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THE OCCURRENCE OF ACYLATED FLAVONOL GLYCOSIDES IN THE CRUCIFERAE

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Key Word Index—*Sisymbrium gilliesii*; *Crambe tataria*; *C. cordifolia*; *C. scaberrima*; Cruciferae; acylated flavonol glycosides.

Abstract—Nine acylated glycosides of kaempferol or quercetin were identified in *Sisymbrium gilliesii*, and in three *Crambe* spp. They were usually present together with the related unacylated glycosides. Acylation is a very common characteristic of the four crucifer species studied.

INTRODUCTION

Acylated flavonoids with aromatic acids, or, less frequently, aliphatic acids have been identified in a number of plant families [1–5]. In the Cruciferae, although several acylated anthocyanins have been reported [6], no acylated flavonols have been described [7, 8]. A survey of the Cruciferae has now shown the presence of several neutral or acylated flavonol glycosides. In this paper we report on the occurrence of nine acylated flavonol glycosides in one species of *Sisymbrium* and three of *Crambe*.

RESULTS

Nine acylated compounds were isolated from *Sisymbrium gilliesii* Romanczuk, *Crambe tataria* Sebeök, *C. cordifolia* Steven and *C. scaberrima* Webb. The leaf material was extracted with 80% methanol and the compounds were purified by PC. Fig. 1 shows the relative positions of the acylated flavonoids and their unacylated glycosides on the 2D-chromatogram. The R_f values and the colour characteristics of the nine compounds are given in Table 1.

Compounds **5a** and **5b** from *S. gilliesii*, **1a** and **1b** from *C. tataria*, **13a** and **13b** from *C. cordifolia* and compound **6** from *C. scaberrima* are all acylated glycosides of kaempferol or quercetin. All (except **5a** and **5b**) showed a UV spectrum with a maximum at *ca* 320 nm for band 1 (Table 3), suggesting the presence of an aromatic acid moiety.

Four compounds were further isolated from component **5** on the 2D-chromatogram (Fig. 1). Two were completely identified (**5a** and **5b**, Table 1). The absorption spectra of the mixture (Table 3) suggest that positions 3 and 4′ are blocked. Kaempferol, quercetin and glucose were found after acid hydrolysis (2 N HCl for 45 min). Furthermore, controlled acid hydrolysis (3 min) liberated kaempferol 4′-glucoside and quercetin 4′-gluocside (Table 2). Component **5** was also treated with β-glucosidase, liberating glucose from the 4′-position and yielding four new acylated compounds. These four are very similar and would not be completely separated.

After isolation and alkaline hydrolysis, **5a** yielded kaempferol 3-glucoside plus 2-hydroxypropionic acid, and **5b** yielded quercetin 3-glucoside plus malonic acid (Tables 2 and 3), indicating that the acids are attached to the 3-*O*-glucose moiety.

It is therefore assumed that **5a** is a kaempferol 3 - (2 - hydroxypropionyl) - glucoside - 4′ - glucoside (Table 1) and that **5b** is a quercetin 3 - malonyl-glucoside - 4′ - glucoside. However, the presence of four compounds after enzymatic hydrolysis suggests that each aglycone occurs combined with both 2-hydroxypropionic acid and malonic acid, i.e. component **5** contains two kaempferol and two quercetin

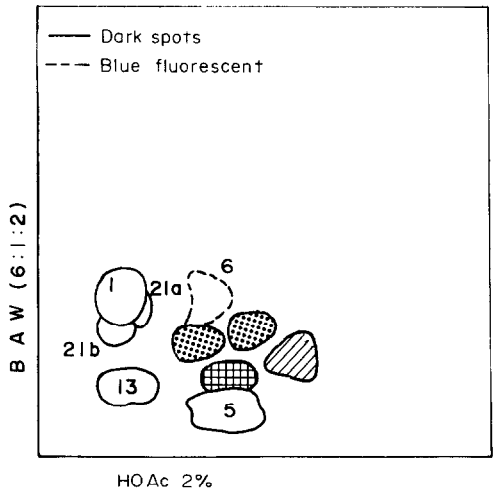

Fig. 1. Pattern of acylated flavonol glycosides in *S. gilliesii*, *C. tataria*, *C. cordifolia*, *C. fruticosa* and their unacylated glycosides. (▨) Unacylated glycoside corresponding to spot **6** (kaempferol 3 - di - glucoside 7 - rhamnoside, Table 2); (▩) unacylated glycosides corresponding to spots **5** and **1** (kaempferol and quercetin 3,4′ - di - glucoside, Table 2); (▦) unacylated glycosides corresponding to spot **21a** and **21b** (kaempferol and quercetin 3,7 - di - glucoside, Table 2).

Table 1. Chromatographic data of nine compounds analysed

Component	Flavonoid	Colour UV/+NH_3	$R_f \times 100$* BAW	$R_f \times 100$* 2% HOAc
5a	Kaempferol 3-(2-hydroxypropionyl)glucoside-4′-glucoside	Dk/Dk	13	38
5b	Quercetin 3-malonylglucoside-4′-glucoside	Dk/Dk	13	40
1a	Kaempferol 3-(*p*-coumaroyl)glucoside-4′-glucoside	Dk/YG	34	18
1b	Quercetin 3-feruloylglucoside-4′-glucoside	Dk/YG	32	18
21a	Kaempferol 3-feruloylglucoside-7-glucoside	Dk/YG	32	21
21b	Quercetin 3-feruloylglucoside-7-glucoside	Dk/Y	28	18
13a	Kaempferol 3-(*p*-coumaroyl)glucoside-7,4′-diglucoside	Dk/Dk	16	20
13b	Quercetin 3-feruloylglucoside-7,4′-diglucoside	Dk/Dk	16	20
6	Kaempferol 3-(*p*-coumaroyl)feruloyl-diglucoside-7-rhamnoside	Fl. Blue/ + Intes.	34	37

*R_f values are measured from 2DPC (Whatman No. 1).
Key: Dk = dark, G = green, Y = yellow, Fl. = fluorescent.

Table 2. Chromatographic data of the main intermediate products from hydrolysis of the nine compounds analysed

Compounds	Colour in UV/+NH_3	PC* BAW	PC* 2% HOAc	TLC† BAW	TLC† 50% HOAc	TLC† 15% HOAc	TLC† Phenol	TLC† EtOH–H_2O–AcCH_2Ac
Kaempferol 3-(*p*-coumaroyl)glucoside(tiliroside)	Dk/YG	75	15	88	80	25	84	14
Quercetin 3-feruloylglucoside	Dk/Y	62	12	82	80	24	80	14
Kaempferol 3-feruloylglucoside	Dk/YG	78	10	82	83	29	72	14
Kaempferol 3-(2-hydroxy-propionyl)glucoside	Dk/YG	65	19	78	—	49	87	12
Quercetin 3-malonylglucoside	Dk/Y	48	17	62	—	45	47	12
Kaempferol 3,4′-diglucoside	Dk/Dk	28	47	41	83	59	53	67
Quercetin 3,4′-diglucoside	Dk/Dk	26	34	36	78	56	40	63
Kaempferol 3-7-diglucoside	Dk/Y	20	43	25	—	73	73	69
Quercetin 3-7-diglucoside	Dk/Y	18	32	23	—	65	62	69
Kaempferol 3-diglucoside-7-rhamnoside	Dk/YG	26	56	42	—	75	73	72

*Whatman No. 1.
†Cellulose.

Table 3. Spectral data of the acylated flavonol glycosides identified

Component	UV λ_{max} (nm)					
	80% MeOH	+ KOH	+ NaOAc	+ NaOAc–H_3BO_3	+ $AlCl_3$	+ $AlCl_3$–HCl
5	267, 288sh 338	278, 372	272, 295 362	268, 348	276, 298sh 342, 394	272, 296sh 340, 394
1	264, 300sh 320	270, 294sh 368	273, 314 380	267, 320	276, 298 324, 390	273, 298 324, 390
21a	268, 330	274, 300sh 384	268, 332 415sh	—	276, 300sh 332, 400	276, 300sh 330, 400
21b	254, 270 332	273, 390	255, 270 335, 415sh	—	276, 300sh 334, 400	276, 300sh 334, 400
13	266, 325	274, 368	269, 360	—	273, 300sh 320, 410	275, 300sh 320, 410
6	265, 325	274, 295sh 382	265, 325 420	—	274, 300 330, 400	274, 300 330, 400

glycosides acylated with 2-hydroxypropionic and malonic acid respectively.

Two compounds, **1a** and **1b**, were found in component 1 (Fig. 1 and Table 1). The basic unacylated glycosides of both compounds were the same as those in **5a** and **5b** respectively, differing only in the nature of the acids attached to them. Kaempferol, quercetin, the intermediate glycosides (kaempferol and quercetin 4′-glucosides) and glucose were obtained after 15 min acid hydrolysis. Alkaline hydrolysis gave *p*-coumaric and ferulic acids, together with the 3,4′-diglucosides of kaempferol and quercetin. Enzymatic hydrolysis liberated the sugar attached at the 4′-position and gave two acylated compounds: one chromatographically identical to kaempferol 3 - (*p* - coumaroyl)glucoside (tiliroside) (Tables 2 and 3) and the other identified after alkaline hydrolysis, as quercetin 3-feruloylglucoside (Tables 2 and 3). Therefore the structures of **1a** and **1b** are kaempferol 3 - (*p* - coumaroyl) - glucoside - 4′ - glucoside and quercetin 3 - feruloylglucoside - 4′ - glucoside respectively.

Component 13 also contains two compounds, **13a** and **13b** (Table 1). Its absorption spectra (Table 3) show that positions 3, 7 and 4′ are substituted. After acid hydrolysis (30 min) it yielded kaempferol, quercetin and glucose, but after partial acid hydrolysis (5 min), it produced both aglycones together with four intermediates identified as kaempferol 4-glucoside, kaempferol 7-glucoside, quercetin 4′-glucoside and quercetin 7-glucoside. Alkaline hydrolysis yielded *p*-coumaric and ferulic acids plus several intermediates. They were the 3,4′-diglucoside of kaempferol and quercetin (because the glucose at position 7 was also partially liberated) and a small amount of triglycosides which presumably are the 3,7,4′ - triglucosides of kaempferol and quercetin. After β-glucosidase treatment, component **13** lost the glucose residues at positions 7 and 4′, giving two acylated products identical to those obtained from **1a** and **1b**: kaempferol 3 - (*p* - coumaroyl) - glucoside and quercetin 3 - feruloylglucoside. It follows that the structure of **13a** is kaempferol 3 - (*p* - coumaroyl) - glucoside - 7, 4′-diglucoside and **13b** is quercetin 3 - feruloylglucoside - 7,4′ - diglucoside.

Compounds **21a** and **21b**, in contrast to the other components, separated on the 2D-chromatogram from which they were isolated. By acid hydrolysis (15 min) **21a** yielded kaempferol and its 7-glucoside, whereas **21b** yielded quercetin and its 7-glucoside. The alkaline hydrolysis of **21a** and **21b** liberated both ferulic acid and kaempferol and quercetin respectively. Treatment with β-glucosidase hydrolysed the glucose from position 7, giving two acylated intermediates: kaempferol 3 - feruloylglucoside and quercetin 3 - feruloylglucoside. Thus **21a** is kaempferol 3 - feruloylglucoside - 7 - glucoside and **21b** is quercetin 3 - feruloylglucoside - 7 - glucoside.

Surprisingly, compound **6** appeared as a blue spot on paper. The reason for this is not obvious. Its absorption spectrum indicates that the hydroxyl groups at positions 3 and 7 are substituted (Table 3). Acid hydrolysis (3 min) liberated kaempferol plus glucose and rhamnose. Complete hydrolysis, accomplished after only 3 min, suggested that rhamnose was attached to the hydroxyl at position 7, because at that position rhamnose is more readily hydrolysed than glucose [9]. After 1 min acid hydrolysis, **6** produced detectable amounts of kaempferol 7-rhamnoside, demonstrating that the acyl group was attached to the sugars at position 3. Its structure was confirmed by alkaline hydrolysis, which yielded a triglycoside, identified as kaempferol 3 - diglucoside - 7 - rhamnoside, plus *p*-coumaric and ferulic acids. Furthermore, **6** was not hydrolysed by β-glucosidase, thus supporting the proposed structure. The intermediate product obtained after alkaline hydrolysis (kaempferol 3 - diglucoside - 7 - rhamnoside) produced kaempferol 7-rhamnoside after 20 hr treatment with β-glucosidase. From these data the structure assigned to **6** is kaempferol 3 - (*p* - coumaroyl) - ferulcyl-diglucoside - 7 - rhamnoside.

DISCUSSION

All nine acylated compounds identified have not been previously reported. All the acylated compounds shown here, except **13a** and **13b** have their unacylated glycosides present in the same species (Fig. 1). For example, the 3,4′-diglucosides of kaempferol and quercetin (Table 2) occur together with their

acylated derivatives (**5a**, **1a**, **5b** and **1b**) in *S. gilliesii*, *C. tataria* and *C. cordifolia*. The same is true for **6**, the unacylated glycoside of which (kaempferol 3 - diglucoside - 7 - rhamnoside) also appears in *C. scaberrima*.

Compounds **13a** and **13b** are of particular interest because they are substituted at positions 3, 7 and 4'. Although the related unacylated glycoside was not detected, their structures are interesting because they are so complex (Table 1).

It was observed that on the 2D-chromatogram those compounds containing cinnamic acids (Fig. 1, compounds **1a**, **1b** and **6**) have R_f values slightly higher in BAW and lower in 2% acetic acid than their corresponding unacylated glycoside (Fig. 1). But the compounds containing an aliphatic acid, as in **5a** and **5b** show chromatographic behaviour similar to that of a more highly glycosylated compound (Fig. 1). Another noteworthy chromatographic characteristic of the acylated compounds is their behaviour on polyamide in EtOH–H_2O–acetylacetone (4:2:1). Under these conditions, they show R_f values significantly lower than the corresponding unacylated glycosides (Table 2).

The high frequency of acylated flavonols in the species examined is of interest because acylation has been previously reported only for anthocyanins in the Cruciferae[6]. Other acylated flavonols were also found in other species, and their complete identification is in progress. On the basis of these results and some preliminary results in other genera, it appears that acylation of the flavonols is a common phenomenon in this plant family.

EXPERIMENTAL

Plant material. The plant material surveyed was dried leaf tissue kindly supplied by Professor C. Gómez Campo from the Universidad Politécnica de Madrid.

Methods of flavonoid isolation. Plant specimens were ground to a powder and extracted with 80% MeOH (×3). Extracts were applied on Whatman 3 MM sheets and chromatographed in 2D using BuOH–HOAc–H_2O (6:1:2) and 2% HOAc. Individual compounds were isolated by elution from the paper chromatograms and purified by PC.

Identification procedures. (a) *Acid hydrolysis.* Routinely carried out with 2M HCl at controlled times. The aglycones and intermediate products were identified by PC, TLC and UV spectra using standard methods[10, 11]. R_f values for all compounds are given in Tables 1 and 2. (b) *Alkaline hydrolysis* with 2M NaOH was carried out at room temp. under vacuum for 4 hr. The product was then passed through a column of Rexyn 101 (H) (fisher) and eluted with H_2O. The eluate was concd and extracted with Et_2O and this extract analysed for the presence of acids (see below). From the remaining aq. soln the intermediate glycosides and the aliphatic acids were identified. (c) *Enzymatic hydrolysis* was carried out with β-glucosidase at 37°.

Identification of acids. p-Coumaric, ferulic and sinapic acids were identified from the Et_2O extract of the akaline hydrolysis product using C_6H_6–HOAc–H_2O (6:7:3) and sodium formate on cellulose. The acids were detected in UV light. The presence of malonic and 2-hydroxypropionic acids was verified by using *n*-BuOH–HCOOH–H_2O (4:1:5) and *n*-PrOH–1M NH_4OH on cellulose[10] and on Si gel using EtOH–$CHCl_3$–NH_4OH–H_2O (53:30:15:1.5)[3]. Acid spots were co-chromatographed with markers and located by spraying with a mixture of $KMnO_4$–Na_2CO_3–bromocresol green–bromothymol blue[12]. Identification of sugars was carried out by TLC on Si gel pretreated according to Hansen[13].

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REFERENCES

1. Harborne, J. B. and Williams, C. A. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds) Chapman & Hall, London.
2. Woldecke, M. and Herrmann (1974) *Z. Naturforsch. Teil. C* **29**, 355.
3. Markham, K. R., Zinsmeister, H. D. and Mues, R. (1978) *Phytochemistry* **17**, 1601.
4. Wagner, H., Iyengar, M. A., Michaeles, E. and Herz, W. (1971) *Phytochemistry* **10**, 2547.
5. Chari, V. M., Harborne, J. B. and Williams, C. A. (1980) *Phytochemistry* **19**, 983.
6. Harborne, J. B. (1967) in *Comparative Biochemistry of Flavonoids*, Academic Press, London.
7. Durkee, A. B. and Harborne, J. B. (1973) *Phytochemistry* **12**, 1085.
8. Bacon, J. and Mabry, T. J. (1976) *Phytochemistry* **15**, 1087.
9. Ribereau-Gayon, P. (1972) *Plant Phenolics*. Oliver & Boyd, Edinburgh.
10. Harborne, J. B. (1973) *Phytochemical Methods*. Chapman & Hall, London.
11. Mabry, T. J., Markhan, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.
12. Paskova, J. and Monk, V. (1960) *J. Chromatogr.* **4**, 241.
13. Hansen, S. A. (1975) *J. Chromatogr.* **107**, 224.