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FLAVONOL GLYCOSIDES IN *BRASSICA* AND *SINAPIS**

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Abstract—The 7-glucosides and 3,7-diglucosides of kaempferol and isorhamnetin were identified in leaves and flowers of *Sinapis arvensis*. Additionally, the 3-sophoroside-7-glucosides of kaempferol, quercetin and isorhamnetin were found in leaves of *S. arvensis* and *Brassica oleracea*. Two dimensional surveys of leaf extracts of 27 species and cultivars of *Brassica* and *Sinapis* showed that the same pattern occurred in most species. *B. tournefortii* and *S. flexuosa* were exceptional in having flavonol 3-monosides and 3-diglycosides instead. The results suggest that it is the glycosidic patterns, rather than the distribution of the flavonol aglycones, which are likely to be of taxonomic value for distinguishing groups of species or genera within the Cruciferae.

INTRODUCTION

A NUMBER of unusual flavonol glycosides, including derivatives of isorhamnetin, have been identified in various members of the Cruciferae but no systematic survey has yet been attempted. Isorhamnetin 3,4'-diglucoside was identified in the seed[1] and kaempferol 3-rhamnosylarabinoside-7-arabinoside in the petal[2] of *Matthiola incana*, while robinin (kaempferol 3-galactosylrhamnoside-7-rhamnoside) has been found in *Cheiranthus cheiri*.[3] In *Brassica napus* and *Sinapis arvensis*, Horhammer *et al.*[4,5] found the 3-glucoside and other glycosides of isorhamnetin. Paris and Charles[6] reported kaempferol 3-glucoside in leaves of *Sinapis alba*, while Francois and Chaix[7] found rutin in the seed of *Brassica rapa* (syn. *B. campestris*).

The purpose of the present investigation was a more systematic study of the flavonoids in *Brassica* and *Sinapis*, especially those species and cultivars of economic importance such as mustards and rapes.

RESULTS AND DISCUSSION

The results of a survey of the flavonol glycosides in 27 species and cultivars of *Brassica* and *Sinapis* conducted by 2-D paper chromatography, are presented in Table 1. These results are based on detailed isolation and identification of flavonol glycosides from 6

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[1] RAHMAN, A. V. and KHAN, M. S. (1962) *Z. Naturforschg.* **17b**, 9.
[2] HARBORNE, J. B. (1965) *Phytochemistry* **4**, 107.
[3] MAKSYUTINA, N. P. (1965) *Chem. Abstr.* **63**, 1858.
[4] HORHAMMER, L., WAGNER, H., ARNDT, H. G., KRAEMER, H. and FARKAS, L. (1966) *Tetrahedron Letters* 567.
[5] HORHAMMER, L. and WAGNER, H., personal communication.
[6] PARIS, R. R. and CHARLES, A. (1962) *Compt. Rend.* **254**, 325.
[7] FRANCOIS, M. T. and CHAIX, L. (1961) *Chem. Abstr.* **55**, 3744.

plants available in some quantity (Table 2). In making these analyses, one problem was the rather low concentrations of flavonol glycosides present, particularly in seeds and in young seedlings. Concomitantly, the very high concentrations of sinapin and related cinnamic acid esters in the plants interfered with separation and identification. Repeated paper chromatographic separations were necessary to obtain the glycosides pure. A second problem was the tendency of the equivalent glycosides of isorhamnetin and quercetin or of isorhamnetin and kaempferol to resist separation (see Table 2 and Experimental) even after chromatography in a range of solvents. This seems to occur whenever isorhamnetin is a major flavonol constituent and has been encountered in similar studies in the Umbelliferae.[8]

TABLE 1. DISTRIBUTION OF FLAVONOL GLYCOSIDES IN *Brassica* AND *Sinapis*

Plant species or cultivar†	Flavonol glycosides* 7-Glucoside	3-Glycoside	3,7-Di-glucoside	3-Sophoroside-7-glucoside
Brassica napus L. cv. Nugget	—	—	—	+
cv. Oro	—	—	—	+
cv. Target	—	—	+	+
cv. Golden	—	—	+	—
cv. Bronowski	+	—	+	+
B. rapa L. cv. Y. Sarson‡	+	—	—	+
cv. 70–419‡	+	—	—	+
cv. 70–502‡	+	—	—	+
cv. Echo	—	—	—	+
cv. Arlo	—	—	+	+
cv. B. Sarson	—	—	+	+
B. carinata L.	—	—	+	—
B. oleracea L. (broccoli)	+	—	+	+
B. juncea (L.) Czern.	—	—	+	+
B. amplexicaulis Desf.	+	—	—	+
B. chinensis L.	—	+	+	+
B. gravinae Ten.	—	—	+	—
B. maurorum Dur.	—	—	—	+
B. nipposinica Bayley	—	—	—	+
B. spirescens Pomel	—	—	—	+
B. tournefortii Gouan	—	+	—	—
Sinapis alba L.	—	—	+	+
S. arvensis L.	+	—	+	+
S. flexuosa Poiret	—	+	—	—

* Aglycones are kaempferol, quercetin and isorhamnetin; details of the 3-glycosides found are given in the text and in Table 2.

† Seedling leaf or mature leaf was surveyed in all cases, seeds being also examined in the case of the cultivars. Three species failed to yield detectable flavonoids: *B. barreleieri* (L) Janka, *B. fruticulosa* Cyr. and *S. pubescens* L.

‡ These are yellow-seeded varieties of rape, the others being brown.

None of the substances isolated from the crucifers (Table 2) is a new glycoside. However, the 3-sophoroside-7-glucosides of kaempferol and quercetin, the 3,7-diglucoside and 7-glucosides of kaempferol are reported in the Cruciferae for the first time. The widespread occurrence (Table 1) of flavonol 3-sophoroside-7-glucosides is of interest, particularly since

[8] HARBORNE, J. B., unpublished results.

a similar pattern (3-sophoroside-5-glucoside) is present in the anthocyanins of red cabbage, *Brassica oleracea* and radish, *Raphanus sativus*.[9]

TABLE 2. FLAVONOL GLYCOSIDES IDENTIFIED IN CRUCIFER SPECIES

Plant species and part	Flavonol(s)
Brassica rapa cv. Echo (rape) seed	3-Sophoroside-7-glucoside of kaempferol
B. oleracea (broccoli) leaf	3-Sophoroside-7-glucoside of quercetin, kaempferol and isorhamnetin
B. chinensis leaf *B. tournefortii leaf*	3-Monoglycosides and 3-diglycosides of quercetin and isorhamnetin
Sinapis arvensis (charlock) leaf and flower	7-Glucosides of kaempferol and isorhamnetin 3,7-Diglucosides of kaempferol and isorhamnetin 3-Glucoside-7-rhamnosides of kaempferol and quercetin* 3-Sophoroside-7-glucoside of quercetin and isorhamnetin (leaf only)
Sinapis flexuosa leaf	3-Diglycoside of isorhamnetin 3-Galactoside of isorhamnetin

* Isolated as inseparable mixture.

The 3-glucosides of isorhamnetin and kaempferol, previously reported in leaf of *Brassica napus*, *Sinapis arvensis* and *S. alba*,[4–6] could neither be found in these species nor did they appear in most of the other species under study (see Table 1). Exceptionally, *S. flexuosa* contains the 3-galactoside and an unidentified 3-bioside of isorhamnetin (Table 2). The only other species with 3-monoglycosides are *B. tournefortii* and *B. chinensis* which contain 3-monosides of quercetin and isorhamnetin. These latter species also have as yet unidentified quercetin and isorhamnetin 3-diglycosides.

Another discrepancy with earlier studies was our failure to detect rutin (quercetin 3-rutinoside), previously reported in seeds of *B. rapa*, in any of 6 cultivars examined; it was also absent from seed of 5 *B. napus* cultivars too (Table 1). Instead, these seeds contained kaempferol-3-sophoroside-7-glucoside. The earlier report of rutin in *Brassica* is, therefore, in doubt, particularly since this glycoside was not found in any of the other material studied. It is certainly not a characteristic glycoside of *Brassica* or *Sinapis*. It may also be noted that robinin (kaempferol 3-rhamnosylgalactoside-7-rhamnoside), previously reported in *Cheiranthus*,[3] was run as a standard chromatographic marker but was not found.

The present survey indicates that the flavonol glycoside patterns in the two genera *Brassica* and *Sinapis* are almost identical which agrees with their close morphological relationship,[10] the fact that intergeneric hybrids can be produced, the similarity in isothiocyanate content[11] and in seed sterol patterns.[12] The extensive serological and enzymic studies of Vaughan *et al.*,[13,14] while showing some differences between *S. alba* and the

[9] HARBORNE, J. B. (1967) *Comparative Biochemistry of the Flavonoids*, Academic Press, London.
[10] BAILEY, L. H. (1930) *Gentes Herb.* **2**, 211.
[11] KJAER, A. (1960) *Fortschr. Chem. Organ. Naturst.* **18**, 169.
[12] KNIGHTS, B. A. and BERRIE, A. M. M. (1971) *Phytochemistry* **10**, 131.
[13] VAUGHAN, J. G. (1968) in *Chemotaxonomy and Serotaxonomy* (HAWKES, J. G., ed.), pp. 103–110, Academic Press, London.
[14] VAUGHAN, J. G. and DENFORD, K. E. (1968) *J. Exp. Botany* **19**, 724.

cultivated *Brassica*, also indicate that there is no absolute distinction between the genera. With regard to the chemical differences between cultivars of rape or mustard or between different hybrids in the *Brassica* complex, there are too few characters to be of much value. However, one may note that the three yellow-seeded rape varieties examined had flavonol 7-glucosides while brown seeded varieties lacked them. Also, the distribution of flavonol glycosides varies between cultivars in both mustard and rape and, after a more extensive survey, it should be possible to separate cultivars into chemical groups based on flavonoid analyses of young seedlings.

Finally, it is suggested that further examination of flavonol glycoside patterns in the Cruciferae, rather than a study of the flavonol aglycones, would be worthwhile. Indeed, the present survey is now being extended to other crucifers in order to see how widespread flavonol 3-sophoroside-7-glucosides are in the family.

TABLE 3. R_fS AND COLOUR REACTIONS OF FLAVONOL GLYCOSIDES

Glycoside	R_f (×100) in* BAW	H_2O	15 HOAc	PhOH	Colour + UV	UV + NH_2
Kaempferol						
7-Glucoside	50	03	12	66	Bright Y	Bright Y
3,7-Diglucoside	40	46	66	65	Dark Br	Intense YG
3-Sophoroside-7-glucoside	22	74	86	48		
Quercetin						
3-Sophoroside-7-glucoside	15	70	78	30	Dark Br	Intense Y
3-Glucoside-7-rhamnoside	32	27	59	52		
Isorhamnetin						
3-Galactoside	64	15	46	70	Dark Br	Bright Y
3-Diglucoside	36	35	45	57		
7-Glucoside	32	03	09	70	Bright Y	Bright Y
3,7-Diglucoside	36	36	66	53	Dark Br	Intense Y
3-Sophoroside-7-glucoside	25	63	78	74		
Kaempferol and isorhamnetin 3-glucoside-7-rhamnosides	36	38	59	70		

* Solvent key: BAW, *n*-BuOH–HOAc–H_2O (4:1:5); 15 HOAc, 15% aq. HOAc; PhOH, PhOH–H_2O (3:1).

† Colour key: Br—brown; Y—yellow; G—green.

EXPERIMENTAL

Plant material. All cultivars were grown from seed supplied by, and verified by, the Canadian Seed Growers' Association, Ottawa, Canada and S. H. Pawlowski, Research Station, Saskatoon, Canada. Wild species were grown from seed provided from the Cruciferae germ plasm collection of the Instituto Nacional de Investigaciones Agrarias, Madrid, Spain. Seed meal of *Brassica rapa* cv. L. V. Echo was provided by the Food Research Institute, Ottawa, Canada. *Sinapis arvensis* was collected locally and verified by Dr. E. V. Watson of this University.

Flavonoid identification. Flavonol glycosides were separated, purified and identified by standard procedures (see e.g.[9]). UV spectra were measured in MeOH and all standard spectral shifts were determined. Chromatography was on paper (Whatman No. 1 and 3 MM) or on plates of MN 300 cellulose. R_f values of purified glycosides are given in Table 3.

Flavonol monoglycosides. Kaempferol and isorhamnetin 7-glucosides were isolated from leaves and flowers of charlock. The spectra (lack of NaOAc shift in band I), colour reactions and R_fs (Table 3) indicated that they were 7-glycosides. Acid hydrolysis yielded glucose as the only sugar, in each case. The aglycones were identified as kaempferol and isorhamnetin by 2-dimensional TLC in 50% HOAc in both directions.

Kaempferol 7-glucoside was confirmed by co-chromatography with an authentic sample. The 7-position of the glucose in the isorhamnetin derivative was confirmed by β-glucosidase hydrolysis, spectral shift and by the failure to detect glucose after H_2O_2 oxidation. Two isorhamnetin glycosides present in *S. flexuosa* were purified with considerable difficulty. One was identified as isorhamnetin 3-galactoside, from general similarity in R_fs to, but separation from, isorhamnetin 3-glucoside. Acid hydrolysis gave isorhamnetin, galactose and traces of glucose which seemed to be an impurity, since H_2O_2 oxidation gave only galactose. The second glycoside was provisionally identified from R_fs, colour reactions and hydrolytic data as an isorhamnetin-3-diglycoside.

Flavonol-3,7-diglycosides. Kaempferol- and isorhamnetin-3,7-diglucosides, isolated from charlock flowers, were identified from spectral and R_f data and from the results of controlled acid and β-glucosidase hydrolysis. The kaempferol derivative was confirmed by co-chromatography with authentic material. Another fraction, from charlock flowers, yielded glucose after 6 min acid hydrolysis and rhamnose after 20 min hydrolysis. The aglycones were isorhamnetin, kaempferol and quercetin and partial acid hydrolysis (6 min) gave three 7-glycosides, two of which agreed in R_f with the 7-rhamnosides of kaempferol and quercetin. Even after extensive purification, the kaempferol and isorhamnetin derivative could not be separated (Table 3). The compounds present were provisionally identified as the 3-glucoside-7-rhamnosides of the two flavonols.

Flavonol-3-sophoroside-7-glucosides. The kaempferol derivative, from *B. rapa*, and the quercetin derivative, from *Sinapis arvensis* and broccoli, were identified without difficulty by standard procedures. That is, β-glucosidase gave 3-sophorosides, identified by co-chromatography with authentic samples, and acid hydrolysis gave the corresponding 7-glucosides (see [2]). The isorhamnetin derivative was present in too small a quantity for rigorous identification, but its R_fs (Table 3) agreed with expected values and the compound has previously been identified in *Brassica* and *Sinapis*.[4]

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