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QUERCETIN AND ITS GLYCOSIDES IN THE FLOWERS OF *ASCLEPIAS SYRIACA* L.

MARIA SIKORSKA and IRENA MATŁAWSKA

Department of Pharmacognosy Karol Marcinkowski University of Medical Sciences
10 Sieroca Str., 61–771 Poznań, Poland

Abstract: The following flavonoid compounds have been isolated and identified from the flowers of *Asclepiac syriaca* L.: quercetin and its glycosides: 7–O–galactoside, 7–O–glucoside, 3–O–β–D–xylopyranoside(1→2) β–D–galactoside and 3–O–β–D–glucopyranoside(1→2) β–D–galactoside. Their structures were established by acid and enzymatic hydrolysis or H_2O_2 oxidation as well as spectral analysis (UV, ^1H and ^{13}C NMR).

Keywords: *Asclepias syriaca*, flowers, flavonoids, quercetin derivatives.

Asclepias syriaca L. (*Asclepiadaceae*) – common milkweed, grows naturally in North America. The cardenolides were found in all parts of the plant (1) and identified as syrioside, syriobioside, uzarin, desglucouzarin (2), uzarigenin, xysmalogenin and syriogenin (3). The presence of fitosterols, proteins and vitamin C was also confirmed (4). The main components of its latex are rubber and resins, as well as oil, waxes, terpens, hydrocarbons and enzymes (6).

In the leaves or/in flowers we identified the following phenolic acids: *p*–hydroxybenzoic, *p*–coumaric, protocatechuic, caffeic, gallic, α–resorcylic, vanilic and chlorogenic acids (7 in press).

Extracts from the leaves and herb or flowers of *A. syriaca* L. are used in folk medicine as expectorants, emetics and antiasthmatics, astringent and antibacterial remedies (8). Tinctures from the common milkweed are used in homeopathy (4).

The aim of this work was isolation and identification of the flavonoids present in flowers of *A. syriaca* L., since according to our knowledge only four flavonoids i.e. pyranoflavone and aglycones: kaempferol, quercetin, isorhamnetin had been identified before (5).

EXPERIMENTAL

Plant material: Flowers of *A. syriaca* were collected from the garden of the Department of Medicinal Plants (K. Marcinkowski University of Medical Sciences, Poznań). A voucher specimen has been deposited at the Department of Pharmacognosy.

Extraction and isolation: The air–dried flowers (650 g) were pulverised and subsequently extracted with hot MeOH. The dry extract was dissolved in H_2O, filtered, washed with $CHCl_3$ and successively extracted with Et_2O, EtOAc, EtOAc/MeOH (8:2). The Et_2O extract was fractionated over cellulose columns by successive elution, first with S_9 next S_{10} and S_{11} and separated by preparative PC with S_3 and S_5 yielded **I** and **IV**.

The EtOAc and EtOAc/MeOH (8:2) extracts were subjected to cellulose columns, eluted with S_{12} and the obtained fractions were separated by preparative PC with S_1, S_4, yielded **IV, VII, X, XIII**. The crude compounds were further purified by CC on Sephadex LH–20 using S_{13}.

Identification

Chromatography, solvent systems:
PC, Whatman No 1 or 3:
S_1 – HOAc–H_2O (3:97); S_2 – HOAc–H_2O (15:85); S_3 – HOAc–H_2O (30:70); S_4 – EtOAc–HCOOH–H_2O (10:2:3) upper phase; S_5 – iso–PrOH–HCOOH–H_2O (2:5:5); S_6 – C_6H_6–HOAc–H_2O (125:72:3);
TLC, Silica gel (Merck):
S_7 – $CHCl_3$–MeOH–H_2O (6:4:1); S_8 – n–PrOH–EtOAc–H_2O (7:2:1);
Chromatograms were analysed in UV_{366} nm before and after spraying with 0.1% NA reagent.
CC, Cellulose Whatman CF11:
S_9 – EtOAc saturated with H_2O; S10 – EtOAc–MeOH–H_2O (100:5:5); S_{11} – EtOAc–MeOH–H_2O (100:10:10); S_{12} – H_2O–MeOH–EtOAc (100:14:10);
CC, Sephadex LH–20 (Pharmacia, Uppsala):
S_{13}–MeOH.
Acid hydrolysis: 2 mg of compounds: 1% HCl, 100°C, 1 h (total); 0.5% HCl, 100°C, $^1/_2$ h (partial), the hydrolysis was monitored by PC in S_2.

The EtOAc extracts of hydrolysates were analysed for aglycones (co–PC in S_5, S_6) whereas the water residues for sugars (co–TLC in S_7, S_8).

Enzymatic hydrolysis with β–glucosidase (Koch–Light): 2 mg of compounds, 1 mg of enzyme in 1 ml of H_2O at room temp., monitored by PC in S_2.

H_2O_2 oxidation: 2 mg of compound **X**; 0.1 M NH_4OH, 2 drops 30% H_2O_2, room temp. 24 h. Products were analysed in S_7, S_8.

Spectral analysis: UV spectra were performed according to the method of Mabry et al. (9), on the Specord UV–VIS spectrometer. ^1H (300 MHz) and ^{13}C (75.5 MHz) NMR spectra were recorded on a Varian Unity–300 instrument with TMS as internal standard.

Quercetin [**I**] yellow needles (3 mg) m.p. 312–314°C, yellow fluorescence in UV.
Rf: 0.11 (S_5), 0.15 (S_6).
UV (MeOH) λ max (nm) 259, (266), 375; +NaOMe 247, 321; +$AlCl_3$ 275, (304), 330, 468; +$AlCl_3$/HCl 265, (301), 357, 425; +NaOAc 272, 329, 395; +NaOAc/H_3BO_3, 263, (303), 387.

Quercetin 7–O–β–glucoside [**IV**] yellow powder (5 mg) m.p. 244–247°C, yellow fluorescence in UV. RF: 0.12 (S_2), 0.31 (S_5).
UV (MeOH) λ max (nm) 259, (267), 378; +NaOMe 291, (241), 367, 456; +$AlCl_3$ 275, (259), (332), 439; +$AlCl_3$/HCl 271, (300), 363, 426; +NaOAc 286, 375, 426; +NaOAc/H_3BO_3 261, (289), 384.
Enzymatic (β–glucosidase) and acid (1% HCl) hydrolysis gave quercetin and glucose (co–PC, TLC).

Quercetin 3–O–β–D–galactopyranoside [**VII**], yellow needles (320 mg) m.p. 230–232°C, brown fluorescence in UV. Rf: 0.33 (S_2), 0.68 (S_4).
UV (MeOH) λ max (nm) (255), 268, (288), 351; +NaOMe 274, 328, 405; +$AlCl_3$ 277, 302, 436; +$AlCl_3$/HCl 274, 297, 360, 403; +NaOAc 275, 325, 389; +NaOAc/H_3BO_3 265, 299, 371.
Acid hydrolysis (1% HCl): quercetin, galactose (co–PC, co–TLC). ^1H and ^{13}C NMR (Table 1 and 2).

Quercetin 3–O–β–D–xylopyranosyl (1→2) β–D–galactopyranoside [**X**] yellow needles (360 mg) m.p. 204–207°C, brown fluorescence in UV. Rf: 0.55 (S_2), 0.30 (S_4).
UV (MeOH) λ max (nm) 255, 263, 365; +NaOMe 274, 420; +$AlCl_3$ 277, 306, 445; +$AlCl_3$/HCl 274, 302, 374, 412; +NaOAc 275, 406; +NaOAc/H_3BO_3, 265, 380.
Total acid hydrolysis (1% HCl): quercetin, galactose, xylose (co–PC, co–TLC).
Partial acid hydrolysis (0.5% HCl): quercetin 3–O–galactoside as secondary heteroside.
H_2O_2 oxidation Rf (TLC): 0.09 (S_7), 0.17 (S_8). ^1H and ^{13}C NMR (Table 1 and 2).

Quercetin 3–O–β–D–glucopyranosyl (1→2) β–D–galactopyranoside [**XIII**] yellow needles (63 mg) m.p. 188–190°C, brown fluorescence in UV. Rf: 0.63 (S_2), 0.35 (S_4).
UV (MeOH) λ max (nm) 254, (260), (330), 360; +NaOMe 271, (320), 410; +$AlCl_3$ 270, (300), 434; +$AlCl_3$/HCl 264, 364, 400; +NaOAc 273, (326), 397; +NaOAc/H_3BO_3 260, 380.
Total acid hydrolysis (1% HCl): quercetin, glucose, galactose (co–PC, co–TLC).
Partial acid hydrolysis (0.5% HCl): quercetin 3–O–galactoside as secondary heteroside.
^1H and ^{13}C NMR (Table 1 and 2).

Table 1. ^1H NMR data of compounds **VII, X, XIII**, DMSO–d_6 + D_2O, δ ppm (J = Hz)

H	Compounds		
	VII	**X**	**XIII**
Quercetin			
6'	7.69 d (2.0)	7.69 d (2.0)	7.68 d (2.0)
2'	7.60 d (2.0)	7.59 d (2.0)	7.59 d (2.0)
5'	6.85 d (8.0)	6.87 d (9.0)	6.87 d (9.0)
8	6.47 d (2.0)	6.46 d (2.0)	6.44 d (2.0)
6	6.24 d (2.0)	6.24 d (2.0)	6.22 d (1.0)
Galactose H–1''	5.35 d (7.0)	5.64 d (8.0)	5.64 d (8.0)
Xylose H–1'''		4.61 d (9.0)	
Glucose H–1'''			4.55 d (9.0)

RESULTS AND DISCUSSION

The methanolic extract from the flowers of *Asclepias syriaca* L. contained fifteen flavonoid compounds.

This mixture was separated on celulose columns then by preparative paper chromatography and purified by CC on Sephadex LH–20. As a result we identified quercetin and their glucosides (**I, IV, VII, X, XIII**). Investigations of other compounds namely the glycosides of kaempferol and isorhamnetin are in progress.

Table 2. ^{13}C NMR data of compounds **VII, X, XIII** (DMSO–d_6, δ ppm)

C	Compounds		
	VII	**X**	**XIII**
Quercetin			
2	156.3	156.2	155.3
3	133.6	133.0	133.0
4	177.4	177.3	177.3
5	161.1	161.1	161.0
6	98.7	98.3	98.5
7	164.3	164.3	163.9
8	93.5	93.3	93.4
9	156.3	156.3	155.3
10	105.5	104.5	104.1
1'	121.1	121.1	121.0
2'	115.1	115.1	115.8
3'	144.7	144.8	144.6
4'	148.4	148.4	148.2
5'	116.0	117.1	115.8
6'	121.9	122.1	122.0
3–Galactose			
1''	102.6	100.5	98.4
2''	71.3	78.7	80.6
3''	73.3	73.8	73.0
4''	67.9	69.4	67.4
5''	75.6	74.1	75.6
6''	60.0	61.2	59.8
2''–Xylose			
1'''		103.9	
2'''		76.0	
3'''		76.2	
4'''		70.1	
5'''		65.6	
2''–Glucose			
1'''			103.9
2'''			74.0
3'''			76.6
4'''			69.6
5'''			76.4
6'''			60.5

The UV spectra indicated the presence of an orthodihydroxyl group at positions C–3' and C–4' in all compounds, a free hydroxyl group at position C–7 in compounds **I, VII, X** and **XIII**, a substituted hydroxyl group at position C–7 OH in compound **IV**.

Compounds **I, IV** basing on their UV spectra, analysis of the products of acid and enzymatic hydrolysis and cochromatogrpahy with standards were identified as quercetin (**I**) and quercetin 7–O–β–glucoside [**IV**].

Compound **VII, X** and **XIII** consisted of quercetin as aglycone; in the sugar part of the compound **VII** was found galactose, of the compound **X** – xylose and galactose, of the compound **XIII** glucose and galactose, what was confirmed by complete hydrolysis.

The partial acid hydrolysis of flavonoid diglicosides [**X, XIII**] gave the secondary heterosides identical with quercetin 3–O–β–galactoside [compound **VII**], what was confirmed by UV analysis and cochromatography of the hydrolysis products with standards.

The ^1H NMR spectra of flavonoids **VII, X** and **XIII** displayed in the aglycone region signals characteristic for 3–O–substituted quercetin (9) with the anomeric proton of galactose at 5.35 ppm in spectrum of compound **VII**; moreover the spectra showed signals at 5.64 ppm for H–1 of galactose [compounds **X, XIII**] and at 4.61 ppm for the H–1 of xylose [compound **X**] and 4.55 ppm for H–1 of glucose [compound **XIII**] (Table 1).

Coupling J=8 Hz for the anomeric signals of galactose and J=9Hz for anomeric proton of D–xylose and D–glucose corresponded to the diaxial coupling, that indicated β–configuration and pyranose form of sugars. Xylose in compound **X**, as well as glucose in compound **XIII**, were linked to C–2'' of galactose as resulted from downfield shifts of the C–2'' signals, i.e. 7.4 ppm for compound **X** and 9.3 ppm for compound **XIII**, and upfield shifts of the C–1'' signals, i.e. 2.1 ppm for compound **X** and 4.2 ppm for compound **XIII**, as compared with the spectrum of quercetin 3–O–galactoside (compound **VII**) (Table 2) (10, 11).

Compound **X** was identified as quercetin 3–O–β–D–xylopyranosyl(1→2)–β–D–galactopyranoside, while compound **XIII** as quercetin 3–O–β–D–glucopyranosyl(1→2)–β–D–galactopyranoside.

Quercetin 3–O–galactosides and 7–O–glucosides have often been found in plant material. However, flavonoids containing xylose in the sugar part are rare

in nature. The flavonoids with galactose and xylose were described for the first time in the leaves of *Lysichiton camtschatcense* L., but the interglycosidic linkage has not been established (12). The structure of O–β–D–xylopyranosyl(1→2)–β–D–galactopyranose as lathyrose was confirmed by Harborne, who studied two flavonoids isolated from the petals of *Lathyrus odoratus* L. (13, 14).

Lathyrose also constitutes the sugar part of cyanidin glycosides found in the shoots and fruits of *Aralia elata* Thum (15), as well as in the fruits of *Fatsia japonica* L. (16). Furthermore, quercetin 3–lathyroside was isolated from the leaves of *Armoracia rusticana* G.M.Sch. (17).

On the other hand, quercetin 3–O–β–D–glucopyranosyl(1→2)β–D–galactopyranoside was isolated from the pollen of *Corylus avellana* (18) and *Phytolacca thyrsiflora* (19) and now from the flowers of *A. syriaca* L.

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