:15:25), spray reagent Ce(SO_4)$_2$ in H_2SO_4]. Fractions 11−12 (**a**; 1.2 g), 13−14 (**b**; 0.9 g), and 15−18 (**c**; 1.2 g) containing the crude compounds were submitted to HPLC using MeOH/H_2O (40:60 for **a** and **b**, 50:50 for **c**) as eluent (flow rate of 2 mL/min) to give **1** (111.7 mg, t_R 42 min) from **a**; **2** (64 mg, t_R 15 min), **3** (55.7 mg, t_R 16 min), and **4** (158 mg, t_R 17 min) from **b**; and **5** (180.6 mg, t_R 8.0 min), **6** (100.8 mg, t_R 18.0 min), and **7** (57.8 mg, t_R 26 min.) from **c**.

Methanolysis. Each compound (1.0 mg) was heated in a vial for 24 h at 80 °C in 2% MeOH/HCl (2 mL). After MeOH

Table 3. NMR Data for Compound 3 (in CD_3OD)

	^{1}H	^{13}C	COSY	HMQC	HMBC
2		158.7			
3		134.0			
4		183.9			
5		162.8			
6	6.62 d 2 Hz	95. 4		95. 4	C10 (105.0)
7		165.6			
8	6.52 d 2 Hz	103.8		103.8	C7 (165.6) C9 (158.7) C10 (105.0)
9		158.7			
10		105.0			
1′		123.1			
2′	7.86 dd 8.9, 2 Hz	129.4	H3′ (6.95)	129.4	C4′ (162.0) C6′ (129.4)
3′	6.95 dd 8.9, 2 Hz	117.1	H2′ (7.86)	117.1	C1′ (123.1) C4′ (162.0) C5′ (117.1)
4′		162.0			
5′	6.95 dd 8.9, 2 Hz	117.1			
6′	7.86 dd 8.9, 2 Hz	129.4		129.4	
glucose					
1″		109.3		109.3	
2″		80.2			
3″		75.3			
4″		82.6			
5″		71.7			
6″		62.8			

Table 4. NMR Data for Compound 4 (in CD_3OD)

	^{1}H	^{13}C	HMQC
2		165.7	
3	6.51	103.9	
4		183.7	
5		161.9	
6		109.1	109.1
7		165.3	
8	6.42	95.4	95.4
9		158.5	
10		105.0	
1′		123.3	
2′	7.36	110.2	110.2
3′		149.3	
4′		152.0	
5′	6.89	116.7	116.7
6′	7.41	121.6	
glucose			
1″	3.53	80.1	80.1
2″	4.24	72.5	
3″	4.92	75.3	
4″	3.52	71.7	
5″	3.43	82.5	
6″	3.95	62.8	
OCH_3	3.92	56.5	

and HCl evaporation in an N_2 stream, Ag_2CO_3 and MeOH were added until CO_2 production stopped. The centrifugate was dried over P_2O_5. The resulting monosaccharide was treated with Trisil-Z (Pierce) [10−30 μL of 1-(trimethylsilyl)imidazole in dry pyridine, 1:1; 15 min at room temperature] and analyzed by GC-MS. Retention times were identical to those of the authentic Trisil-sugar.

RESULTS AND DISCUSSION

Seven flavonoid glycosides (**1**−**7**) were isolated from *B. vulgaris*. The molecular formulas $C_{28}H_{32}O_{16}$ for **1**, $C_{27}H_{30}O_{16}$ for **2**, $C_{21}H_{20}O_{11}$ for **3**, $C_{22}H_{22}O_{11}$ for **4**, $C_{21}H_{20}O_{11}$for **5**, $C_{22}H_{22}O_{11}$ for **6**, and $C_{22}H_{22}O_{12}$ for **7** were determined by negative ion FAB-MS and ^{13}C NMR analysis. Their ^{1}H and ^{13}C NMR spectra indicated that

Table 5. NMR Data for Compound 5 (in CD_3OD)

	^{1}H	^{13}C	HMQC
2		166.2	
3	6.47 d 1.8 Hz	103.8	
4		183.9	
5		161.9	
6		109.1	109.1
7		165.1	
8	6.51 d 1.8 Hz	95.3	95.3
9		158.6	
10		105.1	
1′		123.4	
2′	7.34	114.2	114.2
3′		146.9	
4′		151.0	
5′	6.90 d 8.2 Hz	116.9	116.9
6′	7.37	120.4	
glucose			
1″		80.1	
2″		72.4	
3″		75.2	
4″		71.7	
5″		82.5	
6″		62.8	

Table 6. NMR Data for Compound 6 (in CD_3OD)

	^{1}H	^{13}C	HMQC
2		148.7	
3		137.0	
4		177.5	
5		163.2	
6	6.80 d 1.8 Hz	99.7	99.7
7		162.3	
8	6.47 d 1.8 Hz	95.4	95.4
9		157.7	
10		106.1	
1′		123.0	
2′	7.91	112.6	112.6
3′		150.2	
4′		148.8	
5′	6.96	116.3	116.3
6′	7.79	123.0	
OCH_3	3.91	56.4	
rhamnose			
1″		99.7	99.7
2″		71.8	
3″		72.1	
4″		73.6	
5″		69.4	
6″		18.1	

glycosides **4** and **5** had luteolin as aglycon; **2**, **6**, and **7** had quercetin; and **3** had kaempferol. The aglycons were identified by comparing their ^{1}H and ^{13}C NMR data with published values.

The ^{1}H NMR spectrum showed that **1** was a quercetin derivative. Acid methanolysis of **1** gave glucose and rhamnose in a 1:1 ratio. ^{1}H and ^{13}C NMR data indicated β and α configurations for the glucopyranosyl and rhamnopyranosyl units, respectively (Table 1). The rhamnose was attached to the C-6″−OH of glucose as judged from the downfield shift of the C-6″ signal. The location of rutinose was determined from the downfield shift of the C-3 signal and confirmed by HMBC experiment. The ^{1}H NMR spectrum showed the presence of peak at δ 3.91 (3H, s) ascribable to a methoxyl group. Finally, the 6 ppm downfield shift of the C-3′ signal, in comparison with the corresponding peak of quercetin, indicated a 3′-O-methylation.

Table 7. NMR Data for Compound 7 (in CD_3OD)

	^1H	^{13}C	HMQC
2		158.6	
3		135.5	
4		179.3	
5		163.0	
6	6.24 d 1.8 Hz	100.2	100.2
7		167.0	
8	6.41 d 1.8 Hz	95.4	95.4
9		158.6	
10		105.5	
1′		123.1	
2′	7.95	114.3	114.3
3′		150.6	
4′		148.4	
5′	6.95 d 8.2 Hz	116.3	116.3
6′	7.60	124.0	
OCH_3	3.91	56.8	
glucose			
1″	5.42 d 7.0 Hz	103.7	103.7
2″		75.9	
3″		78.5	
4″		72.1	
5″		78.1	
6″		62.5	

Therefore, the quercetin 3′-*O*-methyl-3-*O*-*β*-D-glucopyranosyl-[(1→6)-α-L-rhamnopyranoside] structure was assigned to **1**.

The ^{13}C NMR spectrum of **2** showed 27 signals, of which 15 were assigned to quercetin and 12 were assigned to the sugar moiety. The sugar structures and their positions were determined by 1D and 2D NMR. The location of each sugar unit was deduced from an HMBC experiment (Table 2). The ^1H and ^{13}C NMR data indicated *β* configuration for the glucopyranosyl and α configuration for the rhamnopyranosyl unit. Thus, **2** was quercetin 3-*O*-*β*-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside.

Analysis of 1D NMR data (Tables 6 and 7) indicated that compounds **6** and **7** differ from **1** only in the absence of the glucopyranosyl and rhamnopyranosyl units, respectively. Therefore, the structures of quercetin 3′-*O*-methyl-7-*O*-α-L-rhamnopyranoside and quercetin 3′-*O*-methyl-3-*O*-*β*-D-glucopyranoside were assigned to **6** and **7**, respectively.

The downfield portion of the 1D ^1H NMR spectrum of **3** showed a 4H AA "XX" system at δ 7.86 and 6.95 and a 2H AX system at 6.62 and 6.52 ppm, which is characteristic of a kaempferol nucleus (Markham, 1978) (Table 3). The sugar moiety was identified as glucofuranose by ^{13}C NMR. Therefore, the structure of kaempferol 3-*O*-*β*-D-glucofuranoside was assigned to compound **3**.

The aglycon moiety of compound **5** was identified by its 1D NMR spectra. The flavonoid character was immediately apparent from one singlet at δ 6.47, in the ^1H NMR spectrum, attributable to H-3, and signals, in the ^{13}C NMR spectrum, at δ 166.2 and 103.8, attributable to C-2 and C-3, respectively. The presence of the 2,3-olefinic bond also led to an upfield shift (~15 ppm) of the carbonyl resonance (C-4), which appeared at 183.9 ppm. The ^{13}C NMR data indicated the presence of a *β*-D-glucopyranose moiety and a C-glycosylation in **6** (Table 5) shown by the C-6 signal downfield shift (~10 ppm). The ^1H NMR spectrum, furthermore, showed a typical ABX system of the B ring protons at δ 7.34, 6.90, and 7.37. Therefore, the structure luteolin-6-*C*-*β*-D-glucopyranoside was assigned to **5**.

1D and 2D spectra of **4** showed the same signals as **5** and one singlet at δ 3.97 characteristic of an $-OCH_3$ group. The downfield shift of C-3′ (~3 ppm) and upfield shifts of C-2′ (~4 ppm) and C-4′ (~1 ppm) indicated a 3′-O-methylation (Table 4). Thus, the structure of **4** was luteolin 3′-*O*-methyl-6-*C*-*β*-D-glucopyranoside.

ACKNOWLEDGMENT

The NMR, GC-MS, and FAB-MS spectra were performed at the Centro di Ricerca Interdipartimentale di Analisi Strumentale of the University Federico II Napoli. The assistance of the staffs is gratefully appreciated.

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Received for review June 14, 1999. Revised manuscript received February 25, 2000. Accepted April 11, 2000. This research was supported by PRIN grants from the Ministero della Ricerca Scientifica e Tecnologica (Italy).

JF990625K