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NEW 6-HYDROXYFLAVONOIDS AND THEIR METHYL ETHERS AND GLYCOSIDES FROM *NEUROLAENA OAXACANA*

AYHAN ULUBELEN,* KATHLEEN M. KERR and TOM J. MABRY

The Department of Botany, The University of Texas at Austin, Austin, TX 78712, U.S.A.

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Key Word Index—*Neurolaena oaxacana*; Compositae; Heliantheae; Galinsoginae; 6-hydroxykaempferol methyl ethers and glycosides; quercetagetin methyl ethers, glycosides and sulfate; 6-hydroxyflavone methyl ether and glycoside.

Abstract—Six new and nine known flavonoids were obtained from *Neurolaena oaxacana*. The known flavonoids are 6-hydroxykaempferol 3,7-dimethyl ether, quercetagetin 3,7-dimethyl ether, quercetin 3-methyl ether, axillarin, nodifloretin, 6-hydroxyluteolin 7-glucoside, kaempferol 3-glucoside, quercetagetin 7-glucoside and patulitrin. The new compounds are 6-hydroxykaempferol 3-methyl ether, quercetagetin 3,7-dimethyl ether 6-galactoside, quercetagetin 3-methyl ether 7-glucoside, the 6- and 7-glucosides of 6-hydroxykaempferol 3-methyl ether and quercetagetin 3-methyl ether 7-sulfate.

INTRODUCTION

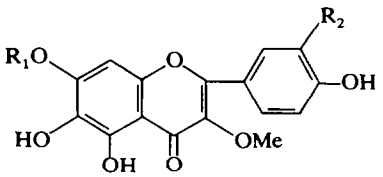
The genus *Neurolaena* consists of ten species of soft-wooded trees, shrubs and perennial herbs. Nine species are endemic and restricted to southern Mexico and adjacent Guatemala, while the distribution of *N. lobata* (L.) R. Br. extends from southern Mexico into South America. Previously, thymol derivatives [1] were described from *N. oaxacana* B. L. Turner, *N. venturana* B. L. Turner and *N. lobata* and sesquiterpene lactones [2] were reported from *N. lobata*. No polyacetylenes were detected in the roots of any of these taxa (Bohlmann, F., private communication). This is the first report of flavonoids from *Neurolaena*. We describe the isolation and characterization of nine known and six new flavonoids from *N. oaxacana*.

RESULTS

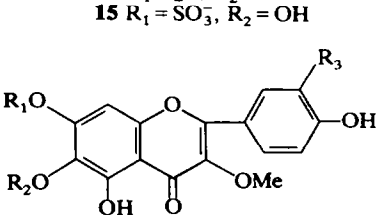
The dried and powdered leaves of *Neurolaena oaxacana* were extracted with aqueous methanol and water, and the concentrated syrup was partitioned between water and three organic solvents: hexane, chloroform and ethyl acetate. Later the hexane and chloroform extracts were combined. The hexane–chloroform extract yielded 6-hydroxykaempferol 3,7-dimethyl ether (**1**) [3], quercetagetin 3,7-dimethyl ether (**2**) [3], quercetin 3-methyl ether (**3**) [4], quercetin 3,6-dimethyl ether (axillarin) (**4**) [5], 6-hydroxyluteolin 3′-methyl ether (**5**) [6], 6-hydroxyluteolin 7-glucoside (**6**) [7], and 6-hydroxykaempferol 3-methyl ether (**7**). From the ethyl acetate extract three new glycosides, namely, quercetagetin 3,7-dimethyl ether 6-galactoside (**8**), 6-hydroxykaempferol 3-methyl ether 6-glucoside (**9**) and quercetagetin 3-methyl ether 7-glucoside (**11**), were obtained together with kaempferol 3-glucoside (**10**). The remaining water layer yielded four flavonoids, two of which were new: 6-hydroxykaempferol 7-glucoside (**12**), the previously known patulitrin (**13**) [8], quercetagetin 7-glucoside (**14**) [9] and quercetagetin 3-methyl ether 7-sulfate (**15**). The spectral properties of all the compounds, as well as colors and R_f values, are reported in Tables 1–4.

6-*Hydroxykaempferol* 3-*methyl ether* (**7**)

In addition to signals for H-8 (δ6.50) and a kaempferol B-ring pattern, the ^{1}H NMR of the TMSi ether

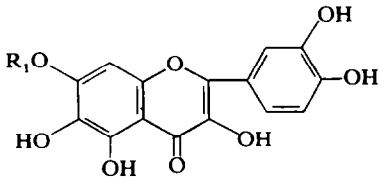


1 R_1 = Me, R_2 = H
2 R_1 = Me, R_2 = OH
7 R_1 = R_2 = H
11 R_1 = glc, R_2 = OH
12 R_1 = glc, R_2 = H
15 R_1 = SO_3^-, R_2 = OH

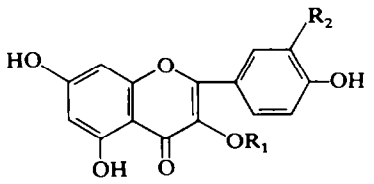


4 R_1 = H, R_2 = Me, R_3 = OH
8 R_1 = Me, R_2 = gal, R_3 = OH
9 R_1 = R_3 = H, R_2 = glc

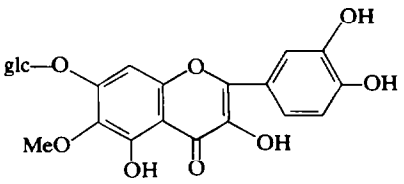
* Permanent address: Faculty of Pharmacy, University of Istanbul, Istanbul, Turkey.



14 R_1 = glc



3 R_1 = Me, R_2 = OH
10 R_1 = glc, R_2 = H



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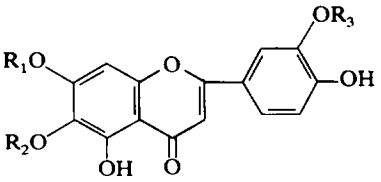

5 R_1 = H, R_2 = H, R_3 = Me
6 R_1 = glc, R_2 = H, R_3 = H

of this new flavonol exhibited one methoxyl signal at 3.84 ppm which shifted with C_6D_6 only to 3.82 ppm typical for a 3-methoxyl group. The UV spectrum with $AlCl_3$–HCl gave a band I bathochromic shift of 27 nm relative to the methanol spectrum indicating a free 6-hydroxyl group and a 3-*O*-substituent [10]. The strong MS fragment at *m/e* 315 (65) for M−H supported the presence of a 6-OH group [11] (for details see Tables 2–4).

Quercetagetin 3,7-dimethyl ether 6-galactoside (**8**)

Hydrolysis of this new glycoside yielded galactose and quercetagetin 3,7-dimethyl ether (UV, MS and TLC comparison with compound **2**). An 18 nm bathochromic shift with $AlCl_3$–HCl relative to the MeOH spectrum suggested a C_6-OR moiety. Since the hydrolysed product exhibited a shift of 28 nm indicating a free C_6-OH group (for spectral data see Tables 2

Table 1. Chromatographic data (R_f s × 100 and colors) for flavonoids of *Neurolaena oaxacana**

Compound	Cellulose				Polyamide		Silica gel		Colors		
	HOAc 15%	HOAc 40%	TBA	*n*-BAW	BMM	BPMM	CAA	BPA	UV	UV/NH_3	UV/NA
6-Hydroxykaempferol 3,7-dimethyl ether (**1**)	11	52	70	80	63	16	78	54	p	p–br	p–br
Quercetagetin 3,7-dimethyl ether (**2**)	10	36	55	70	52	8	45	30	p	p–br	or–r
Quercetin 3-methyl ether (**3**)	12	37	50	66	53	8	45	30	p	y	or–r
Axillarin (**4**)	13	52	72	88	59	11	60	40	p	y	or–r
Nodifloretin (**5**)	9	35	64	87	47	6	6	25	p	p–br	br–y
6-Hydroxyluteolin 7-glc (**6**)	15	20	11	18	13	0	15	5	p	p–br	or
6-Hydroxykaempferol 3-methyl ether (**7**)	11	36	64	88	48	8	0	25	p	p–br	p–br
Quercetagetin 3,7-dimethyl ether 6-gal (**8**)	15	20	10	18	12	0	42	27	p	y†	or–r
6-Hydroxykaempferol 3-methyl ether 6-glc (**9**)	27	58	48	66	40	0	0	—	p	y†	p–br
Kaempferol 3-glc (**10**)	34	62	67	55	—	0	0	—	p	y	y
Quercetagetin 3-methyl ether 7-glc (**11**)	16	34	25	34	9	0	0	—	p	p–br	or
6-Hydroxykaempferol 3-methyl ether 7-glc (**12**)	8	42	69	62	6	0	—	—	p	p–br	p–br
Patulitrin (**13**)	10	31	28	35	20	0	—	—	y	y	or–r
Quercetagetin 7-glc (**14**)	3	7	5	12	6	0	—	—	p	p–br	or–r
Quercetagetin 3-methyl ether 7-SO_3 (**15**)	34	54	33	46	0	0	—	—	p	p–br	or–r
Quercetagetin*	2	10	19	37	1.5	19	—	—	p	p–br	or–r

* TLC data on cellulose (Merck); Polyamide MN (Macharey-Nagel) silica gel G (Merck); for solvent key see Experimental. Colors key: p = purple, y = yellow, or = orange, r = red, br = brown, NA refers to Naturstoffreagenz A in MeOH. The aglycone quercetagetin was not isolated here.

† Trace compounds **8** and **9** were not fumed with NH_3 but should appear yellow.

Table 2. UV data (λ_{max}, nm) for flavonoids from *Neurolaena oaxacana*

	MeOH	NaOMe	$AlCl_3$	$AlCl_3$/HCl	NaOAc	NaOAc/H_3BO_3
6-Hydroxykaempferol 3,7-dimethyl ether (**1**)	341(1)*, 281(0.6)	375(1), 295(sh), 270(sh), 255(0.15)	377(1), 297(0.6)	368(1), 297(0.7)	390(sh), 343(1), 281(1)	335(1), 287(1.3)
Quercetagetin 3,7-dimethyl ether (**2**)	352(1), 281(1), 258(0.9)	388(1), 265(0.5)	440(1), 340(0.05), 295(sh), 281(0.6)	381(1), 290(0.1), 265(0.6)	400(sh), 360(1), 274(1)	366(1), 280(sh), 265(0.15)
Quercetin 3-methyl ether (**3**)	358(1), 295(0.05), 271(sh), 255(1.5)	406(1), 315(0.16), 272(1.4)	440(1), 325(sh), 305(sh), 276(1.5)	400(1), 350(sh), 305(sh), 268(2)	376(1), 322(sh), 273(1.2)	378(1), 305(sh), 260(1.5)
Axillarin (**4**)	356(1), 271(sh), 257(0.9)	408(1), 334(0.15), 270(0.8)	444(1), 335(0.02), 277(0.9)	376(1), 305(sh), 275(sh), 266(1.1)	380(1), 320(sh), 272(1.3)	381(1), 264(1.1)
Nodifloretin (**5**)	344(1), 276(1.1)	385(1), 306(0.15), 260(0.9)	390(1), 302(1), 262(sh)	370(1), 290(1), 255(sh)	366(1), 290(1.5), 265(1.3)	360(1), 290(1.5)
6-Hydroxyluteolin 7-glc (**6**)	348(1), 288(1), 254(sh)	410(1), 300(sh), 280(1.2)	440(1), 300(sh), 280(1.2)	378(1), 294(sh), 266(1.05)	420(sh), 354(1), 285(1), 255(1.05)	365(1), 285(sh), 267(1.2)
6-Hydroxykaempferol 3-methyl ether (**7**)	340(1), 281(0.9)	377(1), 304(0.3) 252(0.35)	375(1), 302(0.8)	367(1), 297(1)	410(sh), 342(1), 285(1.2)	336(1), 285(2.5)
Quercetagetin 3,7-dimethyl ether 6-gal (**8**)	346(1), 300(sh), 255(6)	406(1), 304(sh), 280(8)	436(1), 310(sh), 278(6)	364(1), 262(7)	416(1), 290(sh), 258(5)	360(1), 268(4)
6-Hydroxykaempferol 3-methyl ether 6-glc (**9**)	334(1), 284(5)	376(1), 308(0.3), 260(0.2)	372(1), 305(0.5), 255(sh)	346(1), 298(2)	334(1), 294(0.8)	340(1), 292(0.7), 255(0.2)
Kaempferol 3-glc (**10**)	352(1), 295(sh), 265(1.7)	403(1), 326(0.25), 274(1)	397(1), 350(1), 304(0.8), 274(2)	395(1), 346(1.25), 303(1.15), 274(2)	372(1), 302(0.5), 274(3)	352(1), 295(sh), 265(2)
Quercetagetin 3-methyl ether 7-glc (**11**)	346(1), 282(sh), 262(1.8)	386(1), 269(0.7)	430(1), 304(sh), 296(sh), 279(1.2)	380(1), 290(sh), 270(1.5)	420(sh), 360(1), 296(1.1), 264(sh)	358(1), 266(1.3)
Quercetagetin 3-methyl ether	350(1), 270(sh), 258(0.9)	390(1), 310(sh), 258(1)	426(1), 305(0.5), 274(1.1)	381(1), 276(sh), 262(1.5)	374(1), 260(1.3)	370(1), 284(sh), 265(1.1)
6-Hydroxykacmpferol 3-methyl ether 7-glc (**12**)	340(1), 280(sh), 272(0.6)	387(1), 295(0.1), 268(0.3)	396(sh), 372(1), 299(0.9), 278(0.9)	371(1), 292(0.9), 278(sh)	396(sh), 340(1), 280(1.1)	336(1), 278(1.5)
Patulitrin (**13**)	368(1), 276(sh), 260(sh), 254(1.5)	456(1), 406(sh), 274(2)	458(1), 360(sh), 286(1)	428(1), 384(sh), 270(sh), 264(2)	387(1), 262(2)	386(1), 264(1.8)
Quercetagetin	360(1), 274(sh), 254(0.7)	380(dec)(1), 310(3), 246(4)	442(1), 280(0.7)	414(1), 270(1)	380(1), 262(0.7)	376(1), 292(sh), 266(0.7)
Quercetagetin 7-glc (**14**)	362(1), 276(sh), 258(1.3)	456(dec.), 398(1), 270(1.4)	458(1), 260(0.9)	428(1), 390(1.2) 264(sh), 270(0.8)	392(1), 280(sh), 262(1.5)	386(1), 280(sh), 268(1.5)
Quercetagetin 3-methyl ether 7-SO_3 (**15**)	346(1), 270(sh), 258(1.5) (the same with HCl)	406(1), 276(1.7)	426(1), 276(1.6)	398(sh), 368(1), 278(sh), 270(2)	380(1), 260(1.8)	380(1), 262(2)

* The relative absorptivities are presented following each λ_{max} using the longest wavelength peak as (1).

and 3), the natural product is a 6-*O*-galactoside. MS of the underivatized galactoside gave a strong M−H aglycone peak at *m/e* 345 (80) indicating thermal loss of a 6-*O*-galactosyl moiety.

6-*Hydroxykaempferol* 3-*methyl ether* 6-*glucoside* (**9**)

Hydrolysis of the new glycoside yielded glucose and 6-hydroxykaempferol 3-methyl ether (UV, NMR, MS and TLC comparison with compound **7**). The UV spectrum of **9** with $AlCl_3$–HCl showed a 12 nm bathochromic shift in band I relative to the MeOH spectrum indicating a 6-OR group. After hydrolysis the same reagent gave a 26 nm bathchromic shift for a 6-OH group thus establishing a 6-*O*-glucosyl moiety in the original compound. In the MS of the underivatized glycoside a typical M−H fragment is present at *m/e* 315 in accord for a 6-*O*-glycosyl group. In the ^{1}H NMR of **9**, the signal for H_1 of glucose was centered at δ5.05 and other sugar signals were between 3.4 and 3.85 ppm. Signals for the aromatic protons as well as the methoxyl group were essentially the same as those for compound **7** (see Tables 2–4).

Quercetagetin 3-*methyl ether* 7-*glucoside* (**11**)

The UV spectrum of this new flavonoid with $AlCl_3$–HCl indicated a free 6-OH (29 nm bathochromic shift in band I relative to the MeOH spectrum). The lack of band III in the NaOMe spectrum, a shoulder at 420 nm and no bathochromic shift in band II with NaOAc indicated that the 7-OH was not free. Hydrolysis yielded glucose and quercetagetin 3-methyl ether (UV, ^{1}H NMR and MS) (see Tables 2–4). Therefore, the natural product is quercetagetin 3-methyl ether 7-glucoside.

6-*Hydroxykaempferol* 3-*methyl ether* 7-*glucoside* (**12**)

Hydrolysis of this compound yielded glucose and 6-hydroxykaempferol 3-methyl ether (UV, ^{1}H NMR and TLC comparison with **7**). The UV spectrum of **12** with $AlCl_3$–HCl exhibited a 31 nm bathochromic shift relative to the MeOH spectrum typical for a free 6-OH group. Other UV data established free 5- and 4′-hydroxyl groups. The lack of a band III in the NaOMe spectrum indicated that the glycosyl moiety sugar is attached at C_7 (see Tables 2–4).

Quercetagetin 3-*methyl ether* 7-*sulfate* (**15**)

This compound gave quercetagetin 3-methyl ether and sulfate but no sugar on hydrolysis. Migration in electrophoresis under standard conditions was 1 cm, indicating only one sulfate group. The UV spectrum of this compound in MeOH before and after hydrolysis did not change significantly (see Table 2) indicating

Table 3. ^{1}H NMR data of *Neurolaena oaxacana* flavonoids*

Compound	H-2′	H-6′	H-5′	H-3′	H-6	H-8	H-3	Glycosyl H_1''	Glycosyl H_2''–H_6''	O-Methyls CCl_4 3	CCl_4 6	CCl_4 7	C_6D_6 3	C_6D_6 6	C_6D_6 7
1	7.92 *d* (9.0)	7.92 *d* (9.0)	6.87 *d* (9.0)	6.87 *d* (9.0)	—	6.50 *s*	—	—	—	3.90	—	3.83	3.83	—	3.23
2	7.6 *d* (2.5)	7.5 *dd* (2.5) (9.0)	6.83 *d* (9.0)	—	—	6.50 *s*	—	—	—	3.90	—	3.85	3.85	—	3.23
4	7.6 *d* (2.5)	7.5 *dd* (2.5) (9.0)	6.82 *d* (9.0)	—	—	6.50 *s*	—	—	—	3.85	3.75	—			
6	7.28 *d* (2.5)	7.35 *dd* (2.5) (9.0)	6.84 *d* (9.0)	—	—	6.60 *s*	6.35 *s*	5.02 *d* (8.0)	3.3–3.8 *m*	—	—	—	—	—	—
7	7.95 *d* (9.0)	7.95 *d* (9.0)	6.9 *d* (9.0)	6.9 *d* (9.0)	—	6.50 *s*	—	—	—	3.84			3.82		
9	7.95 *d* (9.0)	7.95 *d* (9.0)	6.9 *d* (9.0)	6.9 *d* (9.0)	—	6.45 *s*	—	5.05 *d* (7.0)	3.3–3.85 *m*	3.9	—	—	—	—	—
10	7.95 *d* (9.0)	7.95 *d* (9.0)	6.85 *d* (9.0)	6.85 *d* (9.0)	6.2 *d* (2.0)	6.30 *d* (2.0)	—	5.78 *d* (7.0)	3.3–3.75 *m*						
11	7.6 *d* (2.5)	7.45 *dd* (2.5) (9.0)	6.8 *d* (9.0)	—	—	6.55 *d*	—	5.05 *m*	3.35–3.8 *m*	3.86			3.75		
12	7.55 *d* (9.0)	7.55 *d* (9.0)	6.85 *d* (9.0)	6.85 *d* (9.0)	—	6.50 *s*	—	5.1 *m*	3.2–3.8 *m*	3.85	—	—	—	—	—
13	7.55 *d* (2.5)	7.6 *dd* (2.5)(9.0)	6.8 *d* (9.0)	—	—	6.6 *s*	—	5.00 *m*	3.3–3.76 *m*	—	3.75	—	—	3.72	—
14	7.54 *d* (2.5)	7.6 *dd* (2.5) (9.0)	6.82 *d* (9.0)	—	—	6.5 *s*		5.05 *d* (7.0)	3.2–3.8 *m*	—	—	—	—	—	—

* Spectra were recorded in CCl_4 and C_6D_6 (only OMe signals are reported for this solvent). Values are given in ppm (δ scale) relative to TMS as an internal standard. Numbers in parentheses denote coupling constants in Hz. Signals: *s* = singlet, *d* = doublet; *dd* = double doublet; *m* = multiplet.

that the sulfate was not at the 3-position [12]. The purple–brown color of the compound when spotted on paper and viewed over UV light established a 6-OH group. Comparison of the UV spectra with $AlCl_3$ and $AlCl_3$–HCl indicated 3′- and 4′-hydroxyl groups. The compound is therefore quercetagetin 3-methyl ether 7-sulfate.

EXPERIMENTAL

Plant material. *N. oaxacana* was collected from the state of Oaxaca, Mexico 23 miles south of Valle Nacional on Hwy 175, in March 1978 by one of us (K.K.). Voucher specimens are deposited in the Herbarium of the Univ. of Texas at Austin (119–78K).

General techniques. Column chromatography employed polyclar [Polyclar AT (GAF Corp.)] and Sephadex LH-20 (Pharmacia), cellulose powder (Merck); PC and electrophoresis were carried out with Whatman 3MM paper. Precoated Polyamide (Macharey–Nagel), cellulose (Merck) and Si gel 60 GF-254 (Merck) plates were used for TLC. The solvents were: BPMM (C_6H_6–petrol (65–110°)–MeCOEt–MeOH, 60:26:7:7); BMM (C_6H_6–MeCOEt–MeOH, 4:3:3); CAA ($CHCl_3$–Me_2CO–HCOOH, 9:2:1); BPA (C_6H_6–Py–HCOOH, 36:9:5); TBA (*t*-BuOH–HOAc–H_2O, 3:1:1) and *n*-BAW, upper layer (*n*-BuOH–HOAc–H_2O, 4:1:5). The flavonoids were visualized by UV light with exposure to NH_3 and by spraying with NA (Naturstoffreagenz-A, Carl Roth, Germany) in MeOH. Hydrolyses were carried out with 0.1 N TFA on a steam cone for 1 hr for both glycosides and sulfates. All the fractions were purified by standard procedures [13, 14] over Sephadex LH-20 using MeOH or 80% aq. MeOH prior to spectral analysis (see Tables 1–4).

Extraction and identification of flavonoids. Ground leaves of *N. oaxacana* (240 g) were extracted with 85% aq. MeOH, followed by 50% aq. MeOH and H_2O until the extracts were almost colorless. The combined extracts were evapd *in vacuo* to 500 ml. The aq. concentrate was successively extracted with *n*-hexane, $CHCl_3$ and EtOAc.

A. $CHCl_3$ extract. The conc $CHCl_3$ extract (5.2 g) was chromatographed over a Polyclar column (6 × 70 cm; 300 g), first eluted with Egger's solvent ($CHCl_3$–MeOH–MeCOEt–Me_2CO, 40:20:5:1), and later the amount of MeOH was increased. 6-Hydroxykaempferol 3,7-dimethyl ether (**1**), 237 mg, eluted first followed by 6-hydroxyquercetin 3,7-dimethyl ether (**2**), 51 mg, and 6-methoxyquercetin 3-methyl ether (axillarin) (**4**), 30 mg, and a mixture of 6-hydroxyluteolin 3′-methyl ether (**5**) and the new compound 6-hydroxykaempferol 3-methyl ether (**7**) (116 mg) which were separated on a Sephadex LH-20 column. Quercetin 3-methyl ether (**3**), 43 mg, and 6-hydroxyluteolin 7-glucoside (**6**), 16 mg, eluted last.

Table 4. MS data of *Neurolaena oaxacana* flavonoids*

Compound	M^+	M^+-H	M^+-Me	M^+-H_2O	M^+-CHO	M^+-COMe	A_1	A_1-Me	A_1-H_2O	B_1	B_2
1	330	329	—	312	301	287	182	—	164	118	121
	(100)	(39)		(20)	(4)	(23)	(4)		(6)	(3)	(23)
2	346	345	—	328	317	303	182	—	164	134	137
	(100)	(80)		(10)	(20)	(10)	(4)		(4)	(4)	(20)
3	316	—	—	—	—	—	152	—	—	134	137
	(40)						(50)			(15)	(100)
4	346	345	331	328	—	303	182	167	—	—	137
	(70)	(30)	(25)	(20)		(27)	(5)	(5)			(20)
5	316	315	—	298	—	273	168	—	—	148	—
	(100)	(80)		(20)		(30)	(15)			(15)	
6†	302	301	—	—	—	—	168	—	—	134	—
							(10)			(5)	
7	316	315	—	298	287	273	168	—	150	118	121
	(85)	(65)		(15)	(10)	(100)	(45)		(2)	(10)	(40)
8†	346	345	—	—	—	303	182	—	—	—	137
	(100)	(80)				(12)	(13)				(40)
9†	316	315	—	298	—	273	168	—	150	118	121
	(100)	(50)		(22)		(35)	(35)		(5)	(15)	(55)
10†	286	287	—	—	257	243	152	—	—	118	121
	(100)	(30)			(15)	(5)	(12)			(5)	(30)
11†	332	331	—	314	303	289	168	—	150	134	137
	(90)	(60)		(20)	(18)	(30)	(30)		(10)	(10)	(10)
14†	318	317	—	—	289	275	168	—	150	134	137
	(100)	(62)			(40)	(5)	(25)		(20)	(5)	(33)

* MS were recorded at 70 eV, source temp. 200° and probe temp. from 250 to 425°. Values are given in *m/e*, in parentheses the % abundance relative to the base peak. The A_1, B_1 and B_2 terminology for the fragments is given in ref. [14].

† M^+ of these compounds corresponds to the aglycone.

B. EtOAc *extract.* Five of the 10 g of the material from the EtOAc extract were chromatographed on a Polyclar column (5 × 50 cm, 200 g) first eluted with MeOH and later H_2O was added. When the H_2O reached 30% several bands migrated rapidly. The compounds obtained from this column were quercetagetin 3,7-dimethyl ether 6-galactoside (**8**), 20 mg, 6-hydroxykaempferol 3-methyl ether 6-glucoside (**9**), 18 mg, kaempferol 3-glucoside (**10**), 11 mg and quercetagetin 3-methyl ether 7-glucoside (**11**), 125 mg.

C. H_2O *extract.* The aq. extract was chromatographed on a Sephadex G-10 column (6 × 40 cm); the elution was initiated with H_2O then MeOH added up to 40%. The sulfated flavonoid was obtained from fractions containing 20% MeOH. The flavonoid glycosides were obtained from fractions containing 20–50% MeOH as a mixture (0.9 g) and were separated on a Polyclar column (4.5 × 14 cm). The column was first eluted with H_2O–MeOH–MeCOEt–Me_2CO, (13:3:3:1) and eventually the H_2O was eliminated. The compds obtained from the H_2O extract were: 6-hydroxykaempferol 7-glucoside (**12**), 8 mg, patulitrin (**23**), 15 mg, quercetagetin 7-glucoside (**14**), 80 mg, and quercetagetin 3-methyl ether 7-sulfate (**15**), 3 mg.

The presence of the sulfate group in **15** was established by high voltage electrophoresis (1.5 kV) on Whatman 3MM paper (27 × 46 cm) for 1.5 hr at pH 1.9 (HCOOH–HOAc–H_2O, 33:147:1820). The compd migrated 1.0 cm from the origin. Acid hydrolysis gave sulfate which was precipitated with $BaCl_2$–H_2O.

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