Acta Poloniae Pharmaceutica – Drug Research, Vol. 60 No. 4 pp. 313–316, 2003
ISSN 0001-6837
Polish Pharmaceutical Society

KAEMPFEROL AND QUERCETIN GLYCOSIDES FROM *RUBUS IDAEUS L.* LEAVES

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Abstract: Quercetin 3–0–β–D–glucoside (**I**), quercetin and kaempferol 3–0–β–D–galactosides (**II**, **III**), kaempferol 3–0–β–L–arabinopyranoside (**IV**), kaempferol 3–0–β–D–(6''–E–p–coumaroyl)–glucoside (tiliroside) (**V**) and methyl gallate (**VI**) were isolated from *Rubus idaeus L.* subspecies culture of Norna leaves and fully characterized.

Keywords: *Rubus idaeus, Rosaceae,* kaempferol and quercetin glycosides, methyl gallate.

Our previous chemical investigation of the flavonoid compounds present in the leaves of *Rubus idaeus L.*, subspecies culture of Norna, dealt with: quercetin, kaempferol, quercetin 3–0–α–L–arabinopyranoside, kaempferol 3–0–β–D–glucuronide and ellagic acid, which were isolated and fully characterized (1).

As a continuation of that work, the isolation and characterization of a few next flavonoid compounds and methyl gallate present in the same source is described.

EXPERIMENTAL

Plant material and extraction have been described previously (1)

Chromatography

PC: Whatman No. 1 Solvent systems: S–1 n–BuOH – HOAc – H_2O (4:1:5) upper phase; S–2 15% HOAc; S–3 HOAc – conc HCl – H_2O (30:3:10); S–4 C_6H_6– HOAc – H_2O (6:7:3) upper phase.

TLC: plates cellulose precoated (Merck) – TLC_c; plates silica gel 60 precoated (Merck) – TLC_g Solvent systems: S–5 EtOAc– 85% HCOOH – H_2O (18:1:1); S–6 n–BuOH–pyridine–H_2O (6:4:3). CC was achieved on polyamid (Roth). Solvent systems: S–7 H_2O–MeOH; S–8 C_6H_6–MeOH (both steep gradient).

The spots of flavonoids were visualized under UV light (366 nm) before and after spraying with 2%$AlCl_3$ in MeOH and 0.5% NA–reagent in MeOH. Sugars were visualised by spraying with aniline phthalate and heating at 105°C.

Isolation

Compounds **V** (40 mg) and **VI** (50 mg) have been obtained from the etheral extract after column chromatography separation (polyamide, S–7).

Compound **V** was eluted with 60% aqueous MeOH as a solvent system. Compound **VI** was eluated with water and further purified on a polyamide column (S–8).

Compounds **I** (45 mg), **II** (140 mg), **III** (15 mg) and **IV** (40 mg) have been obtained from ethyl acetate extract after separation on the polyamide column (S–7).

Coumpound **I**, **II** and **III** were eluted with 40% aqueous MeOH (as the first fractions) and further purified on the polyamide column (S–8).

Compounds **IV** and some amount of quercetin 3–0–α–L–arabinopyranoside have been found in further fractions eluted with 40% aqueous MeOH and were further purified on the polyamide column (S–8).

Identification

Melting points (m.p.) uncorrected were determined on a Boetius apparatus.

Flavonoids were identified by chromatographic analysis of acid hydrolysates and by spectroscopic methods. Total acid hydrolysis was carried out with 5% HCl for 3 h under reflux. EtOAc extracts of hydrolysates were analysed for aglycones (PC, S–3) and H_2O residues for sugars (TLC_c, S–6). The UV spectra (Unicam SP800) of flavonoids were recorded in (a) MeOH, also after the addition (b) NaOMe, (c) $AlCl_3$, (d) $AlCl_3$/HCl, (e) NaOAc, (f) NaOAc/H_3BO_3 according to Mabry et al. (2).

IR spectra were recorded on an ATI – Mattson FTIR apparatus (compounds **V**, **VI**). ^{1}H NMR spectra were recorded on a Bruker MSL 300

(compounds **I** – **IV**) and DRX 500 (compounds **V**, **VI**), ^{13}C NMR on DRX 500 (compounds **IV**, **V**); ^{1}H NMR at 300.13 MHz and 500.13 MHz, ^{13}C NMR at 125.75 MHz respectively (TMS as internal standard). Additionally, for compound **V** 2D NMR – HMQC the spectrum was recorded on a DRX 500 apparatus.

Quercetin 3–0–β–D–glucopyranoside (**I**), yellow needles (MeOH), m.p. 186–189°C.

Rf: TLC$_g$ S–5, 0.52. UV λ max: a) 255, (268), (300), 358; b) 270, 325, 410; c) 270, (307), (330), 438; d) 265, (300), (358), 402; e) 271, 322, 384; f) 260, (298), 382.

^{1}H NMR (DMSO – d$_6$) δ ppm: 12.63 (s, 1H, OH–5), 7.58 (dd J_1=2.1 Hz, J_2=8.1 Hz, 1H, H–6'), 7.57 (d, J=2.1 Hz, 1H, H–2'), 6.83 (d, J=8.5 Hz, 1H, H–5'), 6.39 (d, J=2.0 Hz, 1H, H–8), 6.19 (d, J=2.0 Hz, 1H, H–6), 5.46 (d, J=7.5 Hz, 1H, H–1''), 3.59 – 3.07 (m, 6H of glucose + H_2O).

Acid hydrolysis: quercetin, glucose

Quercetin 3–0–β–D–galactopyranoside (**II**), yellow needles (MeOH), m.p. 247–248 °C.

Rf: TLC$_g$, S–5, 0.47. UV λ max: a) 257, (270), (297), 360; b) 271, 330, 410; c) 274, (307), (331), 440; d) 267, (300), (360), 406; e) 272, 322, 386; f) 261, (300), 381.

^{1}H NMR (DMSO–d$_6$) δ ppm: 12.63(s, 1H, OH–5), 7.66 (dd, J_1=2.2 Hz, J_2=8.5 Hz, 1H, H–6'), 7.52 (d, J=2.2 Hz, 1H, H–2'), 6.81 (d, J=8.5 Hz, 1H, H–5'), 6.40 (d, J=2.0 Hz, 1H, H–8), 6.19 (d, J=2.0 Hz, 1H, H–6), 5.37 (d, J=7.7 Hz, 1H, H–1''), 3.77 – 3.16 (m, 6H of galactose + H_20).

Acid hydrolysis: quercetin, galactose

Kaempferol 3–0–β–D–galactopyranoside (**III**), pale yellow needles – (MeOH), m.p. 255–257°C.

Rf: TLC$_g$, S–5, 0.48. UV λ max: a) 263, (293), (319), 349; b) 270, (325), 405; c) 272, (303), 345, 400; d) 272, (301), 343, 400; e) 270, (300), 370; f) 265, (291), (320), 350.

^{1}H NMR (DMSO–d$_6$) δ ppm: 12.61 (s, 1H, OH–5), 8.06 (d, J=8.9 Hz, 2H, H–2', H–6'), 6.86 (d, J=8.9 Hz, 2H, H–3', H–5'), 6.43 (d, J=2.0 Hz, 1H, H–8), 6.20 (d, J=2.0 Hz, 1H, H–6), 5.40 (d, J=7.6 Hz, 1H, H–1''), 3.66 – 3.27 (m, 6H of galactose + H_20).

Acid hydrolysis: kaempferol, galactose

Kaempferol 3–0–α–L–arabinopyranoside (**IV**), pale yellow needles (MeOH), m.p. 199–202°C.

Rf: TLC$_g$, S–5, 0.60. UV λ max: a) 265, (293), (322), 350; b) 274, (325), 404; c) 273, (303), 349, 400; d) 273, (304), 347, 400; e) 274, (302), 369; f) 268, (294), (321), 348.

^{1}H NMR (DMSO–d$_6$) δ ppm: 12.62 (s, 1H, OH–5), 8.07 (d, J=8.9 Hz, 2H, H–2', H–6'), 6.87 (d, J=8.9 Hz, 2H, H–3', H–5'), 6.43 (d, J=2.0 Hz, 1H, H–8), 6.19 (d, J=2,0 Hz, 1H, H–6), 5.33 (d, J=5.1 Hz, 1H, H–1''), 3.76 – 3.17 (m, 5H of arabinose + H_2O).

^{13}C NMR (DMSO–d$_6$) δ ppm: 177.58 (C–4), 164.29 (C–7), 161.24 (C–5), 160.09 (C–4'), 156.38 (C–9), 156.25 (C–2), 133.58 (C–3), 131.03 (C–2', 6'), 120.71 (C–1'), 115.32 (C–3', 5'), 103.97 (C–10), 101.25 (C–1''), 98.76 (C–6), 93.73 (C–8), 71.60 (C–2''), 70.82 (C–3''), 66.06 (C–4''), 64.26 (C–5'').

Acid hydrolysis: kaempferol, arabinose

Kaempferol 3–0–β–D (6''–E–*p*–coumaroyl)–glucopyranoside (tiliroside) (**V**), pale yellow needles (70% MeOH), m.p. 263–266°C.

Rf: PC, S–1, 0.90; S–2, 0.32. UV λ max: a) 267, (302), 315, (360); b) 275, (312), 370; c) 275, 308, (322), 398; d) 276, 307, (322), 397; e) 276, (298), 313, 370; f) 268, (302), 316, (360).

IR ν max (KBr) 1680 (ester C=O), 1650 cm^{-1} (γ–pyron C=O).

^{1}H NMR (DMSO–d$_6$) δ ppm: 12.57 (s, 1H, OH–5), 7.98 (d, J=8.7 Hz, 2H, H– 2', 6'), 7.36 (d, J=8.7 Hz, 2H, H–2''', 6'''), 7.33 (d, J=16 Hz, 1H, H–7'''–β), 6.85 (d, J=8.7 Hz, 2H, H–3', 5'), 6.78 (d, J=8.7 Hz, 2H, H–3''', 5'''), 6.38 (d, J=2.0 Hz, 1H, H–8), 6.14 (d, J=2.0 Hz, 1H, H–6), 6.10 (d, J=15.9 Hz, 1H, H–8'''–α), 5.44 (d, J=7.5 Hz, 1H, H–1'').

^{13}C NMR (DMSO–d$_6$) δ ppm: 177.43 (C–4), 166.20 (C–9'''), 164.19 (C–7), 161.17 (C–5), 160.01 (C–4'), 159.81 (C–4'''), 156.46 (C–2), 156.38 (C–9), 144.62 (C–7'''–β), 133.07 (C–3), 130.85 (C–2', 6'), 130.18 (C–2''', 6'''), 124.94 (C–1'''), 129.79 (C–1'), 115.78 (C–3''', 5'''), 115.11 (C–3', 5'), 113.65 (C–8'''–α), 103.89 (C–10), 100.97 (C–1''), 98.79 (C–6), 93.69 (C–8), 76.23 (C–3''), 74.25 (C–2''), 74.14 (C–5''), 69.98 (C–4''), 62.98 (C–6'').

Acid hydrolysis: kaempferol, glucose and *p*–coumaric acid; (PC – S–4) red color after visualization with a mixture of 0.5% diazotized sulphanilic acid and 10% Na_2CO_3.

Methyl gallate (**VI**), amorphous, white powder (MeOH), m.p. 195–199°C.

Rf: PC S–1 – 0.77; S–2 – 0.70, violet–brown: under UV; dark–blue after spraying 2% $FeCl_3$. UV λ max (MeOH): 220, 276 nm.

IR (KBr) ν max – 1691.8 cm^{-1}(C=O, ester).

^{1}H NMR (CD_3OD) δ ppm; 7.03 (s, 2H), 3.81 (s, 3H – Me ester).

RESULTS AND DISCUSSION

Five flavonoid compounds (**I** – **V**) and methyl gallate (**VI**) were isolated from the leaves of *Rubus idaeus L.* subspecies culture of Norna using a multistep chromatography technique.

Flavonoids **I** and **II** are derivatives of quercetin; their sugar part is composed of glucose (**I**) and galactose (**II**) as was evident from the chromatographic analysis of acid hydrolysate; both sugars were connected to C–3 of the aglycone as followed from the UV spectra. The ^{1}H NMR spectra of these compounds showed in the aglycone region signals typical for quercetin as well as the presence of the anomeric protons of glucose (**I**) and galactose (**II**) with the coupling constants characteristic for β–configuration (2,3). Compound **I** was identified as quercetin 3–0–β–D–glucopyranoside, and compound **II** as quercetin 3–0–β–D–galactopyranoside. Flavonoids **III** and **IV** were derivatives of kaempferol: their sugar part was composed of galactose (**III**) and arabinose (**IV**), both sugars were connected to C–3 of the aglycone as followed from the UV spectra. ^{1}H NMR spectrum of **III** confirmed that it was kaempferol 3–0–β–D–galactopyranoside. ^{13}C NMR spectrum of **IV** was characteristic in the sugar part for α–L–arabinopyranosides (4). Compound **IV** was kaempferol 3–0–α–L–arabinopyranoside.

The ^{1}H and ^{13}C NMR spectra of compound **V** were in accordance with tiliroside kaempferol 3–0–β–D (6''–E–*p*–coumaroyl)–glucoside. The ^{1}H NMR spectrum showed a doublet for the anomeric proton of glucose with a large coupling constant, which revealed that glucose was β–linked. Two doublets at 7.33 and 6.10 ppm with the coupling constant J=16 Hz indicated trans configuration of *p*–coumaric acid.

The ^{13}C NMR spectrum confirmed that p–coumaric acid was attached to C–6'' of glucose. Two dimensional (HMQC) spectrum let us precisely design signals in ^{1}H and ^{13}C NMR spectrum. Tiliroside has been described as a chemical compound from the plant family *Tiliaceae* and *Malvaceae*, as well a component of some species of genus *Rubus* (5–8).

^{1}H NMR spectrum of **VI** showed two signals; two proton singlets at 7.03 ppm and three proton singlet at 3.81 ppm charecteristic of gallic acid and its methyl ester (3). Compound **VI** was recognized as a methyl gallate.

REFERENCES

1. Gudej J., Rychlińska I.: Herba Polon. 42, 257 (1966).
2. Mabry T.J., Markham K.R., Thomas M.B.: The Systematic Identification of Flavonoids, Springer Verl., Berlin–Heidelberg–New York, 1970.
3. Harborne J.B.: The Flavonoids advances in research since 1986, Chapman & Hall, London–Glasgow–New York–Tokyo–Melbourne–Madras, 1994.
4. Markham K.R., Ternai B., Stanley R., Geiger H., Mabry T.J.: Tetrahedron 34, 1389 (1978).
5. Hörhammer L., Stich L., Wagner H.: Naturwiss. 46, 358 (1959).
6. Gudej J.: Acta Polon. Pharm. 45, 340 (1988).
7. Matławska I.: Acta Polon. Pharm. 58, 127 (2001).
8. Gudej J., Tomczyk M., Urban E., Tomczykowa M.: Herba Polon. 44, 340 (1998).

Received: 21.11.2002