Pergamon

PII: S0031-9422(97)00091-5

Phytochemistry, Vol. 45, No. 5, pp. 1063–1067, 1997

0031-9422/97 $17.00+0.00

QUERCETIN GLYCOSIDES FROM EUROPEAN AQUATIC *RANUNCULUS* SPECIES OF SUBGENUS *BATRACHIUM*

K. GLUCHOFF-FIASSON,*† J. L. FIASSON† and H. WATON‡

†Laboratoire de Biologie Micromoléculaire et Phytochimie, Université Lyon-I, 43 Bd. 11 Nov., F-69622 Villeurbanne Cédex, France; ‡Service Central d'Analyses du C.N.R.S., B.P. 22, F-69390 Vernaison, France

(*Received in revised form* 25 *November* 1996)

Key Word Index—*Ranunculus*; *Batrachium*; Ranunculaceae; quercetin glycosides; acylated quercetin glycosides; quercetin 3-(6″-malonylglucoside)-7-glucoside.

Abstract—A chemical investigation of the leaves of aquatic *Ranunculus* species yielded six quercetin glycosides and eight acylated quercetin glycosides, three of which are reported for the first time: quercetin 3-(6″-malonylglucoside)-7-glucoside and the partially characterized quercetin 3-caffeylsophoroside-7-caffeylglucoside and quercetin 3-caffeylsophoroside-7-ferulylglucoside. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In the course of a chemotaxonomic study of European representatives of the genus *Ranunculus* subgenus *Batrachium*, several leaf flavonoid chemotypes were identified [1]. A previous investigation of this aquatic group [2] based only on R_f values and spot colours revealed the presence of quercetin glycosides, but none were fully identified. In the present paper we describe the structural elucidation of 12 of these glycosides and partial characterization of two minor constituents.

RESULTS AND DISCUSSION

The 14 quercetin glycosides isolated from *Batrachium* species are listed in Table 1.

The similarity between the chromatographic behaviour, UV spectral properties, hydrolysis products and $[M]^+$ values from electrospray mass spectrometry (ES-MS) (Tables 2–4) of compounds **1**, **2**, **5**, **6** and **8** and those of flavonoids from Antarctic *Ranunculus* species [3] led us to assign them the structures presented in Table 1. The structure of **8**, which was previously suggested to be quercetin 3-sophoroside [3], has now been confirmed by ^1H and ^{13}C NMR spectral data (Tables 5 and 6). Thus, the shifts of the glucose anomeric protons as well as the downfield shift of the C-2″ prove the (1 → 2) linkage within the diglucoside at position 3.

UV spectral properties of compounds **11** and **14**, as well as obvious differences in chromatographic data, compared with those of **2**, **5** and **6**, indicated that they were also quercetin 3,7 polyglycosides esterified by at least one additional cinnamic acid (Tables 2 and 3). Indeed alkaline hydrolysis of **11** and **14** yielded quercetin 3-sophoroside-7-glucoside (**1**) and caffeic acid, and **1**, caffeic + ferulic acids, respectively (Table 4).

Table 1. Free and acylated quercetin glycosides from *Batrachium* sp.

	Number on HPLC
Triglycosides	
Quercetin 3-sophoroside-7-glucoside	1
Quercetin 3-(2‴-caffeylsophoroside)-7-glucoside	2
Quercetin 3-ferulylsophoroside-7-glucoside	5
Quercetin 3-*p*-coumarylsophoroside-7-glucoside	6
Quercetin 3-caffeylsophoroside-7-caffeylglucoside	11
Quercetin 3-caffeylsophoroside-7-ferulylglucoside	14
Quercetin 3-rutinoside-7-glucoside	4
Diglycosides	
Quercetin 3-sophoroside	8
Quercetin 3-(2‴-caffeylsophoroside)	9
Quercetin 3-glucoside-7-glucoside	3
Quercetin 3-(6″-malonylglucoside)-7-glucoside	7
Quercetin 3-rutinoside	10
Monoglycosides	
Quercetin 3-glucoside	12
Quercetin 3-(X″-malonylglucoside)	13

* Author to whom correspondence should be addressed.

Table 2. Chromatographic and ES-mass spectrometry data of quercetin glycosides (**1**–**14**)

Compound	TLC $R_f \times 100$ in: P1*	P2*	C1†	C2†	C3†	C4†	HPLC‡ R_t (min)	ES-MS $[M]^+$
1	24	86	6	76			21.6	788
2	16	71	28	69	69	54	26	950
3	38	71	26	48	38	23	32.8	626
4	41	80	9	66			33.6	772
5	41	80	33	79			34.4	964
6	28	71	41	75			35.8	934
7	1	14	22	53	45	94	40	712
8	43	60	45	56	44	27	52	626
9	22	34	51	61	55	37	53.2	788
10	51	44	46	45			61.8	610
11	11	40	38	43			64.8	1112
12	54	25	57	26	12	6	65.6	464
13	4	0	81	36	22	76	72	550
14	25	40	47	47			75.4	1126

*On polyamide TLC 6; P1: toluene–MeOH–MeCOEt–*n*-BuOH (2:4:4:0.05): twice; P2: H_2O–EtOH–MeCOEt–CH_2AC_2 (13:3:3:1).

†On cellulose TLC; C1: *n*-BuOH–HOAc–H_2O (12:3:5); C2: HOAc–H_2O (1.5:8.5); C3: HOAc–H_2O (0.5:9.5); C4: H_2O.

‡HPLC: reverse phase hypersil ultrabase C18 UB 225 μm, L: 25 cm; 70 min gradient from 1.5–36% of acetonitrile in water in presence of 2% HOAc, flux 0.65 ml min^{-1}, detection at 340 nm.

Table 3. UV spectral properties (λ_{max} nm) of quercetin glycosides (**1**–**14**)

Compound	MeOH	NaOAc "+H_3BO_3	$AlCl_3$ "+HCl	NaOH
1, **3**, **4**, **7**	256, (267), 357	262, 408 260, 378	275, (330), 435 269, (300), (360), 400	269, 400
2	255, (266), (300), 339	260, 384 258, 360	274, (300), 359, 438 273, 297, 339, 402	271, 387
5	254, (266), (300), 334	257, 384 257, 331, 375		
6	256, (266), 318, (354)	259, 372 257, (309), 354	275, 300, (314), 434 275, 300, 321, 399	271, 369
8, **10**, **12**, **13**	257, (266), (300), 358	270, (312), 400 260, (307), 380	274, (300), (327), 434 269, (296), (360), 400	271, 320, 410
9	255, (266), (300), 339	267, (300), 382 255, (300), 359	274, 304, 359, 433 274, 300, 337, 400	272, 313, 396
11	253, (266), 332			271, 388
14	253, (266), 332	258, 378 257, 297, 345	276, 300, 329, 436 276, 300, 327, 401	267, 382

ES-MS (Table 2) provided M_r indicating that **11** and **14** were a dicaffeoyltriglucoside and a caffeoylferulytriglucoside, respectively. β-Glucosidase hydrolysis left both compounds unchanged unlike quercetin 3-(2‴-caffeylsophoroside-7-glucoside (**2**), indicating that a second acyl group was attached to the glucose at position 7. Although the amounts isolated did not allow further characterization, it seems likely, in the biosynthetic context, that **11** and **14** derived from **2** by a further caffeylation and ferulylation of the 7-glucose, respectively.

Compound **9** showed the same UV spectrum as **2** but gave diagnostic shifts indicative of a 3-substituted quercetin (Table 3). Alkaline hydrolysis of **9** yielded caffeic acid and a compound identical to **8** (Table 4). ES-MS confirmed **9** as a monocaffeyldiglucoside (Table 2). ^1H NMR of **9** (Table 5) validated these conclusions and also indicated, from the vicinal coupling constants of the anomeric protons, a β-configuration for the sugars, and from those of the α- and β-protons of the caffeyl residue, a *trans* configuration. Moreover, the downfield shift (4.66 ppm) of H-2‴ located the acylation at the 2‴ position [4]. ^{13}C NMR (Table 6) of **9** confirmed the position of the caffeyl residue at C-2‴ which induced upfield shifts in the C-1‴ and C-3‴ signals compared with those in **8**. It additionally defined the stereochemistry of the sugars and the (1 → 2) linkage within the diglucoside as in **8**

Table 4. Hydrolysis products of quercetin glycosides (**1**–**14**)

Compound	Acid hydrolysis	Alkaline hydrolysis	β-Glucosidase hydrolysis
Triglycosides			
1	Q + Glc	—	—
2	Q + Glc + Caffeyl	**1** + Caffeyl	**9**
4	Q + Glc + Rha	—	**10**
5	Q + Glc + Ferulyl	**1** + Ferulyl	—
6	Q + Glc + *p*-Coumaryl	**1** + *p*-Coumaryl	—
11	Q + Glc + Caffeyl	**1** + Caffeyl	**11**
14	Q + Glc + Caffeyl + Ferulyl	**1** + Caffeyl + Ferulyl	**14**
Diglycosides			
3	Q + Glc	—	—
7	Q + Glc	**3**	**13**
8	Q + Glc	—	—
9	Q + Glc + Caffeyl	**8** + Caffeyl	—
10	Q + Glc + Rha	—	—
Monoglycosides			
12	Q + Glc	—	—
13	Q + Glc	**12**	—

Q—quercetin; Glc—glucose; Rha—rhamnose.

Table 5. ^{1}H NMR chemical shifts of quercetin diglycosides **7**, **8**, **9** and **10***

Compound	H	**7**	**8**	**9**	**10**
Quercetin	6	6.44 *d*(2.1)	6.18 *d*(2.1)	6.18 *d*(1.8)	6.20 *d*(2.1)
	8	6.75 *d*(2.1)	6.41 *d*(1.5)	6.36 *d*(1.8)	6.40 *d*(1.8)
	2′	7.54 *d*(2.1)	7.55 *d*(2.4)	7.48 *d*(2.1)	7.55 *d*(2.1)
	5′	6.85 *d*(8.5)	6.87 *d*(8.5)	6.88 *d*(8.5)	6.85 *d*(8.8)
	6′	7.44 *dd*(2.1, 8, 5)	7.60 *dd*(2.1, 8.5)	7.57 *dd*(2.1, 8.5)	7.55 *dd*(2.1, 8.8)
Glucose at C3	1″	5.37 *d*(7.0)	5.69 *d*(7.0)	5.70 *d*(7.3)	5.34 *d*(7.3)
	6″a	4.18 *dd*(11.6			
	6″b	3.97 *dd*(11.9, 5.2)			
Glucose at C2″	1‴		4.60 *d*(7.9)	5.05 *d*(8.2)	
	2‴			4.66 *d*(8.7)	
Rhamnose at C6″	1‴				4.38 *d*(1.5)
Glucose at C7	1⁗	5.07 *d*(7.3)			
Caffeyl	α			6.23 *d*(16.2)	
	β			7.41 *d*(15,9)	
	2‴			7.0 *d*(1.8)	
	5⁗			6.70 *d*(8.2)	
	6⁗			6.88 *dd*(1.9, 8.2)	
Malonyl	2‴	3.15			

*At 400 MHz in DMSO-d_6 δ values (ppm) are followed by multiplicity (coupling constants Hz).

[5]. Therefore, **9** is quercetin 3-*O*-(2″-*O*-(2‴-*trans*-caffeyl)-β-D-glucopyranosyl)-β-D-glucopyranoside (Table 1). This structure has been previously described from *Petunia hybrida* cultivars [6]. With regard to the biosynthetic background, the occurrence of two pairs of quercetin derivatives with the same glycosylation pattern (**1** and **2**, **8** and **9**), without and with caffeylation of C-2‴, is not surprising.

UV spectral properties of **10** indicated it was a non-acylated 3-substituted quercetin glycoside (Table 3). Acid hydrolysis and ES-MS (Tables 4 and 2) suggested a rutin-like structure which corroborated HPLC and TLC co-chromatography. ^{1}H NMR of **10** (Table 5) gave glucose and rhamnose H-1 signals at 5.34 and 4.38 ppm, respectively, confirming a (1 → 6)(rutinoside) instead of a (1 → 2)(neohesperidoside)-linked rhamnoglucoside, although the latter could be envisaged in the biogenetic context [3]. Thus, the structure of quercetin 3-rutinoside could be assigned to **10** (Table 1).

Compound **4** showed UV spectral and chromatographic data (Tables 3 and 2) indicating a quercetin 3,7-triglycoside. Acid hydrolysis gave glucose and rhamnose (Table 4) and enzyme hydrolysis with β-glucosidase gave rutin **10** and ES-mass spectrometry (Tables 4 and 2) confirmed the structure of **4** as quer-

Table 6. ^{13}C NMR chemical shifts of quercetin diglycosides **7**, **8**, and **9***

	Quercetin					Caffeic acid					Malonic acid		
Compound	C-6	C-8	C-2′	C-5′	C-6′	C-α	C-β	C-2‴	C-5‴	C-6‴	C-1‴	C-2‴	C-3‴
7	99.8	92.9	115.5	117.0	121.3						167.9	43.1	168.6
8	98.9	93.7	115.6	116.2	122.0								
9	98.8	93.6	115.6	115.9	121.0	114.9	144.7	115.1	115.9	122.2			

Sugars

		†	C-1	C-2	C-3	C-4	C-5	C-6
7	Glucose	7	100.3	73.5	76.6	69.8	77.6	61.0
		3	101.5	74.5	76.8	70.0	74.3	63.5
8	Glucose	3	98.2	82.9	76.8	69.7	77.7	60.8
		2″	104.4	74.6	76.7	69.7	77.0	60.8
9	Glucose	3	97.6	79.8	76.8	70.5	77.5	61.0
		2″	99.8	73.9	74.7	70.3	77.0	61.0

*DEPT profiles at 100 MHz in DMSO-d_6; δ values in ppm.
†Glycosylation site.

cetin 3-rutinoside 7-glucoside, previously described from spindle tree [7].

The special chromatographic features (strong retention upon polyamide, high migration on cellulose in water—Table 2) of **7** and **13** displayed an acidic nature for these molecules. Ultra-violet spectral analyses (Table 3) indicated that they were 3,7 and 3-substituted quercetin derivatives, respectively, without acylation by an aromatic acid. Acid hydrolysis yielded in both cases glucose, while alkaline hydrolysis gave **3** from **7** and **12** from **13** (Table 4). The natural products **3** and **12** were characterized by UV spectral and ES-mass spectrometry data (Tables 3 and 2) as quercetin 3,7 diglucoside and quercetin 3-glucoside, respectively. ES-MS provided M_r indicating that **7** and **13** were malonyl esters of **3** and **12**, respectively (Table 2). Moreover enzyme hydrolysis of **7** with β-glucosidase (Table 4) yielded a compound identical to **13** (HPLC and TLC co-chromatography, M_r from ES-MS, UV spectral properties), ensuring the position of malonic ester on the glucose at the 3 position. NMR spectra of **7** validated these conclusions (Tables 5 and 6). In the ^{13}C NMR spectrum, the methylene and carbonyl groups of the malonyl moiety were evident by signals at δ 43.1 and 167.9–168.6 ppm, respectively. The site of malonylation was defined as the 6″-hydroxyl by the 2.5 ppm downfield shift of the glucose C-6″ as well as the 3 ppm upfield shift of the glucose C-5″ [8, 9]. This was confirmed by the ^1H NMR spectrum which showed the methylene protons of the malonyl moiety at δ 3.15 ppm and the typical low field H-6″a H-6″b of the malonylated glucose [8, 9]. Thus, the structure of **7** was clearly defined as quercetin 3-*O*-(6″-*O*-malonyl-β-D-glucopyranoside)-7-*O*-β-D-glucopyranoside, a new compound. Although **13** was isolated in insufficient amounts for NMR studies it seemed likely, as it co-occurred with **7**, that the malonic acid is located at the 6″ position. Thus, it is tentatively identified as quercetin 3-*O*-(6″-*O*-malonyl)-β-D-glucopyranoside, which has been described previously from various plants [8, 9].

EXPERIMENTAL

Plant material. Emerged leaves of *R. peltatus* subsp. *peltatus* were collected in May 1993 (near Lyon) on a Dombes' pond, F-01, France. Laminar leaves of *R. trichophyllus* from Öland (Sweden) were collected in 1994 by Prof. G. Dahlgren. Laminar leaves of *R. fluitans* were collected in June 1995 in the Rhone river (Lafayette bridge, F-69, Lyon, France).

Isolation procedure. The 12 neutral flavonoids were isolated from *R. peltatus* subsp. *peltatus* (leaves dried in the dark at room temp.: *ca* 29 g dry wt) according to the procedure previously described for antarctic *Ranunculus* species [3]. But in some cases final isolation required prep. TLC on cellulose with 5 or 15% HOAc or BAW 12.3.5. Isolated amounts were *ca* 2.5–13 mg for **2**, **8**, **9**, **10** and **12** and 0.1–0.5 mg for **1**, **3**, **4**, **5**, **6**, **11** and **14**.

The malonyl esters **7** and **13** were obtained from *R. fluitans* and *R. trichophyllus*, respectively, the two species in which they were the most abundant [1]. Given the acidic and more hydrophilic nature of these molecules, the previous isolation procedure was modified as follows: the concd 80% EtOH extracts (from dried leaves: 13 g ps of *R. fluitans* and 0.5 g ps of *R. trichophyllus*) were taken up in the minimum vol. of H_2O. Compounds were sepd by prep. TLC on cellulose with 5% HOAc and eluted with H_2O; after filtration on cellulose and purification on sephadex LH 20 with H_2O, isolated amounts were *ca* 13 mg for **7** and 0.5 mg for **13**.

Hydrolytic procedures. As described in ref. [3].

Acknowledgements—We are indebted to Professor G. Dahlgren (Department of Systematic Botany, Lund University, Sweden) for collecting, determining and

sending *Ranunculus* samples and to M.-M. Flament (C.N.R.S., F-69-Vernaison, France) for mass spectral measurements.

REFERENCES

1. Fiasson, J. L., Gluchoff-Fiasson, K. and Dahlgren, G., *Biochemical Systematics and Ecology*, in press.
2. Webster, S. D., *Aquatic Botany*, 1991, **40**, 11.
3. Gluchoff-Fiasson, K., Fiasson, J. L. and Favre-Bonvin, J., *Phytochemistry*, 1994, **37**, 1629.
4. Markham, K. R. and Geiger, H., in *The Flavonoids—Advances in Research Since* 1986, ed. J. B. Harborne, Chapman and Hall, London, 1994, p. 441.
5. Markham, K. R., Mabry, T. J. and Chari, M., in *The Flavonoids—Advances in Research*, ed. J. B. Harborne and T. J. Mabry, Chapman and Hall, London, 1982, p. 19.
6. Griesbach, R. J. and Asen, S., *Plant Science*, 1990, **70**, 49.
7. Gonnet, J. F. and Lebreton, Ph., *Plantes Médicin. et Phytotherapie*, 1971, **5**, 255.
8. Geslin, M. and Verbist, J. F., *Journal of Natural Products*, 1985, **48**, 111.
9. Wald, B., Wray, V., Galensa, R. and Herrmann, K., *Phytochemistry*, 1989, **28**, 663.