Flavonoids from the bird's foot trefoil seeds (*Lotus corniculatus* L.)

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Abstract

In the seeds of bird's foot trefoil (*Lotus corniculatus* L.) the presence of quercetin- and kaempferol heterosides was found. Eight crystalline compounds and one chromatographically homogeneous fraction were obtained from the flavonoid mixture using column chromatography on polyamide and cellulose. By means of TL-chromatography, elemental analysis and UV-spectrometry seven of the isolated compounds were identified to be quercetin, quercetin 3-arabopyranoside (guajaverin), quercetin 3-galactoside, quercetin 3-rhamnoside, kaempferol 3,7-diglucoside, kaempferol 3,7-dirhamnoside kaempferol 3-glucosyl-7-rhamnoside and two remaining ones were only partly characterized a snearer indefinite kaempferol heterosides.

INTRODUCTION

In the flowers of bird's foot trefoil (*Lotus corniculatus* L.) Harborne (1969) found the presence of gossypetin derivatives, compounds interesting from the point of view of chemotaxonomy. Lately Nielsen (1969) isolated from the same material a new flavonol compound (corniculatin) which he defined to be 3-O- β -D-galactoside of 5,6,3',4'-pentahydroxy-8-methoxy-flavonol (corniculatusin). In the leaves of that species the presence of kaempferol 3,7-dirhamnoside was reported by Nakasaki (1956).

So far the flavonoids from the bird's foot trefoil seeds have not been investigated and are the object of the present work.

MATERIAL AND METHODS

Chromatography: a) paper (PC) — Whatman Nr 1 and Nr 2 filter paper; b) thin-layer (TLC)-DC-Alufolien Cellulose, Whatman CC 41 Cellulose; c) column chromatography — Polyamide Woelm, Cellulose CF₁₁ Whatman.

Solvent systems: S₁ — water, acetone, ethyl acetate — 105:28:2; S₂ — acetic acid, water — 15:85; S₃ — ethyl acetate, methanol, water — 100:10:20 (organic phase); S₄ — ethyl acetate, methanol, acetone, water — 100:8:2:20 (o.p.); S₅ — butanol, acetic acid, water — 40:10:50 (o.p.); S₆ — butanol, pirydine, benzene, water — 5:3:1:3 (o.p.); S₇ — acetic acid, hydrochloric acid, water — 15:3:82.

Detection reagents: a) for flavonoids — ammonia vapors, 2% methanolic solution of AlCl₃ and 2% meth. sol. of ZrOCl₂; b) for sugars — aniline phtalate.

Isolation of crude flavonoid fractions

1kg of the ground bird's foot trefoil seeds (v. Skrzyszowicka) was defatted by petroleum ether and extracted 5 times with 1 l of 90% ethanol on a water bath for one hour. The combined extracts were concentrated in a rotary-vacuum evaporator untill all alcohol was removed and the residue was two-dimensionally TLC chromatographed (on DC-Alufolien Cellulose) in S₁ and S₂ solvent systems. After spraying with chromogenic reagents the chromatogram was examined in UV light (Fig. 1).

Fig 1. Fig 1. Two-dimensional TL chromatogram of crude extract from *Lotus corniculatus* L. (visible in UV light)

I dimension — water, acetone, ethyl acetate — 105:28:2; II — 15% acetic acid A-I — flavonoids, 1 and 1 — blue fluorescent components

The concentrated water extract was first extracted completely with di-ethyl ether in a separatory funnel (fraction I) and then with ethyl acetate (fraction II). Fractions I, II were analysed by TLC as above. The presence of compounds A, B, C, D was found in fraction I and compounds E, F, G, H, I as well as two blue in UV fluorescent components (1,2) were observed in fraction II.

Purification and separation of flavonoid fractions on polyamide and cellulose

Fraction I was chromatographed on a polyamide column using water (1 l), ethyl acetate (2 l) and methanol (1 l) successively. Flavonoids were found in the ethyl acetate eluate (A, B, D, trace C) and in the methanol eluate (C, trace B). The mixture of flavonoids was crystallized from the concentrated eluates and separated on a cellulose column using S_1 solvent system. Similar fractions were combined and concentrated. After crystallisation 7 mg of A, 5 mg of B, 78 mg of C and 824 mg of D flavonoids were obtained.

Fraction II was eluted with water from the polyamide column. The compounds E, F, G, H, I and blue in UV fluorescent components were found in the water eluate. The water eluate was extracted with butanol in a separatory funnel. The butanol phase was concentrated and the residue was separated on a cellulose column using S_3 solvent system. According to the three in UV-light visible on the column bands three eluates (1,2,3) were obtained. Eluate 1 was concentrated and chromatographed preparatively on cellulose plates using S_2 solvent system. The chromatographically homogeneous fraction of compound I was obtained. After concentration of eluate 2 flavonoid G (65 mg) was crystallised from a water-ethanol solution (7:3). Eluate 3 was chromatographed on the cellulose column using S_4 solvent system, and compounds E (about 18 mg), F (ca 12 mg) and H (ca 2 mg) were obtained.

Identification of flavonoids

The chromatographic characteristic of the isolated compounds is reported in Table 1. UV spectra were determined in Unicam SP 800 spectrophotometer, melting points — in Boetius hot stage microscope. Investigations of the presence or absence of a substituent at C_3 -OH and C_7 -OH were conducted like by Mabry et al. (1970).

Quantitative hydrolysis — the investigated substance was dried, carefully weighed (0.02 g) and hydrolysed with 3 ml of 5% H_2SO_4 for two hours on a water bath. The precipitated aglycones were washed

with water until neutral washing, dried and then weighed. The percentages of the aglycones were calculated from the glycoside weight.

Partial hydrolysis — 1 ml of 0.5% methanol solution of the investigated compound was refluxed with 2.5 ml of 1% HCl in a methanol-water (1:1) solution and heated on a water bath for 3 minutes. The products of hydrolysis were investigated with standards by PC using S_6 and S_7 solvent system.

Compound A — yellow needle crystals m.p. 306—307°, λ max. UV 258.357 nm, heated with 5% H_2SO_4 did not hydrolyse. The elemental analysis results were the same as for quercetin. The co-chromatography of compound A with quercetin confirmed the identity of the substances.

Compound B — yellow needle crystals m.p. 225° (ethanol), λ max. UV 259.350 nm. The products of acid hydrolysis were identified by TLC as quercetin and arabinose. The glycosidic bond was found at C_3 -OH (zirconium test). Co-chromatography with isomeric quercetin 3-monoarabinosides, avicularin (3- α -L-arabofuranoside) and guajaverin (3- α -L-arabopyranoside) showed the identity of compound B with guajaverin.

Compound C — yellow needle crystals m.p. 237°, λ max. UV 257, 312, 362, nm. The product of acid hydrolysis contained galactose and quercetin (TLC). The quantitative hydrolysis yielded results corresponding to those calculated for quercetin monoglucoside. The sugar bond was established in the C_3 -OH position (zirconium test). Co-chromatography of compound C and hyperoside gave identical results.

Compound D — yellow needle crystals m.p. 181—182°, λ max. UV 260.352 nm. The products of acid hydrolysis contained quercetin and rhamnose (TLC). Quantitative hydrolysis and elemental analysis correspond with the data given for the monorhamnoside of quercetin. Zirconium test showed the presence of the sugar moiety in the C_3 position. Co-chromatography of compound D with quercetin 3-rhamnoside (quercitrine) confirmed the identity of both substances.

Compound E — yellow needle crystals m.p. 220—222°, λ max. 247,350 nm (methanol). The hydrolysate contained kaempferol and glucose (TLC). The results of the quantitative hydrolysis indicated compound D to be kaempferol diglucoside. The glucosidic band in the C_3 position was established by the characteristic colour of the spot of compound E in UV-light before and after detection with the chromogenic reagents. The presence of the sugar moiety in the C_7 position was determined after spectral analysis in UV (no bathochromic shift of maximum II in sodium acetate). The conducted experiment found compound E to be kaempferol 3,7-diglucoside.

Compound F — needle crystals m.p. 246—247°, λ max. UV (in methanol) 267,353 nm (no bathochromic shift of max. II in sodium acetate). The product of acid hydrolysis contained kaempferol, glucose and

rhamnose (TLC). The glycosidic bonds at the C₃ and C₇ positions were determined as above. Partial hydrolysis of compound F and kaempferol 3-glucosyl-7-rhamnoside (Fig. 2) gave the same results. After co-chromatography with standard, compound F was identified as kaempferol 3-β-D-glucosyl-7-α-rhamnoside.

Fig 2. PC chromatogram of partial hydrolysis of compound F in S solvent system

I — compound F, II — standard (kaempferol 3 — glucosyl-7-rhamnoside 1. kaempferol 3-glucose-7-rhamnoside, 2. kaempferol 3-glucoside, 3. kaempferol 7-rhamnoside, 4. kaempferol

Compound G. — yellow needle crystals m.p. 189—191°, max. UV 266,347 nm. In the product of acid hydrolysis kaempferol and rhamnose were found using TLC. Quantitative hydrolysis yielded results corresponding to those of kaempferol dirhamnoside. The C₃ and C₇ positions of the glycosidic bonds were established using the zirconium test and UV spectrum analysis (no bathochromic shift of max. II in sodium acetate). Co-chromatography with kaempferol 3,7-dirhamnoside obtained from *Celastrus orbiculata* Thunb. by Rządowska-Bodalska (1970) and compound G showed the identity of both substances.

Compound H — m.p. 190—191°, max. UV 268,350 nm (267,356 nm in sodium acetate). The acid hydrolysate contained kaempferol, glucose, rhamnose and arabinose. The presence of substituents in C₃ and C₇ positions was defined by UV spectrum analysis and chromogenic reactions. Accurate identification was not possible because of too small amount of compound H.

Compound I — green fluorescence after reaction with AlCl₃ (UV). In the product of acid hydrolysis only kaempferol and glucose were found. The lack of the glycosidic bond in the C₃ position was established by zirconium test. The high R_f values in S₁ and S₂ solvent systems suggest that compound I contains more than one glucose moiety. The accurate identification of compound I was not performed because of the lack of standard and too small quantity of the investigated substance.

Table 1

The chromatographic characteristic of the flavonoids isolated from the seeds of *Lotus corniculatus* L.

Compounds	R _f values		
	S ₁	S ₂	S ₅
A: quercetin	0.05	0.02	0.72
B: quercetin 3-arabopyranoside	0.29	0.20	0.73
C: quercetin 3-galactoside	0.40	0.29	0.70
D: quercetin 3-rhamnoside	0.60	0.45	0.85
E: kaempferol 3,7-diglucoside	0.58	0.56	0.74
F: kaempferol 3-glucosyl-7-rhamnoside	0.65	0.65	0.69
G: kaempferol 3,7-dirhamnoside	0.72	0.69	0.83
H: kaempferol heteroside	0.75	0.73	0.57
I: kaempferol 7-glucoside (di?)	0.91	0.87	0.82

RESULTS

The investigation of the flavonoid fraction from the seeds of *Lotus corniculatus* L. showed the presence of kaempferol and quercetin heterosides. Gossypetin and its derivatives were not found. The following flavonoids were isolated and identified: quercetin (A), quercetin 3-arabopyranoside (B), quercetin 3-galactoside (C), quercetin 3-rhamnoside (D), kaempferol 3,7-diglucoside (E), kaempferol 3-glucosyl-7-rhamnoside (F), kaempferol 3,7-dirhamnoside (G), and two partly characterized compounds — kaempferol-rhamnosyl-arabinosyl-glucoside (H) m.p. 190—191° showing the presence of the substituents in C₃ and C₇ positions, and kaempferol 7-glucoside (I) (probably diglucoside).

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*Flawonoidy nasion komonicy różkowej
(Lotus corniculatus L.)*

Streszczenie

W nasionach komonicy różkowej (*Lotus corniculatus* L.) stwierdzono obecność heterozydów flawonolowych pochodnych kemferolu i kwercetyny. Z mieszaniny flawonoidowej wydzielono drogą chromatografii kolumnowej na poliamidzie i celulozie 8 związków krystalicznych, oraz jeden związek w postaci chromatograficznie jednorodnej frakcji.

Wydzielone związki zidentyfikowano jako: kwercetynę, 3-arabopyranozyd kwercetyny, 3-galaktozyd kwercetyny, 3-ramnozyd kwercetyny (3,7-dwuglukozyd kemferolu, 3-glukozylo-7-ramnozyd kemferolu, 3,7-dwuramnozyd kemferolu, bliżej nie scharakteryzowany ramnozylo-arabinozylo-glukozyd kemferolu wykazujący obecność podstawników przy C₃ i C₇ układu chromonu oraz prawdopodobnie 7-dwuglukozyd kemferolu.