

KAEMPFEROL AND ITS GLYCOSIDES IN THE SEEDS HAIR OF *ASCLEPIAS SYRIACA* L.

MARIA SIKORSKA, IRENA MATŁAWSKA and RAFAŁ FRAŃSKI¹

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

¹ Faculty of Chemistry, A. Mickiewicz University,
6 Grunwaldzka Str., 60-780 Poznań, Poland

Abstract: The following flavonoid compounds have been isolated and identified from the seeds hair of *Asclepias syriaca* L.: kaempferol, kaempferol 3-O-β-galactopyranoside, kaempferol 3-O-β-xylopyranosyl (1→2)-β-galactopyranoside, kaempferol 3-O-β-glucoxyranosyl (1→2)-β-galactopyranoside, kaempferol 3-O-α-rhamnopyranosyl (1→2)-β-galactopyranoside. Their structures were established by acid hydrolysis, H₂O₂ oxidation and spectral analysis (UV, ¹H, ¹³C NMR, MS).

Keywords: *Asclepias syriaca* L., seeds hair flavonoids, kaempferol glycosides.

Asclepias syriaca L. (Asclepiadaceae) – common milkweed, grows naturally in North America, it is cultivated in Europe as a garden plant (1, 2).

The presence of the cardenolides in all parts of the plant (3, 4, 5) were determined. Seeds hair are a source of cellulose fiber and contain: α-,β-, γ-cellulose, lignin and hemicellulose (6). Our previous studies on the *A. syriaca* L. showed the presence of glycosides of quercetin (7) kaempferol and isorhamnetin (8) in the flowers, whereas phenolic acids (9) we found both, in the leaves and flowers of this plant.

In this paper we report on the isolation and identification of the flavonoids present in the seeds hair of *Asclepias syriaca* L.

EXPERIMENTAL

Plant material

Asclepias syriaca L. seeds hair were collected in the Botanic Garden of A. Mickiewicz University, Poznań, Poland. A voucher specimen has been deposited at the Department of Pharmacognosy.

Extraction and isolation

Seeds hair (45.8 g) were extracted with hot MeOH (6 x) and 50% MeOH (5 x). The extracts were combined, concentrated, and subjected to CC (cellulose Whatman CF 11). The column was eluted with S₇ (compounds **I**, **II** and a mixture of **III**, **IV**, **V**). The fractions containing **III**, **IV** and **V** were separated by preparative PC (Whatman No. 3, S₁ and S₂), yielded compound **III**, **IV** and **V**.

Purification of **I–V** was carried out on CC (Sephadex LH-20), eluted by MeOH.

Identification

Chromatography, solvent systems:

PC, Whatman No. 1 or 3: S₁ – HOAc–H₂O (15:85); S₂ – EtOAc–HCOOH–H₂O (10:2:3) upper phase; S₃ – iso-PrOH–HCOOH–H₂O (2:5:5); S₄ – C₆H₆–HOAc–H₂O (125:72:3)

TLC, Silica gel (Merck):

S₅ – n-PrOH–EtOAc–H₂O (7:2:1); S₆ – CHCl₃–MeOH–H₂O (6:4:1)

CC, Cellulose Whatman CF 11:

S₇ – EtOAc–MeOH–H₂O (100:6:20) upper phase
CC, Sephadex LH-20 (Pharmacia, Uppsala):

S₈ – MeOH

Acid hydrolysis: 1% HCl, 100°C, 1 h (total); 0.5% HCl, 100°C, 1/2 h (partial), the hydrolysis was monitored by PC in S₁.

The products of hydrolysis (aglycones and secondary heterosides) were identified by co-PC (Whatman No. 1) with the standards in solvent systems S_{1–4}.

Sugars, obtained on hydrolysis, were analysed by co-TLC (Silica gel, Merck) with the standards in solvent systems S₅, S₆. The spots of sugars were visualized by spraying with aniline hydrogen phthalate and heating at 105°C.

H₂O₂ oxidation: 2 mg of compound **III**, 0.1M NH₄OH, 2 drops of 30% H₂O₂, room temp., 24 h. Products were analysed in S₅, S₆.

Spectral analysis:

UV spectra were performed on a Specord UV-VIS spectrometer according to the methods by Mabry et al. (10).

¹H (300 MHz) and ¹³C (75.5 MHz) NMR spectra were recorded on a Varian Unity-300 instrument with TMS as internal standard.

MS spectrometry

LSI mass spectra were performed on an AMD 604 (Germany) mass spectrometer, which was fitted with a cesium gun (12 keV) and an accelerating voltage of 8 kV. Samples were dissolved in glycerol as a matrix. GC/MS analyses were performed on a Hewlett-Packard Model 5890II gas chromatograph with a Model 5971 mass-selective detector. An XTI-5 fused-silica capillary column (30 m × 0.25 mm i.d.) (Restek, USA) and helium as carrier gas were used. The column temperature was programmed from 140°C (2 min) at 5°C min⁻¹ to 300°C (5 min). Standards of kaempferol, galactose and rhamnose were obtained from Sigma Chemical (USA). Methylation, methanolysis and acetylation were performed according to the procedure described elsewhere (14, 15).

Kaempferol [I] dull yellow needles (1.5 mg) m.p. 276–278°C, yellow fluorescence in UV₃₆₆ PC Rf: S₃=0.20; S₄=0.51

UV λ max MeOH: 266, (194), (323), 366; +NaOMe 278, 316, 419; +AlCl₃ 268, (302), 350, 423; +AlCl₃/HCl 269, (302), 347, 425; +NaOAc 277, 302, 388; +NaOAc/H₃BO₃ 266, (297), (319), 323 nm.

Kaempferol 3-O-β-galactopyranoside [II] dull yellow needles (42 mg) m.p. 241–243°C, brown fluorescence in UV, PC Rf: S₁=0.36, S₂=0.80

UV λ max MeOH: 265, 352; +NaOMe 277, 327, 405; +AlCl₃ 275, (305), 348, 398; +AlCl₃/HCl 275, (302), 346, 397; +NaOAc 274, 305, 373; +NaOAc/H₃BO₃ 266, 353 nm.

Acid hydrolysis (1% HCl): kaempferol, galactose (co-PC, TLC)

¹H and ¹³C NMR Table 1 and 2

Kaempferol 3-O-β-Xylopyranosyl (1→2)-β-galactopyranoside [III] yellow needles (62 mg) m.p. 205–208°C, brown fluorescence in UV, PC Rf: S₁=0.57; S₂=0.35

UV λ max MeOH: 266, (300), 355; +NaOMe 277, 327, 400; +AlCl₃ 275, (305), 355, 409; +AlCl₃/HCl 277, (303), 349, 408; +NaOAc 274, (313), 397; +NaOAc/H₃BO₃ 270, (305), 360 nm.

Acid hydrolysis total (1% HCl): kaempferol, galactose, xylose. Acid hydrolysis partial (0.5% HCl) and/whereas kaempferol 3-O-galactoside as a secondary heteroside (co-PC, TLC)

H₂O₂ oxidation TLC: Rf S₅=0.17; S₆=0.09

¹H and ¹³C NMR Table 1 and 2

Kaempferol 3-O-β-glucopyranosyl (1→2)-β-galactopyranoside [IV], dull yellow needles (32 mg) m.p. 182–184°C, brown fluorescence in UV, PC Rf: S₁=0.65; S₂=0.38

UV λ max MeOH: 265, (300), 354; +NaOMe 275, 325, 406; +AlCl₃ 275, (305), 356, 410; +AlCl₃/HCl 276, (305), 347, 408; +NaOAc 276, (315), 396; +NaOAc/H₃BO₃ 266, 380 nm.

Acid hydrolysis total (1% HCl): kaempferol, galactose, glucose. Acid hydrolysis partial (0.5% HCl) and kaempferol 3-O-galactoside as secondary heteroside (co-PC, TLC)

¹H and ¹³C NMR Table 1 and 2.

Table 1. ¹H NMR data of compounds **II**, **III**, **IV** and **V** (DMSO-d₆ + D₂O, δ ppm, J=Hz)

H	II	III	IV	V
Kaempferol				
6	6.05 d (2.0)	6.23 d (2.0)	6.22 d (2.0)	6.17 d (2.0)
8	6.25 d (2.0)	6.48 d (2.0)	6.40 d (2.0)	6.39 d (2.0)
2'	8.01 d (8.0)	8.12 d (8.0)	8.15 d (8.0)	8.07 d (8.3)
3'	6.86 d (8.3)	6.90 d (8.0)	6.80 d (8.0)	6.85 d (8.3)
5'	6.86 d (8.3)	6.90 d (8.0)	6.80 d (8.0)	6.85 d (8.3)
6'	8.01 d (8.0)	8.12 d (2.0)	8.15 d (8.0)	8.07 d (8.3)
1''' galactose	5.32 d (7.0)	5.64 d (8.0)	5.63 d (8.0)	5.64 d (7.3)
1''' xylose		4.57 d (9.0)	–	
1''' glucose			4.51 d (9.0)	
1''' rhamnose				5.04 s
CH ₃ of rhamnose				0.73 d (5.4)

Table 2. ^{13}C NMR data of compounds **II**, **III**, **IV** (DMSO- d_6 , δ ppm)

C	II	III	IV
Kaempferol			
2	156.4	156.9	156.2
3	132.6	134.3	133.6
4	177.5	177.3	177.7
5	161.2	161.0	161.5
6	98.9	98.2	98.5
7	165.0	164.5	164.4
8	93.8	93.5	93.9
9	156.5	156.1	156.5
10	103.2	104.2	104.4
1'	120.8	120.6	120.8
2'	130.6	131.6	131.5
3'	115.2	115.8	115.5
4'	159.7	159.3	160.0
5'	115.2	115.8	115.5
6'	130.6	130.8	130.7
3-Galactose			
1''	102.6	98.8	98.7
2''	71.5	78.9	80.6
3''	73.4	74.0	73.5
4''	68.3	69.4	67.9
5''	75.5	74.4	76.0
6''	60.4	61.1	61.1
2''Xylose			
1'''		104.2	
2'''		75.8	
3'''		76.4	
4'''		70.1	
5'''		66.0	
2''Glucose			
1'''			104.1
2'''			74.6
3'''			76.8
4'''			70.0
5'''			77.2
6'''			60.2

Kaempferol 3-O- α -rhamnopyranosyl (1 \rightarrow 2)- β -galactopyranoside [V], yellow needles (18 mg) m.p. 202–204°C, brown fluorescence in UV, PC Rf: $S_1=0.67$; $S_2=0.63$

UV λ max MeOH: 262, (300), 350; +NaOMe 270, 322, 395; +AlCl₃ 272, (300), 345, 405; +AlCl₃/HCL 264, (300), 350, 403; +NaOAc 271, (300), 349; +NaOAc/H₃BO₃ 265, (320), 350 nm.

Acid hydrolysis total (1% HCl): kaempferol, galactose, rhamnose. Acid hydrolysis partial (0.5% HCl) and kaempferol 3-O-galactoside as second

dary heteroside (co-PC, TLC) ^1H NMR Table 1, MS spectrometry Table 3, Figures 1–5.

RESULTS AND DISCUSSION

Opposite to the flowers of *A. syriaca* L. from which we have isolated many of the derivatives of quercetin, isorhamnetin and kaempferol; the seeds hair contained only derivatives of kaempferol. Basing on the UV spectra and chromatography with standards, compound **I** with yellow fluorescence under UV₃₆₆ nm was identified as kaempferol.

Compounds **II**, **III**, **IV** were identified by UV, ^1H and ^{13}C NMR analysis as the 3-O-kaempferol derivatives: 3-O- β -galactopyranoside; 3-O- β -xylopyranosyl (1 \rightarrow 2) β -galactopyranoside; 3-O- β -glucopyranosyl (1 \rightarrow 2) β -galactopyranoside respectively. They were identical with the compounds **VIII**, **XI**, **XIV**, isolated earlier from the flowers of *A. syriaca* (8). Compound **V** released galactose, rhamnose and kaempferol on hydrolysis. The attachment of the sugars in C-3 was established by the UV spectra.

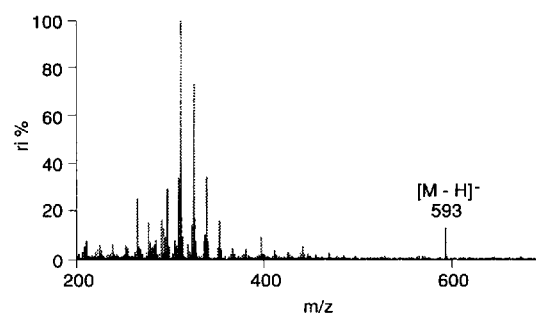
Signals in the ^1H NMR spectra of **II**, **III**, **IV** and **V** were in agreement with the literature date for kaempferol-3-O-substituted (11).

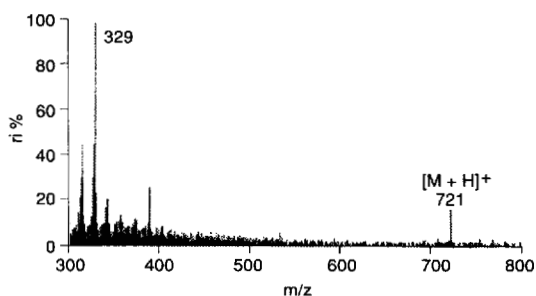
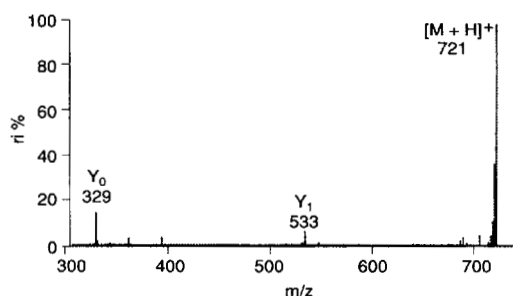
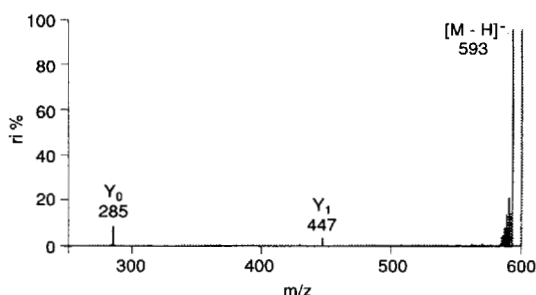
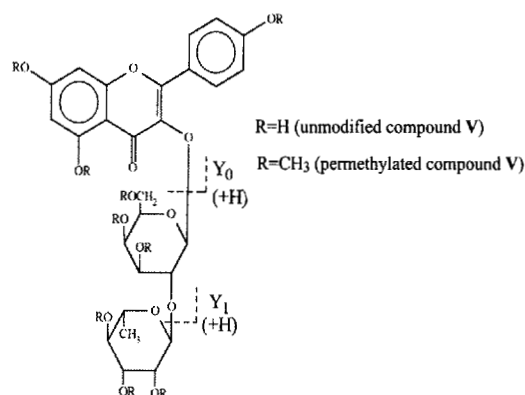
Doublets at δ 5.32 ppm, $J=7.0$ Hz (compound **II**); 5.64 ppm, $J=8.0$ Hz (compound **III**); 5.63 ppm, $J=8.0$ Hz (compound **IV**) and 5.64 ppm, $J=7.3$ Hz (compound **V**) were assigned to galactose in pyranose forms β -linked to the kaempferol moiety.

Signals of the terminal sugars in compounds **III** and **IV** appeared as the doublets at 4.57 ppm, $J=9.0$ Hz for xylose and 4.51 ppm, $J=9.0$ Hz for glucose respectively, indicated β -configurations and pyranose forms.

Singlet at 5.04 ppm and doublet at δ 0.73 ppm, $J=5.4$ Hz corresponded with anomeric proton of the α -rhamnose in pyranose form (12).

The linkage of xylose (compound **III**) and glucose (compound **IV**) to the C-2 position of the

Figure 1. LSI mass spectrum of unmodified compound **V**.

Figure 2. LSI mass spectrum of permethylated compound **V**.Figure 4. B/E mass spectrum of permethylated compound **V**.Figure 3. B/E mass spectrum of unmodified compound **V**.Figure 5. Fragmentation pattern of compound **V**.

galactose moiety was determined on the basis of ^{13}C NMR spectra. Comparison of the ^{13}C NMR spectra of compounds **III** and **IV** with kaempferol 3-O- β -galactopyranoside (compound **I**) revealed downfield shifts of the galactose carbons for compounds **III** and **IV** at C-2 (7.4 and 9.1 ppm respectively) Table 2 (13).

Because of the fact that compound **V** was obtained in too small amount for recording ^{13}C NMR spectra, we decide to use MS spectrometry, to elucidate interglycosidic linkage. We use various mass spectrometric techniques according to methodological approach, described elsewhere (14, 15). For registration of normal and B/E mass spectrum of unmodified and permethylated compound **V** desorption ionization (LSIMS) was used. Normal mass spectrum of this compound, recorded in negative mode, allowed to define its molecular mass, based on the $m/z=593$ value of deprotonated molecule $[\text{M}-\text{H}]^-$ (Figure 1).

Mass spectrum of the permethylated sample was recorded in positive mode producing protonated molecular ion $[\text{M}+\text{H}]^+$ (Figure 2).

The difference between the molecular mass of the unmodified and permethylated compound **V** indicated the presence of nine free hydroxyl group:

three of the flavonoid skeleton, three of the rhamnose and three of the galactose moiety.

The B/E linked-scan mass spectra gave structural information which was not obtainable from normal LSI spectrum. In the B/E mass spectrum of $[\text{M}-\text{H}]^-$ unmodified compound (Figure 3) and $[\text{M}+\text{H}]^+$ permethylated compound (Figure 4) fragment ions of Y_1 and Y_0 type were observed (nomenclature proposed for glycoconjugate fragmentation according to Domon and Costello (16)).

That permitted the definition of the type of sugar moiety: deoxyhexose (rhamnose) and hexose (galactose) and the mass of aglycone (Figure 5).

The m/z value of Y_1 ions correspond to the elimination of the deoxyhexose (rhamnose) ring and Y_0 ions correspond to the mass of aglycone, deprotonated aglycone for unmodified compound and protonated aglycone in the case of permethylated conjugate. Differences between Y_1 and Y_0 indicate the presence of the hexose (galactose).

Methylated sample after methanolysis and further derivatization (methylation or acetylation) was

Table 3. Conjugates identified after GC/MS analysis of methylated, methanolyses and *de novo* methylated^a or acetylated^b compound V.

Conjugate	Retention time (min)
3,4',5,7-Tetra-O-methylkaempferol ^a	32.06
1,2,3,4,6-Penta-O-methylgalactopyranoside ^a	4.15/4.75
1,2,3,4,-Tetra-O-methylrhamnopyranoside ^a	2.59/2.82
3-O-Acetyl-4',5,7-Tetra-O-methylkaempferol ^b	34.25
2-O-acetyl-1,3,4,6-Penta-O-methylgalactopyranoside ^b	6.75/7.54
1,2,3,4,-Tetra-O-methylrhamnopyranoside ^b	2.59/2.82

analysed with gas chromatography/mass spectrometry (Table 3).

GC/MS analyse of the methylated products of methanolysis (this sequence of chemical reactions led to permethylated derivatives of sugars and aglycone) permitted objective identification of sugars and aglycone by comparison of the mass spectra and retention times which were identical as for respective standards of permethylated derivatives of kaempferol, galactose and rhamnose. Information about glycosidic linkages between sugars and substitution of sugars to aglycone was obtained following GC/MS analyse of methanolized and acetylated permethylated compound V. Diagnostic fragment ions were registered in the mass spectra of the mono-acetylated methyl ether of galactose and kaempferol as it was described elsewhere (14, 15). The presented above data allow to draw the conclusion that compound V is kaempferol 3-O- α -rhamnopyranosyl (1 \rightarrow 2) β -galactopyranoside.

Compound with this structure was found previously from the leaves and stems of *Blackstonia perfoliate* (L.) Hudson (17) and from the whole plant of *Lisymachia mauritiana* (18).

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