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SHORT COMMUNICATIONS

RUTINOSIDES AND VICIANOSIDES OF QUERCETIN, APIGENIN AND LUTEOLIN FROM *SYMPHORICARPUS ALBUS* L. BLAKE (*CAPRIFOLIACEAE*)

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Symphoricarpus albus (L.) Blake is used in folk medicine as emetics, laxatives and antiinflammatory agents, also in homeopathy. The fruits are toxic; especially for little children even after eating several fruits (1).

The presence of chlorogenic, quinic, aminobutyric and glutamic acids as well as serine, sugars, pectins, and also malic, tartaric and citric acids was confirmed in the investigated species (1). Sangwinarin, chelidonin and choline were also present. Triterpenoid alcohols, like lanosterol, α– and β–amyrin also free sterols (their esters, glycosides and acylated glycosides), ursolic, 2–α–hydroxyursolic and oleanolic acids as well as alkanes and aliphatic alcohols were found in the petroleum ether fraction from the fruits. The leaves contained coumarins: fraxin, esculin, and iridoids: i.e. secologanin (2, 3, 4).

Our previous studies (4) led to identification (co–chromatogrpahy, hydrolysis, UV spectra, ^{1}H NMR spectra for comounds **D, K, F**) of the following flavonoid compounds: quercetin, apigenin, luteolin, quercetin 3–O–glucoside, apigenin 7–O–glucoside, luteolin 7–O–glucoside, quercetin 3–O–rhamnosylglucoside, quercetin 3–O–arabinosylglucoside, apigenin 7–O–rhamnosylglucoside, apigenin 7–O–arabinosylglucoside, and also luteolin 7–O–rhamnosylglucoside and luteolin 7–O–arabinosylglucoside (without determination of interglycosidic linkages between sugar units).

Herein presented analysis of ^{1}H and ^{13}C NMR spectra of all isolated flavonoid diglycosides enables their full identification.

EXPERIMENTAL

Plant material

The fruits of *Symphoricarpus albus* (L.) Blake were collected from the PAN Arboretum in Kórnik in 1995. A voucher specimen is deposited in the author's laboratory.

Extraction, isolation and identification

The fresh fruits (5 kg) were extracted with hot methanol (6x) and the extract was concentrated, treated with hot water and filtered. Six flavonoid diglycosides marked as **D, K, H, J. F** and **G** were isolated from the methanolic extract from the fruits of *Symphhoricarpus albus* (L.) Blake using the previously described method, identified by classical methods, chromatography, hydrolyses and analysis of their UV spectra and ^{1}H NMR for compounds **D, K, F** (4).

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were registered on a Varian Unity 300 apparatus in d_6–DMSO with TMS as internal standard. The ^{1}H NMR spectra were also recorded with the addition of D_2O. Chemical shifts are given in ppm (Table 1 and 2).

RESULTS AND DISCUSSION

The present investigations by ^{1}H and ^{13}C NMR of flavonoid diglycosides **D, K, H, J, F, G** were aimed identification of these comounds including determination of the interglycosidic linkages.

The ^{1}H and ^{13}C NMR spectra (Table 1 and Table 2) showed the expected signals in the aromatic region for the luteolin (**D** and **K**), quercetin (**H, J**) and apigenin moiety in the flavonoids **G** and **F**.

In the ^{1}H NMR spectra (Table 1) of compounds **D, F** and **H**, two signals were observed in the region characteristic for anomeric protons of sugars. Doublets at δ 5.07 ppm (J = 7.23 Hz, compound **D**), 5.06 ppm (J = 7.19 Hz, compound **F**) and 5.34 ppm (J = 7.60 Hz, compound **H**) were assigned to glucose β–linked to the aglycone. Signals at δ 4.55 ppm (J = 1.20 Hz, compound **D**), 4.55 ppm (J = 1.05 Hz, compound **F**) and 4.39 ppm (J = 1.26 Hz, compound **H**) corresponded to the anomeric proton of the α–linked rhamnose (5, 6).

In the ^{13}C NMR spectra (Table 2) of compounds **D, F** and **H** signals corresponding to the anomeric carbons of glucose were found at δ 99.76; 99.81 and 100.63 ppm, respectively, and those corresponding to rhamnose were seen at 100.42; 100.43 and 101.06 ppm. A downfield shift of 5–6 ppm of the C–6 glucose signal accompanied by a minor (0.3–0.4 ppm) upfield shift of the C–5 glucose signal shows that the anomeric carbon of rhamnopyranose is linked to the C–6 position of the glucopyranosyl residue which is in accordance with reported data for rutinosides (5, 6). Compound **D** is therefore considered to be luteolin 7–O–α–L–rhamnopyranosyl(1→6)–β–D–glucopyranoside, **F** – apigenin 7–O–α–L–rhamnopyranosyl (1→6)–β–D–glucopyranoside and **H** – quercetin 3–O–α–L–rhamnopyranosyl(1→6) –β–D–glucopyranoside.

In the ^{1}H NMR spectra (Table 1) of compounds **K, G** and **J** doublets of the anomeric protons of β–D–glucopyranose were seen at δ 5.10 (J = 7.20 Hz), 5.06 (J = 7.19 Hz) and 5.34 ppm (J = 7.60 Hz), respectively. The anomeric protons of the terminal sugar were observed as doublets at δ 4.18, 4.20 and 3.95 ppm for compounds **K, G** and **J**; their coupling constants matched the values for α–L–arabinopyranose (7, 8) and amounted to J = 5.99 Hz, J = 5.94 Hz and J = 6.81 Hz, respectively; those for arabinofuranose would have been in a 1–2 Hz range (7, 9, 10, 11).

The ^{13}C NMR spectra of compounds **K, G** and **J** also showed that the β–D–glucopyranose unit was directly attached to the aglycone and α–L–arabinopyranose was a terminal sugar (Table 2). A significant downfield shift of about 3–4 ppm for the C–6 signal of glucose revealed that the interglycosidic linkage was in this position, like in vicianosides. The carbon signals of arabinose were in

Table 1. ^{1}H NMR spectral data of flavonoids **D, K, H, J, F** and **G** in d_6–DMSO δ (ppm), J (Hz)

Proton	D	K	H	J	F	G
6'	7.46 *dd* 2.28; 10.71	7.48 *dd* 2.15; 10.64	7.56 *dd* 2.26; 10.00	7.60 *dd* 2.08; 9.45	7.96 *d* 8.90	8.00 *d* 8.85
2'	7.42 *d* 2.15	7.45 *d* 2.25	7.56 *dd* 2.26; 10.00	7.60 *dd* 2.08; 9.45	7.96 *d* 8.90	8.00 *d* 8.85
5'	6.95 *d* 8.30	6.92 *d* 8.30	6.86 *d* 8.59	6.88 *d* 8.76	6.98 *d* 8.90	6.98 *d* 8.85
3'					6.98 *d* 8.90	6.98 *d* 8.85
8	6.76 *d* 2.15	6.80 *d* 2.14	6.41 *d* 1.98	6.43 *d* 1.94	6.79 *d* 2.0	6.84 *d* 2.21
3	6.75 *s*	6.76 *s*			6.86 *s*	6.87 *s*
6	6.47 *d* 2.00	6.46 *d* 2.11	6.22 *d* 2.11	6.22 *d* 2.09	6.46 *d* 2.21	6.45 *d* 2.07
Glc–1''	5.07 *d* 7.23	5.10 *d* 7.20	5.34 *d* 7.60	5.37 *d* 7.37	5.06 *d* 7.19	5.08 *d* 7.19
Rha–1'''	4.55 *d* 1.20		4.39 *d* 1.26		4.55 *d* 1.05	
Ara–1'''		4.18 *d* 5.99		3.95 *d* 6.81		4.20 *d* 5.94
Glc–6''	3.86 *br d* 10.31	3.98 *br d* 10.2	3.70 *br d* 9.17	3.90 *br d* 10.1	3.96 *br d* 10.01	3.96 *br d* 9.95
Rha–Me	1.07 *d* 6.2		1.00 *d* 6.2		1.07 *d* 6.20	
2'', 3'', 4'', 5'', 2''', 3''', 4''', 5'''	3.66–3.12 *m*	3.70–3.28 *m*	3.39–3.04 *m*	3.51–3.09 *m*	3.87–3.28 *m*	3.64–3.25 *m*

Table 2. ^{13}C NMR spectral data of flavonoids **D, K, H, J, F** and **G** in d_6–DMSO, δ (ppm)

Carbon	D	K	H	J	F	G
Aglycone						
4	181.76	181.78	177.24	177.25	181.87	181.89
2	164.46	164.46	156.48	156.24	164.27	164.31
7	162.76	162.78	163.94	164.00	162.78	162.78
5	161.12	161.01	161.11	161.13	161.08	161.35
9	156.79	156.88	156.29	156.17	156.82	156.89
4'	149.83	149.81	148.28	148.38	161.21	160.94
3'	145.63	145.63	144.63	144.70	115.97	115.95
1'	121.27	121.27	121.48	121.48	120.94	120.83
6'	119.12	119.18	121.06	121.48	128.53	128.58
5'	115.96	115.93	116.15	116.05	115.97	115.95
2'	113.45	113.50	115.10	115.15	128.53	128.58
10	105.27	105.30	103.85	103.91	105.29	105.31
3	103.08	103.03	133.18	133.18	130.02	102.89
6	99.41	99.50	98.55	98.54	99.45	99.57
8	94.65	94.61	93.46	93.36	94.69	94.65
Glc						
1''	99.76	99.75	100.63	100.70	99.81	99.78
2''	72.99	72.96	73.95	73.80	72.98	72.95
3''	75.46	75.47	75.79	76.19	75.53	75.52
4''	70.63	69.33	70.44	69.96	70.65	69.67
5''	76.16	76.05	76.32	76.75	76.18	76.11
6''	65.93	67.46	66.88	67.16	65.96	67.48
Rha						
1'''	100.42		101.06		100.43	
2'''	70.19		70.26		70.23	
3'''	69.45		69.88		69.48	
4'''	71.93		71.73		71.96	
5'''	68.23		68.13		68.23	
6'''	17.69		17.62		17.71	
Ara						
1'''		102.97		102.59		102.89
2'''		72.33		72.35		72.33
3'''		70.37		70.36		70.37
4'''		67.01		67.16		66.88
5'''		64.45		64.71		64.95

agreement with published data for α–L–arabinopyranose (7, 8, 9); if the sugar had been in the furanose form the chemical shifts would have had appeared at 108.5, 83.9, 82.0, 77.2 and 61.3 ppm (9, 10, 11). Thus compound **K** is luteolin 7–O–α–arabinopyranosyl(1→6)–β–D→glucopyranoside, **G** is apigenin 7–O–α–L–arabinopyranosyl(1→6)–β–D–glucopyranoside, and **J** is quercetin 3–O–α–L–arabinopyranosyl(1→6)–β–D–glucopyranoside.

Arabinose, as the part of a sugar unit of flavonoid O–diglycosides exists more often in the furanose (10, 11, 12) than in pyranose form (8, 13). The respective flavonoid arabinofuranosides and arabinopyranosides are distinguishable by comparing both their Rf values on chromatograms chemical shifts and coupling constants in ^{1}H and ^{13}C NMR spectra (7, 9, 14, 15).

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