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NATURAL DRUGS

FLAVONOID COMPOUNDS FROM *PYRUS COMMUNIS L.* FLOWERS*

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Abstract: Five flavonoid glycosides: kaempferol 3-O-β-D-glucopyranoside (**IV**), isorhamnetin 3-O-β-D-glucopyranoside (**V**), isorhamnetin 3-O-β-D-(6''-O-α-L-rhamnopyranosyl)-glucopyranoside (**VIII**), 8-methoxykaempferol 3-O-β-D-(2''-O-α-D-glucopyranosyl)-glucopyranoside (**VI**), 8-methoxykaempferol 3-O-β-D-(2''-O-α-L-rhamnopyranosyl)-glucopyranoside (**VII**) and chlorogenic acid (**IX**) were isolated from the flowers naturally growing *Pyrus communis L.* They were identified by chemical and spectroscopic methods.

Keywords: *Pyrus communis* L. flowers, flavonoids, chlorogenic acid.

Pyrus communis L. grows in the moderate climate zone and is cultivated in many varieties. The flowers of common pear are used in folk medicine as components of analgesic and spasmolytic drugs. In our previous studies on *Pyrus communis* L. flowers we have isolated and identified flavonoids: quercetin 3-O-β-D-glucopyranoside, kaempferol 3-O-β-D (6''-O-α-L-rhamnopyranosyl)-glucopyranoside and quercetin 3-O-β-D-(6''-O-α-L-rhamnopyranosyl)-glucopyranoside and we have identified chromatographically hydroquinone derivatives (arbutin, pyroside, free hydroquinone), sterols and triterpenes (β-sitosterol and α-amyrin), phenolic acids and coumarins (1). In this report we present the isolation and the identification of the next flavonoid compounds and chlorogenic acid from *Pyrus communis* L. flowers.

EXPERIMENTAL

Plant material and extraction

The flowers of common pear were collected near Łódź in the beginning of May 1997 and dried in natural condition. A sample is deposited in the herbarium in the Department of Pharmacognosy, Institute of Technology and Chemistry of Drugs, Medical University of Łódź. Plant material was extracted using the method described in our previous report (1).

The melting points (m.p.) were determined on a Boetius apparatus and are uncorrected. PC was carried out on a Whatman No. 1 paper; TLC was carried out on a DC-Fertigplatten Kieselgel G 60 F_{254} (TLCg) and DC-Fertigplatten Cellulose (Merck) (TLCc); a CC stationary phase was polyamide (Roth)(CCp) and Cellulose-Pulver (S&S) (CCc). The solvent systems: S-1 H_2O:MeOH (both step gradient); S-2 C_6H_6:MeOH (both step gradient); S-3 n-BuOH/H_2O; S-4 20% MeOH: S-5 n-BuOH:AcOH:H_2O (4:1:5); S-6 15% AcOH; S-7 AcOEt:85%HCOOH:H_2O (18:1:1); S-8 n-BuOH:pyridine:H_2O (6:4:3).

Chromatograms were analyzed in daylight and in UV at 366nm and 254nm after spraying with 1% methanolic $AlCl_3$. Spectra were recorded with the following instruments: NMR Bruker ^1H

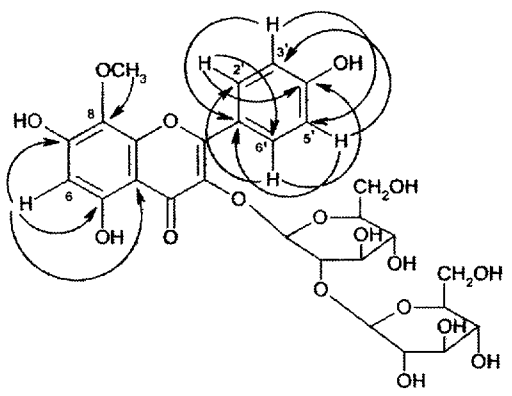

Figure 1. HMBC correlation of compound VI

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DRX 500, ^{13}C DRX 600, HSQC DRX 500, HMBC DRX 500 (in d_6–DMSO, TMS as internal standard); negative and positive mode LSIMS on Finnigan MAT95; UV Unicam SP 800 according to Mabry et al. – a) 0.1 mg of flavonoid compound in 10 ml MeOH, b) a+MeONa, c) a+$AlCl_3$, d) c+HCl, e) a+AcCONa, f) e+H_3BO_3. Acid hydrolysis: 5% HCl, boiling water bath, 3h. All solvents from the fractions were evaporated to dryness using a rotatory evaporator.

Isolation and identification of flavonoid compounds.

Compounds **IV, V, IX** were obtained from the AcOEt extract. The AcOEt extract was separated on the polyamide column using S–1 solvent system. The fractions eluted with 20–40% and 90% MeOH both contained mixtures of flavonoids. The fractions eluted with 20–40% MeOH were separated on the polyamide column using S–2 solvent system. Compound **IV** (250mg) was obtained from the fraction eluted with the mixture C_6H_6–MeOH (7.5:3.5). The fractions eluted with the mixture C_6H_6–MeOH (8:2) were further fractionated on the polyamide column using S–2 solvent system. The fractions eluted with C_6H_6:MeOH (1:1) gave compound **V** (30 mg).

The fractions eluted with 90% MeOH were further fractionated on the cellulose column using S–3 solvent system. Further fractions gave compound **IX** (50 mg).

Compounds **VI, VII, VIII** were obtained from the n–BuOH extract. The n–BuOH extract was separated on the polyamide column using S–1 solvent system. The fractions eluted with 20% and 60% MeOH both contained mixtures of flavonoids. The fractions eluted with 20% MeOH were separated on the polyamide column using S–2 solvent system. The initial fractions eluted with the mixture C_6H_6 : MeOH (6:4) were further fractionated on the cellulose column using S–4 solvent system and gave compound **VII** (5 mg). The final fractions eluted with the mixture C_6H_6 : MeOH (6:4) were further fractionated on the cellulose column using S–3 solvent system and gave compound **VI** (10 mg).

The fractions eluted with 60% MeOH were further fractionated on the polyamide column using S–2 solvent system. The fractions eluted with the mixture C_6H_6 : MeOH (7:3) gave the compound **VIII** (60 mg).

Compounds **IV, V, VIII, IX** were identified by comparison of its UV, ^1H and ^{13}C NMR and MS data with published values.

Compound **IV** was identified as kaempferol 3–O–β–D–glucopyranoside (2,3).

Compound **V** was identified as isorhamnetin 3–O–β–D–glucopyranoside (2,4).

Compound **VIII** was identified as isorhamnetin 3–O–β–D–(6''–O–α–L–rhamnopyranosyl)–glucopyranoside (2,4).

Compound **IX** was identified as chlorogenic acid (identical with that of standard of chlorogenic acid – Fluka).

Compound **VI** 8–methoxykaempferol 3–O–β–D–(2''–O–α–D–glucopyranosyl)–glucopyranoside: pale yellow needles, m.p. 182–5 °C, Rf PC: 0.63 (S–5); 0.79(S–6); TLCg– 0.07 (S–7): UV λ_{max} nm: a) 272, 325, 355 b) 282, 330, 405 c) 282, 312, 354, 410 d) 282, 312, 354, 410 e) 279, 310, 380 f) 272, 327, 353. ^1H and ^{13}C NMR see Table 1.

Aglycone **VI** Rf PC: 0.76 (S–5); 0.04 (S–6); UV λ_{max} nm: a) 274, 324, 376 b) 287, 336, 428 c) 275, 312, 358, 433 d) 274, 311, 357, 433 e) 281, 320, 402 f) 274, 323, 383.

Sugars: Rf value (TLCc) (S–8) identical with that of standard of glucose. LSIMS (glycerol) m/z positive ion 641.3 [M+1], 479.4 [M–162 +1], 317.2 [M–324+1], negative ion 639.3 [M–1], 477.4 [M–162–1], 315.2 [M–324–1] (calcd formula for $C_{28}H_{32}O_{17}$).

Compound **VII** 8–methoxykaempferol 3–O–β–D–(2''–O–β–L–rhamnopyranosyl)–glucopyranoside: pale yellow needles, m.p. 174–6 °C, Rf PC: 0.52 (S–5); 0.85(S–6); TLCg– 0.14 (S–7): UV λ_{max} nm: a) 273, 326, 355 b) 283, 332, 406 c) 283, 314, 355, 411 d) 283, 314, 355, 411 e) 280, 310, 381 f) 272, 328, 355.

Aglycone **VII** identical with aglycone **VI**. Sugars: Rf value (TLCc) (S–8) identical with that of standard of glucose and rhamnose. ^1H and ^{13}C NMR see Table 1.

DISCUSSION

Using the multistep column chromatography technique with various developing phases next five chromatographically homogeneous flavonoid compounds and the chlorogenic acid were isolated from *Pyrus communis* L. flowers. Their structure was determined by the study of acid hydrolysis products and the analysis of UV, ^1H, ^{13}C, NMR, MS spectra. UV–spectral analysis has indicated the glycosidation in flavonoid compounds **IV–VII** has occurred at the hydroxyl group at C–3 (2). Acid hydrolysis of compound **IV** gave aglycone kaempferol and glucose.

^1H NMR spectrum confirmed that compound **IV** was identical with kaempferol 3–O–β–D–glucopyranoside (3,4). Acid hydrolysis of compound **V** gave isorhamnetin and glucose,

Table 1. ^{1}H NMR DRX-500 (500.13 MHz in DMSO-d_6) and ^{13}C NMR DRX-600(150.91MHz in DMSO-d_6) spectral data compounds VI and VII

Carbon	VI			VII	
	^{1}H	^{13}C	HMBC	^{1}H	^{13}C
2		155.9			156.8
3		132.9			133.0
4		177.6			177.8
5		155.4			156.3
6	6.28 (s)	98.9	C-5,7,10	6.28 (s)	99.1
7		156.7			156.3
8		127.4			127.9
9		148.5			148.9
10		103.8			104.3
1'		121.0			121.4
2'	8.06 (d,8.9 Hz)	130.8	C-4',6'	8.05 (d,8.5 Hz)	129.1
3'	6.94 (d,8.9 Hz)	115.4	C-1',5'	6.91 (d,8.5 Hz)	113.7
4'		159.9			161.6
5'	6.94 (d,8.9 Hz)	115.9	C-1',3'	6.91 (d,8.5 Hz)	113.7
6'	8.06 (d,8.9 Hz)	130.8	C-2',4'	8.05 (d,8.5 Hz)	129.1
glucose					
1''	5.71 (d,7.3 Hz)	97.9		5.67 (d,7.6 Hz)	98.8
2''		82.4			77.9
3''		76.5			77.5
4''		69.7			70.5
5''		77.5			77.8
6''		60.8			61.4
glucose					
1'''	4.61 (d,7.8 Hz)	104.1			
2'''		74.3			
3'''		76.6			
4'''		69.6			
5'''		76.9			
6''		60.9			
8-OMe	3.82 (s)	60.5	C-8	3.81 (s)	60.9
rhamnose					
1'''				5.08 (s)	101.1
2'''					70.8
3'''					71.0
4'''					72.1
5'''					68.7
6'''				0.75 (d,6.0 Hz)	17.4

while that of compound **VIII** gave isorhamnetin, glucose and rhamnose. The NMR spectra of compounds **V** and **VIII** differed from each other only in sugar rests. The ^{1}H NMR spectra confirmed that the aglycone of compounds **V** and **VIII** was identical with isorhamnetin (3). In ^{1}H NMR spectrum of compound **V** was visible the doublet at 5,57 ppm (J=7.3Hz) being assigned to the anomeric proton of glucose. In ^{1}H NMR spectrum of compound **VIII** was visible the single doublet at 5.4 ppm (J=7.3Hz) and singlet at 4.4 ppm assigned to the anomeric protons of glucose and rhamnose. ^{13}C NMR spectra confirmed that compound **V** was isorhamnetin 3-O-β-D-glucopyranoside, while compound **VIII** was isorhamnetin 3-O-β-D-(6''-O-α-L-rhamnopyranosyl)-glucopyranoside (3,5). Compounds **V** and **VIII** have been described as components of fruits and leaves of the pear, while compound **IV** as component of pear leaves (6,7,8). ^{1}H and ^{13}C NMR spectra confirmed that compound **IX** was chlorogenic acid (identical with that of standard of chlorogenic acid – Fluka).

Acid hydrolysis of compounds **VI**, **VII** gave

the same aglycone and sugars: **VI** – glucose and **VII** – glucose and rhamnose. UV analysis of compounds **VI** and **VII** reveals the presence of free hydroxyls at 5,7,4' (2). The ^{1}H MNR spectra revealed the presence of 2',6' and 3',5' protons on the B–ring and the single isolated proton (6.28 ppm) on the A–ring, a more usual position for a C–6 proton. Methoxyl protons were evident at 3.81 ppm. HMQC, HMBC and MS spectra confirmed siting of the A–ring proton at C–6 and correlated the methoxyl with C–8 (Figure 1) (5). In the ^{1}H MNR spectrum of sugar rests of the compound **VI** there were visible two doublets at 4.61 ppm (J=7.8 Hz) and 5.71 ppm (J=7.3Hz) for the anomeric protons of glucoses. In the ^{1}H NMR spectrum of sugar rests of the compound **VII** there were visible the following signals : doublet at 5.67 ppm (J=7.6 Hz) and one singlet at 5.08 for the anomeric protons of glucose and rhamnose. ^{13}C NMR spectra confirmed that compound **VI** was 8–methoxykaempferol 3–O–β–D–(2''–O–β–D–glucopyranosyl)–glucopyranoside (sexangularetin 3–O–β–sophoroside), while compound **VII** was identified as 8–methoxykaempferol 3–O–β–D–(2''–O–α–L–rhamnopyranosyl)–glucopyranoside (sexangularetin 3–O–neohesperidoside). Compounds **VI** and **VII** are very rare flavonoids in plant material. Compound **VI** has been found only in the pollen of *Ulex europaeus* and in *Peonia tenuifolia* (9,10). Compound **VII** has been found only once before in the pollen of *Crataegus monogyna* (11).

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