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FLAVONOIDS OF *CHRYSOSPLENIUM TETRANDRUM**

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Key Word Index—*Chrysosplenium tetrandrum*; Saxifragaceae; poly-*O*-methylated flavones; kaempferol; quercetin; chemotaxonomy.

Abstract—*Chrysosplenium tetrandrum*, from northern British Columbia, accumulates a variety of flavonoid glycosides. Several kaempferol and quercetin mono- and diglycosides were identified. The major flavonoid fraction consisted of *O*-methylated compounds having an hydroxyl or methoxyl substituent at position-6. Aglycones identified were 5,4′-dihydroxy-3,6,7-trimethoxyflavone, 5,6,7,3′,4′-pentahydroxy-3-methoxyflavone, 5,6,3′,4′-tetrahydroxy-3,7-dimethoxyflavone, 5,6,4′-trihydroxy-3,7,3′-trimethoxyflavone, 5,3′,4′-trihydroxy-3,6,7-trimethoxyflavone, and 5,4′-dihydroxy-3,6,7,3′-tetramethoxyflavone. All occurred as glucosides. The occurrence of 6-substitution and the preponderance of *O*-methylated flavonoids supports removal of *Chrysosplenium* from Engler's Saxifraginae.

INTRODUCTION

Chrysosplenium L., consisting of about 55 species [1], occurs widely in the northern hemisphere with two localized species from southern South America. Six species occur in North America: (1) *C. wrightii*, central Alaska; (2) *C. americanum*, northeastern United States and adjacent Canada; (3) *C. glechomaefolium*, coastal region of northwestern United States; (4) *C. iowense*, central United States and north into Alberta, Saskatchewan and Manitoba; (5) *C. rosendahlii*, southern islands of the Canadian Arctic Archipelago and adjacent mainland; and (6) *C. tetrandrum* which occurs in North American and Eurasian arctic and subarctic regions and eastern Greenland [1–3].

As part of our chemotaxonomic study of the Saxifragaceae we examined several *Chrysosplenium* species for flavonoids and found that a wide variety of flavonoids were present. Published reports on flavonoids of *Chrysosplenium* describe a few compounds only from each species studied. This paper describes the isolation of no less than 28 compounds from *C. tetrandum* and the detailed structural elucidation of most of them.

RESULTS

The isolated flavonoids were either glycosides of kaempferol and quercetin or glucosides of *O*-methylated flavonols having a 6-hydroxyl function. Kaempferol and quercetin occurred as 3-*O*-arabinosides, 3-*O*-glucosides, 3-*O*-glucosylarabinosides, 3-*O*-glucosylglucosides, and 3-*O*-rutinosides. Two 3-*O*-diglycosides of quercetin were detected but were not identified due to insufficient material. Small amounts of the aglycones were seen. Partial hydrolyses of the diglycosides gave the expected monoglycosides. The position of attachment of the outer sugar and stereochemistry of the linkage were not determined for the glucosylarabinosides and glucosylglucosides.

All of the *O*-methylated flavonol glycosides yielded glucose as the only sugar. The anomeric protons were seen consistently in the region δ 4.73–4.84 with J = 8.0–8.5 Hz (Table 1). These values are characteristic of *trans*-diaxial coupling of $H_{1''}$ and $H_{2''}$ protons of β-D-glucopyranosides [5]. The positions of attachment of the glucose are described in each case below.

Six *O*-methylated aglycones were identified. In the first type the mass spectrum showed the highest mass ion m/e = 344. This is consistent with a flavonoid having three *O*-methyl groups ($C_{18}H_{16}O_7$). The NMR spectrum (Table 1) allowed five aromatic protons to be assigned as follows: a singlet at δ 6.27 for H-8; a doublet δ 7.48 (J = 9 Hz) for H-2′ and H-6′; and a doublet at δ 6.75 (J = 9 Hz) for H-3′ and H-5′. A B-ring fragment ion with m/e = 121 (16%) indicated the presence of an hydroxyl group at C-4′. Sodium acetate failed to produce a shift in Band II of the UV spectrum of the aglucone which, coupled with the observation of a +0.59 ppm benzene-induced shift in one of the *O*-methyl peaks allows placement of an *O*-methyl function at C-7. The 5-hydroxyl group must be unsubstituted as judged by the $AlCl_3$ shifts in the UV spectra of both aglucone and glucoside. The remaining available positions for *O*-methyl groups are C-3 and C-6 which assignments are supported by small benzene-induced shifts (+0.11 and +0.13 ppm) [6]. Sodium acetate gave no shifts in the UV spectrum of the glucoside but did give a small bathochromic shift of Band I of the aglucone supporting placement of the sugar at position-4′. The compounds are thus 5,4′-dihydroxy-3,6,7-trimethoxyflavone and its 4′-*O*-β-D-glucopyranoside. The literature mp for this glucoside is 175–176° [7] in agreement with mp 176–178° observed in this study. The mp of the aglucone, 182–184°, was considerably lower than the reported value of 216–217°

* Part 7 in the series 'Chemotaxonomic Studies in the Saxifragaceae s.l. for Part 6, see Bohm, B. A. and Wilkins, C. K. (1975) *Phytochemistry* 15, 2012.

Table 1. Nuclear magnetic resonance characteristics of flavonoids of *Chrysosplenium tetrandrum*

Compound	CCl_4 as Solvent										Benzene-d_6 as solvent			
	H-6'	H-2'	H-5'	H'3'	H-8	H-1''	C-3 OMe	C-3' OMe	C-6 OMe	C-7 OMe	C-3 OMe	C-3' OMe	C-6 OMe	C-7 OMe
5,4'-Dihydroxy-3,6,7-trimethoxyflavone 4'-*O*-β-D-glucopyranoside	7.48 *d* *J*=9	7.48 *d* *J*=9	6.75 *d* *J*=9	6 75 *d* *J*=9	6.27 *s*	4.79 *d* *J*=8.5	3.75	—	3 63	3 79	3.64 +0.11	—	3 50 +0.13	3.20 +0.59
5,6,7,3',4'-Pentahydroxy-3-methoxyflavone 6-*O*-β-D-glucopyranoside	7.41 *'m'* *J*=9	7.39 *'s'*	6 65 *d* *J*=9	—	6.28 *s*	4.79 *d* *J*=8.5	3.70	—	—	—	3.72 −0.02	—	—	—
5,6,3',4'-Tetrahydroxy-3,7-dimethoxyflavone 6-*O*-β-D-glucopyranoside	7.42 *'m'* *J*=9	7.34 *'s'*	6.60 *d* *J*=9	—	6.27 *s*	4.73 *d* *J*=8.5	3.67	—	—	3.77	3.70 −0.03	—	—	3.18 +0.59
5,6,4'-Trihydroxy-3,7,3'-trimethoxyflavone 6-*O*-β-D-glucopyranoside	7.30 *dd* *J*=2 5 *J*=9	7 42 *d* *J*=2.5 *J*=9	6.60 *d*	—	6.29 *s*	4.80 *d* *J*=8	3.62	3.68	—	3.70	3.53 +0.09	3.05 +0.63	—	3.19 +0.51
5,3',4'-Trihydroxy-3,6,7-trimethoxyflavone 4'-*O*-β-D-glucopyranoside	7.31 *dd* *J*=2 5 *J*=9	7.39 *d* *J*=2.5	6.73 *d* *J*=9	—	6.28 *s*	4.84 *d* *J*=8.5	3.58	—	3.73	3.80	3.59 −0.01	—	3.69 +0.04	3.20 +0.60
5,4'-Dihydroxy-3,6,7,3'-tetramethoxyflavone	7.42 *dd* *J*=2.5 *J*=9	7.56 *d* *J*=2.5 *J*=9	6.78 *d*	—	6.43 *s*	—	3.74	3.96	3.86	3.90	3.70 +0 04	3.46 +0.50	3.80 +0.06	3.28 +0.62
5,4'-Dihydroxy-3,6,7,3'-tetramethoxyflavone 4'-*O*-β-D-glucopyranoside	7 21 *dd* *J*=2.5 *J*=9	7.37 *d* *J*=2.5	6.59 *d* *J*=9	—	6.24 *s*	4.81 *d* *J*=8	3.54	3.78	3.66	3 71	not determined			

s = singlet; *d* = doublet; *dd* = double doublet; *'m'* = one peak partially obscured; *'s'* = overlap with another peak.
Chemical shift data are expressed as a relative to TMS; *J* values are expressed in Hz.
Spectra were obtained on a Varian HA-100 instrument.

[7]. This discrepancy was likely due to the necessity of working with very small amounts of wet compound.

The second aglucone gave the highest mass ion *m/e* = 332 consistent with $C_{16}H_{12}O_8$ which indicates a single *O*-methyl group. Four aromatic protons were assigned as follows: a singlet at δ 6.28 for H-8; a doublet at δ 6.65 (*J* = 9 Hz) for H-5'; a slightly broadened singlet at δ 7.39 for H-2'; and a doublet at δ 7.41 (*J* = 9 Hz) for H-6'. The existence of 3',4'-dihydroxylation in both aglucone and glucoside was indicated by borate shifts in the UV spectra of both. The mass spectrum showed the expected (B^+) fragment *m/e* = 137 (16%) for such a B-ring pattern.

The UV data showed unsubstituted 5- and 7-hydroxyl groups in the glucoside and were similar to patuletin 3-*O*-glycosides. The aglucone had a similar appearance suggesting that the 3-*O*-substituent was not removed during acid hydrolysis. The presence of a 3-*O*-methyl group is supported by the observation of a 0.02 ppm benzene-induced shift in the *O*-methyl proton peak. The aglucone is thus 5,6,7,3',4'-pentahydroxy-3-methoxyflavone. The glucoside is the 6-*O*-β-D-glucopyranoside. The observed mp were glucoside 197–199° and aglucone 218–220°. No comparative data were available.

The aglucone of group three gave the highest mass ion *m/e* = 346, consistent with a hexaoxygenated system with two *O*-methyl groups ($C_{17}H_{12}O_8$). Four aromatic protons were present with resonances essentially identical to those discussed above. Lack of an acetate shift of Band II in the UV spectrum coupled with a +0.59 ppm benzene-induced shift of one of the *O*-methyl signals places a methoxyl function at C-7. The aglucone behaviour in UV light again suggested the presence of a 3-*O*-methyl group, an assignment supported by the benzene-induced shift. The aglycone and one of its glycosylated derivatives displayed characteristic borate shifts indicating unsubstituted 3',4'-dihydroxylation. This is supported by a (B^+) fragment with *m/e* = 137 (20%). The aglucone must be 5,6,3',4'-tetrahydroxy-3,7-dimethoxyflavone in agreement with Jay and Voirin [8]. The monoglucoside is 6-*O*-β-D-glucopyranoside. A compound having the chromatographic characteristics of a dimonside was also isolated which gave the above monoside plus another on partial hydrolysis and the aglucone and glucose on total hydrolysis. The UV spectrum was characteristic of a flavonol with substituted 3- and 4'-hydroxyl groups. The dimonoside is most probably the 6,4'-di-*O*-β-D-glucopyranoside. Melting points are 184–188° for the aglucone and 190–192° for the 6-*O*-glucoside.

The fourth *O*-methylated flavonol also exists as a monoglucoside and a dimonoglucoside. The aglucone had the highest mass ion *m/e* = 360 indicative of a hexaoxygenated structure with three *O*-methyl groups ($C_{18}H_{16}O_8$). Four aromatic protons could be assigned to H-8, H-2', H-6' and H-5'. Two of the *O*-methyl groups behaved as did the two from the aglucone above. The third *O*-methyl resonance exhibited a +0.63 ppm benzene-induced shift which could place it at either the 3'- or 4'-position. This is clearly supported by the absence of a borate shift in Band I of the UV spectrum and by the appearance of a mass spectral fragment *m/e* = 151 (12%) representing (B^+). A substantial shift of Band I of the aglucone with sodium acetate argues for an unsubstituted 4'-hydroxyl group. The aglucone is thus 5,6,4'-trihydroxy-3,7,3'-trimethoxyflavone. These data are in agreement with those of Jay and Voirin [8]. Melting point of the isolated aglucone was 214–216° [9]. The monoglucoside has unsubstituted 4'- and 5-hydroxyl groups as shown by its UV spectra; it must be the 6-β-D-glucopyranoside. The observed mp was 224–225° (softens 160–165°) while the reported value is 206–207° [9]. We are confident in the purity of our samples; it seems that the differences in mps are due to slight impurities in the material of Shimizu and Morita [9]. The diglucoside showed the presence of an unsubstituted 5-hydroxyl group and is most likely the 6,4'-di-β-D-glucopyranoside.

The aglucone of group five had a mass ion *m/e* = 374 consistent with a hexaoxygenated system having four

Table 2. *O*-Methylated flavonoids of *Chrysosplenium* species

Compounds	Species	Group*	Reference
5,4′-Dihydroxy-3,6,7-trimethoxyflavone	*alternifolium*	Alt.	[15]
	tetrandrum	Alt.	Present work
5,4′-Dihydroxy-3,6,7-trimethoxyflavone-4′-glucoside	*flagelliferum*	Alt.	[7]
	tetrandrum	Alt.	Present work
5,6,7,3′,4′-Pentahydroxy-3-methoxyflavone	*tetrandrum*	Alt.	Present work
5,6,7,3′,4′-Pentahydroxy-3-methoxyflavone-6-glucoside	*tetrandrum*	Alt.	Present work
5,6,3′,4′-Tetrahydroxy-3,7-dimethoxyflavone	*alternifolium*	Alt.	[15]
	tetrandrum	Alt.	Present work
	oppositifolium	Opp.	[8]
5,6,3′,4′-Tetrahydroxy-3,7-dimethoxyflavone-6-glucoside	*tetrandrum*	Alt.	Present work
5,6,3′,4′-Tetrahydroxy-3,7-dimethoxyflavone-6,4′-diglucoside	*tetrandrum*	Alt.	Present work
5,6,4′-Trihydroxy-3,7,3′-trimethoxyflavone	*tetrandrum*	Alt.	Present work
	oppositifolium	Opp.	[8]
5,6,4′-Trihydroxy-3,7,3′-trimethoxyflavone-6-glucoside	*tetrandrum*	Alt.	Present work
	maximowiczii	Opp.	[9]
5,6,4′-Trihydroxy-3,7,3′-trimethoxyflavone-6,4′-diglucoside	*tetrandrum*	Alt.	Present work
5,3′,4′-Trihydroxy-3,6,7-trimethoxyflavone	*alternifolium*	Alt.	[15]
	tetrandrum	Alt.	Present work
5,3′,4′-Trihydroxy-3,6,7-trimethoxyflavone-4′-glucoside	*tetrandrum*	Alt.	Present work
	japonicum	Alt.	[11–13]
5,4′-Dihydroxy-3,6,7,3′-tetramethoxyflavone	*alternifolium*	Alt.	[15]
	tetrandrum	Alt.	Present work
	oppositiofolium	Opp.	[8]
5,4′-Dihydroxy-3,6,7,3′-tetramethoxyflavone-4′-glucoside	*tetrandrum*	Alt.	Present work
	tosaense	Alt.	[10]
	japonicum	Alt.	[11–13]
5,4′-Dihydroxy-3,6,7,2′-tetramethoxyflavone	*pseudo-fauriei*	Opp.	[14]
5,2′,5′-Trihydroxy-3,7,4′-trimethoxyflavone-2′-glucoside	*grayanum*	Opp.	[7]
5,2′-Dihydroxy-3,7,4′,5′-tetramethoxyflavone-2′-glucoside	*grayanum*	Opp.	[7]

* Alt. = Alternifolia, Opp. = Oppositifolia.

O-methyl groups ($C_{19}H_{18}O_8$). Four aromatic protons were placed at H-8, H-2′, H-6′ and H-5′ as discussed above. A (B^+) fragment of m/e = 151 (15%) indicates a B-ring with one hydroxyl and one methoxyl. An (A^+) fragment of m/e = 196 (5%) indicates the presence of two methoxyl groups in the A-ring. UV data suggest the presence of a methoxyl group at C-3. An acetate-derived shift in Band I of the aglucone suggests an unsubstituted 4′-hydroxyl group. An unsubstituted 5-hydroxyl group is indicated by the $AlCl_3$ shift. The aglucone is thus 5,4′-dihydroxy-3,6,7,3′-tetramethoxyflavone, in agreement with Jay and Voirin [8]. Mp of the isolated aglucone was 182–185°; literature mp 186–187° [10]. There is no sodium acetate shift of Band I of the glucoside. The natural glucoside is the 4′-*O*-β-D-glucopyranoside, mp 224–226°; lit. mp 226–228° [10].

The last aglucone gave a maximum mass ion m/e = 360 consistent with $C_{18}H_{16}O_8$, a hexaoxygenated system with three *O*-methyl groups. The aromatic protons were assigned to H-8, H-2′, H-6′ and H-5′. A (B^+) fragment in the mass spectrum with m/e = 137 (24%) and a 28 nm borate shift of Band I established the existence of 3′,4′-dihydroxylation. The *O*-methyl groups are located at C-3, C-6 and C-7 which gives 5,3′,4′-trihydroxy-3,6,7-trimethoxyflavone as the structure of the aglucone. The absence of a bathochromic shift of Band I with sodium acetate for the glucoside places the sugar at position-4′. Mp for the isolated glucoside was 164–166°; lit. mp 163–165° [11]. The aglucone had mp 243–245°; lit. mp 236–238° [11].

DISCUSSION

The flavonoid chemistry of the genus *Chrysosplenium* first attracted the attention of Japanese workers who studied *C. flagelliferum* [7], *C. grayanum* [7], *C. japonicum* [12, 13] *C. maximowiczii* [9] and *C. tosaense* [10]. Russian workers added *C. pseudo-fauriei* [14] while Jay and coworkers [8, 15] have recently reported studies of European collections of *C. alternifolium* and *C. oppositifolium*. Noteworthy characteristics of the flavonoids reported from the genus are a high degree of *O*-methylation and the presence of 6- and/or 2′-substitution. The structures known from the genus, including the present results, are presented in Table 2.

The present description of the flavonoids of *C. tetrandrum* represents the first report of a North American member of the genus although this species is not restricted to this continent [1]. This study also apparently represents the first attempt to examine the total flavonoid complement of a member of the genus. The major flavonoids of *C. tetrandrum* did indeed consist of *O*-methylated compounds but kaempferol and quercetin were isolated as their 3-*O*-arabinosides, 3-*O*-glucosides, 3-*O*-glucosylarabinosides, 3-*O*-glucosylglucosides, and 3-*O*-rutinosides.

Jay and Voirin [8] compared the degree of *O*-methylation of flavonoids from the two sections and concluded that members of section Oppositifolia possessed a higher degree of *O*-methylation on the B-ring than did members of section Alternifolia. Two other structural features

deserve comment. One is the apparent restriction of 2'-hydroxylation to members of section Oppositifolia. These compounds have so far been reported from *C. grayanum* [7] and *C. pseudo-fauriei* [14] and have now been found in *C. americanum* (Collins, unpublished) and *C. glechomaefolium* (Bohm, unpublished). The second feature is the restriction of flavonoids lacking substitution at position-6 to section Oppositifolia [7,14, Collins unpublished, Bohm unpublished]. As Jay and Voirin [8] point out, however, too few members of the genus have been examined chemically to give such conclusions firm foundation.

Chrysosplenium has consistently been placed in tribe Saxifrageae DC., subtribe Saxifraginae Engler [16]. But, as Spongberg [3] has pointed out, its affinities within this group are unclear. Of the members of the Saxifraginae (*sensu* Engler) studied to date only *Chrysosplenium* possesses the capacity to perform 2'- and/or 6-hydroxylation of the flavonoid nucleus and to make a variety of *O*-methylated derivatives. These characters do indeed allow a chemotaxonomist to question the taxonomic placement of the genus [8].

The question, however, is not a straightforward one involving *O*-methylation in *Chrysosplenium* and its absence from all other members of the group. Our work with *Heuchera micrantha* var. *diversifolia* [17] and *H. cylindrica* var. *glabella* (Bohm and Wilkins, unpublished) show that these taxa have the capacity to make small quantities of isorhamnetin (3'-*O*-methylquercetin), laricitrin (3'-*O*-methylmyricetin) and syringetin (3',5'-di-*O*-methylmyricetin). Thus, we have shown that *bona fide* members of the subtribe (representing a genus thought by some to be ancestral to the other members of the group) have the capacity for flavonol *O*-methylation. How widely this capacity is distributed in the family is unknown. It is also important to point out that the genus *Chrysosplenium* accumulates simple mono- and diglycosides of kaempferol and quercetin of the same sort seen in other members of the subtribe [17].

EXPERIMENTAL

Plant material. Chrysosplenium tetrandrum (Lund) T. Fries was collected on 6 July 1973 11 miles south of the junction of the Stewart–Watson Lake Road and the Alaska Highway at an elevation of 975 m. A voucher specimen has been deposited in UBC (No. 730268).

Isolation and purification. Plant material was exhaustively extracted with 80% MeOH. The extract was evapd to a small vol. and chromatographed preparatively on Polyamide DC 6.6 plates using toluene–EtOAc–EtOH (2:1:1). Bands were located in UV light (366 nm), scraped from the plates and eluted with MeOH. These fractions were rechromatographed on the same medium in one or more of the following solvent systems: (1) C_6H_6–MeOH–MeCOEt–H_2O (55:20:23:2); (2) toluene–HCO_2Et–EtOH–H_2O (60:20:19:1); (3) cyclohexane–HCO_2Et–*n*-butyl acetate–HCO_2H (25:50:23:2); and (4) H_2O–*n*-BuOH–Me_2CO–dioxane (14:3:2:1). Bands were marked, scraped and eluted with MeOH as above. Final purification of chromatographically homogeneous compounds was achieved by crystallization from MeOH. Chromatographic characteristics and a visual estimate of conc of the compounds are presented in Table 3.

Spectroscopic methods. UV spectral methods used were standard procedures [5]. PMR spectra were obtained using CCl_4 or C_6D_6 as solvent with TMS as internal standard. MS were deter-

Table 3. Chromatographic data for *C. tetrandrum* flavonoids

Compound	Amount present*	$R_f \times 100$†			
		1	2	3	4
Kaempferol	1	65	20	10	3
Kaempferol 3-*O*-arabinoside	2	55	18		35
Kaempferol 3-*O*-glucoside	2	48	15		41
Kaempferol 3-*O*-glucosylarabinoside	1	43	9		58
Kaempferol 3-*O*-glucosylglucoside	1	35	7		68
Kaempferol 3-*O*-rhamnosylglucoside	1	41	8		61
Quercetin	1	41	12	5	2
Quercetin 3-*O*-arabinoside	2	30	10		32
Quercetin 3-*O*-glucoside	1	26	8		39
Quercetin 3-*O*-glucosylarabinoside	1	16	4		55
Quercetin 3-*O*-glucosylglucoside	1	14	3		66
Quercetin 3-*O*-rhamnosylglucoside	2	15	3		61
5,4'-Dihydroxy-3,6,7-trimethoxyflavone	1	98	82	56	41
5,4'-Dihydroxy-3,6,7-trimethoxyflavone 4'-glucoside	2	84	44		78
5,6,7,3',4'-Pentahydroxy-3-methoxyflavone	1	41	40	29	2
5,6,7,3',4'-Pentahydroxy-3-methoxyflavone 6-glucoside	2	22	8		27
5,6,3',4'-Tetrahydroxy-3,7-dimethoxyflavone	3	71	37	19	20
5,6,3',4'-Tetrahydroxy-3,7-dimethoxyflavone 6-glucoside	5	25	10		71
5,6,3',4'-Tetrahydroxy-3,7-dimethoxyflavone 6,4'-diglucoside	2	6	0		80
5,6,4'-Trihydroxy-3,7,3'-trimethoxyflavone	2	89	69	46	36
5,6,4'-Trihydroxy-3,7,3'-trimethoxyflavone 6-glucoside	4	56	36		79
5,6,4'-Trihydroxy-3,7,3'-trimethoxyflavone 6,4'-diglucoside	2	10	0		88
5,3',4'-Trihydroxy-3,6,7-trimethoxyflavone	4	93	65	38	30
5,3',4'-Trihydroxy-3,6,7-trimethoxyflavone 4'-glucoside	3	61	32		69
5,4'-Dihydroxy-3,6,7,3'-tetramethoxyflavone	4	99	88	70	51
5,4'-Dihydroxy-3,6,7,3'-tetramethoxyflavone 4'-glucoside	3	74	38		63

* Estimate of amounts of material present; 1 is smallest amount, 5 is the largest.

† For solvent key, see Experimental.

mined using a direct inlet solids probe at 70 eV ionization potential and trap current of 100 μA. The source temp. was sufficiently high (*ca* 300°) to effect cleavage between the sugar and aglucone portion of the flavonoids. Under these conditions the MS were those of the aglucones free of interfering carbohydrate fragment ions.

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