FLAVONOIDS IN THE FLOWERS OF WILD DOGWOOD (*CORNUS MAS* L.)

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Cornelian flowers (dogwood tree – *Cornus mas* L.) are rich in flavonoids that are during developing into fruits and their ripening modified into anthocyanins. This report describes the isolation and identification of polyphenolic compounds, esp. flavonoids present in the flowers of dogwood tree of Bohemian origin and compares the composition of flavonoid complex with the flowers, buds and leaves of the dogwood tree of other provenance and with other *Cornus* species. On the basis of TLC, spectral data and acid hydrolysis there were identified 4 flavonoid glycosides. The most manifested was isoquercitrin and rutin, in lesser amounts are presented quercetin-3-diglycosides and triglycosides (quercetin-3-diglucoside and 3-glucorutinoside). Likewise in the flowers of the dogwood tree of Rumanian provenance, in the flowers of *C. canadensis* and *C. florida* there were found in the flowers of *C. mas* of Central Bohemian origin glycosides derived from quercetin. The most simplest and in highest concentration represented isoquercitrin can be formed by partial hydrolysis of rutin and other di- or triglycosides of quercetin.

Cornus mas L.; cornelian flowers; composition; flavonoid glycosides

INTRODUCTION

In recent time the interest has been attracted to different non-traditional sources rich in ascorbic acid, polyphenolic compounds, esp. anthocyanins and flavonoids for food-processing industry. One of such indigenous and non-traditional plants is the dogwood tree, *Cornus mas* L. Its fruits – cornelian cherries – are rich in anthocyanins and ascorbic acid and its flowers in flavonoids.

The flavonoid complex was previously studied only in flowers and buds. Delaveau and Paris (1961) have isolated from flowers of *C. mas* L. rutin and derivatives of gallic acid; rutin was also found in lesser amounts in leaves. This fact was confirmed by Egger and Keil (1961) who by the column chromatography on polyamide have obtained rutin and little amounts

of isoquercitrin (quercetin-3-glucoside) and querciturone (quercetin-3-glucuronide). Grigorescu et al. (1972) have found in flowers and buds of the dogwood of Rumanian provenance rutin and its analogue kaempferol-3--rhamnoglucoside. The glycosides of quercetin and kaempferol are typical also for other species of *Cornus* – in the flowers of *C. canadensis* were found quercetin-3-glucoside, galactoside, sophoroside and gentiobioside and kaempferol-3-glucoside and arabinoside (Bain, Denford, 1979), in the leaves and flowers of *C. florida* quercetin- and kaempferol-3-galactoside (Mudry, Schilling, 1983).

In this report we tried to isolate and identify the flavonoids contained in the flowers of *C. mas* of our provenance and to compare the composition of the complex with other species reported previously.

MATERIAL AND METHODS

Plant material. Flowers of dogwood *(Cornus mas* L.) were collected in the area of the Czech University of Agriculture in Prague-Suchdol and dried at the room temperature.

Extraction. 10 g of dried flowers were extracted in Soxhlet extractor at the temperature of b.p. of solvent for 12 hours. Preextraction was performed with ethylacetate and extraction with 80% aqueous methanol.

TLC. Flavonoids were separated by means of TLC on cellulose and silicagel pre-coated sheets:

a) 0,1 mm Cellulose MN 300 Polygram Cel 300 Macherey-Nagel plastic sheets;
b) DC-glass pre-coated sheets Cellulose F (100 x 200 mm, 0.1 mm) Merck;
c) DC-aluminium pre-coated silicagel sheets $60F_{254}$ (200 x 200 mm, 0.1 mm) Merck.

Compounds were laid in the form of points or line segments and developed in next solvent systems:

S_1: TBA = tert. butanol-water-acetic acid (3 : 1 : 1 V/V/V)
S_2: 5% AcOH = acetic acid-water (5 : 95 V/V)
S_3: 15% AcOH = acetic acid-water (15 : 85 V/V)
S_4: BAW = n-butanol-acetic acid-water (4 : 1 : 2 V/V/V)
S_5: conc. HCl-acetic acid-water (3 : 10 : 30 V/V/V)
S_6: chloroform-methanol-n-propanol-water (9 : 12 : 1 : 8 V/V/V/V)

Water-methanolic thickened extract was applied on the DC-glass pre-coated sheets with the 0,1 mm cellulose F layer (100 x 200 mm) Merck in the form of linear segments and developed in the system S_3. After separation of individual compounds into bands compounds were eluated with methanol and after thickening of eluates under vacuum were re-chromatographied in the same manner. Then separated compounds were eluated with methanol (for UV spectroscopy) and measured by UV spectroscopy methods.

Spectral analyses. Spectra of separated compounds were measured on the SPECORD UV-VIS spectrophotometer (Carl Zeiss Jena) in methanolic solutions and after addition of diagnostic reagents natriummethanolate, aluminium chloride, hydrochloric acid, natrium-acetate and hydroboric acid causing characteristic bathochromic and hypsochromic shifts. Systematic analysis was performed after Mabry et al. (1970).

Acid hydrolysis. Methanolic solution of flavonoid glycoside (0.3 mm) was after addition of 6 ml 6% HCl heated in the boiling bath under reflux condenser for 45 min. After cooling was hydrolysate applied to polyamide column (10 x 100 mm) and eluated with 50 ml water and then with 50 ml methanol. Saccharides were in thickened water eluate identified by means of co-chromatography with authentic samples, aglycons were identified chromatographically and spectrally.

Detection of saccharides. Saccharides in the thickened water eluate were identified by co-chromatography with authentic markers using descending PC on Whatman chromatographic paper No. 1 with the solvent system 1-butanol--acetic acid-water (4 : 1 : 5 V/V/V) and detection was made by benzidin and anthrone agents, anilinium hydrogen sulphate, aniline diphenylamine-trihydrogenphosphoric acid and neotetrazolium blue agent (LACHEMA Brno).

Authentic markers. As authentic preparates for co-chromatography and comparison of spectral data were used rutin (Galena Opava), quercetin, quercitrin (FLUKA Chemie AG Switzerland). Authentic samples of D-glucose and L-rhamnose were manufactured by LACHEMA Brno.

RESULTS AND DISCUSSION

On the plastic cellulose sheets Cellulose Polygram Cel 300 MN with 0.1 mm cellulose layer were in the developing systems S_2 and S_5 two dominant flavonoid glycosides observed, meanwhile in the solvent system S_3 were on chromatograms in UV light further two spots 3 and 4 visible.

On the glass cellulose sheets DC-Cellulose F Merck with 0,1 mm cellulose layer were other two spots 5 and 6 apparent. All these compounds were visible as light yellowish. Their R_F values and colours in UV light and in ammonia vapour are given in Tab. I.

Water-methanolic extract was then chromatographied by preparative PC on the papers Whatman No. 4 in the solvent system S_3 after 10 hours saturation with developing system. This system seemed to be the best for the separation of individual compounds.

I. Flavonoids and polyphenols in the *C. mas* flowers – R_F values and colours

Compound		Colour			R_F x 100		
No.	type of glycoside	VIS	UV	UV + NH_3	S_2	S_3	S_5
1	isoquercitrin	Y	B	LY	24	41	52
2	rutin	Y	B	LY	49	55	69
3	unidentified	–	Bl	GB	–	77	–
4	unidentified	–	Bl	Bl	–	88	–
5	quercetin-3–glu, glu	Y	B	LY	–	65	–
6	quercetin-3–glu, rut	Y	B	LY	–	79	–

B – brown, Y – yellow, LY – bright lemon yellow, Bl – blue, GB – green-blue

Spectral data of separated compounds are given in Tab. II.

The flavonoid glycoside 1 coloured in UV light brown has changed in ammonia vapour its colour to bright yellow and was from all present flavonoid glycosides on chromatograms manifested in the largest amount (approx. 75%). This glycoside was chromatographied on silicagel sheets in the solvent system S_6 and was laid on chromatograms in the form of line segments. Flavonoid glycoside has been separated during preparation steps into brown coloured spot in UV light and a bright blue new spot with a lower R_F value. The blue spot was eluated with methanol and rechromatographied on silicagel sheets in two phases solvent system S_6. Blue compound was showing in the lower layer acidified with formic acid (5 ml + 0.1 ml HCOOH) lower R_F values, in upper lesser layer also acidified with 0.1 ml HCOOH higher R_F values.

Basic spectrum of the brown coloured compound was characteristic for quercetin and R_F value was typical for monoglycosides with the saccharides attached in position 3, what is confirmed by the changing of the colour in UV light in the presence of ammonia vapour. By comparison of R_F values with literary data and products of acid hydrolysis it was confirmed that the flavonoid is isoquercitrin, i.e. quercetin-3-glucoside.

The flavonoid glycoside 2 coloured in UV light brown has changed its colour in ammonia vapour to lemon bright yellow. Its basic spectrum and characteristic shifts after addition of diagnostic reagents have confirmed that the pattern of glycoside is quercetin with sugar moitie attached in position 3. R_F values have confirmed a diglycoside, co-chromatography with rutin has shown the same R_F values. By acid hydrolysis was obtained quercetin, D-glucose and L-rhamnose. Glycoside manifested in concentration about 20% we could identify as rutin, i.e. quercetin-3-rutinoside.

II. Spectral data of flavonoids in the cornelian flowers

Comp. No.	MeOH	NaOMe
1	259, 268sh, 300sh, 360	272, 327, 410
2	258, 267sh, 299sh, 359	272, 327, 408
5	256, 268sh, 300sh, 358	274, 330, 405
6	257, 269sh, 298sh, 357	274, 328, 396
Comp. No.	$AlCl_3$	$AlCl_3$/HCl
1	275, 305sh, 332sh, 436	271, 302sh, 364sh, 404
2	274, 304sh, 330sh, 434	270, 301sh, 360sh, 402
5	275, 298sh, 330sh, 441	270, 298sh, 364sh, 403
6	277, 301sh, 332sh, 440	271, 296sh, 366sh, 405
Comp. No.	NaOAc	NaOAc/H_3BO_3
1	272, 325sh, 378	262, 300sh, 375
2	271, 324sh, 383	262, 299sh, 379
5	275, 327sh, 381	262, 298sh, 382
6	70, 326sh, 379	262, 300sh, 383

The compound 5 was manifested only in approximately 5% of the total content of flavonoids. In UV light it was coloured brown and in ammonia vapours its colour has changed to yellow. The basic spectrum was derived from quercetin with the hydroxyl groups glycosylated in position 3. R_F values indicate that the compound is quercetin-3-diglucoside.

The compound 6 was represented only in traces with basic spectrum derived from quercetin glycosylated in position 3. R_F values indicated that the glycoside is quercetin-3-triglycoside, probably quercetin-3-glucorutinoside.

It is to be noted that in the flowers of dogwood of our provenance is present rutin that has been found by many other authors (Delaveau, Paris, 1961; Egger, Keil, 1969; Grigorescu et al., 1972) in the flowers of *C. mas* of North American and Rumanian provenance. In contradiction to their results it was contained as a second dominant compound in respect to concentration of flavonoid glycosides (approx. 20% of total content of flavonoids). It is in accordance with information of Delaveau and

Paris (1961) who have found rutin in the leaves of dogwood in lesser amounts. The most manifested glycoside was isoquercitrin which formed nearly 75% of total content of flavonoid complex contained in the flowers of dogwood. This statement is in accordance with the results of Egger and Keil (1969) who have found in the flowers of *C. mas* lesser amounts of isoquercitrin and quercituron. These compounds have very similar structure to rutin and could be formed by its partial hydrolysis (isoquercitrin by splitting of L-rhamnose from rutin off and quercituron by partial oxidation of isoquercitrin). Only in little amounts or traces are manifested quercetin-3-di- and triglycosides (quercetin-3-diglucoside and 3-glucorutinoside). Likewise in the flowers of the dogwood tree of Rumanian provenance (Grigorescu et al., 1972), in the flowers of *C. canadensis* (Bain, Denford, 1979) and *C. florida* (Mudry, Schilling, 1983) we could find in the flowers of *C. mas* the glycosides of quercetin.

Whereas for the fruits of the dogwood – cornelian cherries – are characteristic anthocyanins-pelargonidine-3-galactoside and rhamnosylgalactoside, cyanidine-3-galactoside and empetrin (Lachman et al., 1995), for the cornelian flowers are characteristic flavonoid glycosides of quercetin, mainly isoquercitrin and rutin. During the development of flowers to fruits and their ripening they are converted into anthocyanins.

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Flavonoidy květů svídy dřínu (*Cornus mas* L.).

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V květech dřínu obecného (*Cornus mas* L.) jsou významně zastoupeny flavonoidní glykosidy, které se během zrání postupně přeměňují na anthokyanová barviva obsažená převážně v plodech. Proto bylo sledováno složení flavonoidního komplexu květů dřínu obecného středočeského původu a provedeno srovnání se složením flavonoidního komplexu květů různých druhů dřínu. Ethanolický extrakt květů byl podroben TLC na celulózových deskách Macherey-Nagel a Merck v řadě vyvíjecích soustav a glykosidy byly podrobeny kyselé hydrolýze. Produkty kyselé hydrolýzy po separaci na koloncе polyamidu byly rozděleny pomocí TLC a PC kochromatografií s autentickými preparáty – flavonoidy FLUKA (Chemie AG Buchs) a monosacharidy (LACHEMA Brno). Flavonoidy byly identifikovány na základě spektrální analýzy v methanolu a charakteristických diagnostických posuvů za přídavku natriummethanolátu, chloridu hlinitého, kyseliny chlorovodíkové, natriumacetátu a kyseliny trihydrogenboritě na spektrofotometru SPECORD UV VIS Zeiss Jena.

Na chromatogramech bylo přítomno celkově šest látek polyfenolického charakteru,z nichž byly identifikovány čtyři flavonoidní glykosidy. Nejvíce zastoupeným glykosidem (cca 75 %) byl isokvercitrin, méně zastoupený (20 %) byl rutin. V malých až stopových množstvích byl nalezen 3-0-diglukosid kvercetinu a 3-0-triglykosid kvercetinu, pravděpodobně 3-0-glukorutinosid kvercetinu.

Ze složení flavonoidního komplexu květů dřínu vyplývá, že všechny glykosidy jsou odvozeny od kvercetinu glykosylací v poloze 3. Jednodušší monoglykosidy mohou vznikat parciální hydrolýzou složitějších di- a triglykosidů, např. isokvercitrin z rutinu.

Cornus mas L.; květy svídy dřínu; složení; flavonoidní glykosidy

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