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COMPARATIVE BIOCHEMISTRY OF FLAVONOIDS—V.

LUTEOLIN 5-GLUCOSIDE AND ITS OCCURRENCE IN THE UMBELLIFERAE*

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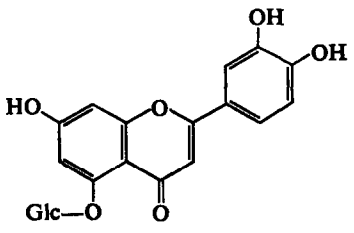
Abstract—A substance thought to be the 5-glucoside of luteolin, which was isolated from petals of *Dahlia variabilis* in 1953 and which agreed in its properties with a similar constituent in seed of *Galega officinalis* the first known source, is now shown to be free luteolin. The genuine 5-glucoside has now been discovered in leaves of *Torilis arvensis*, *T. nodosa* and *Chaetosciadum trichospermum* and re-examination of *Galega* seed has confirmed that it contains the 5-glucoside as well as the free aglycone. Luteolin 5-glucoside has a very distinctive blue fluorescence in u.v. light, has characteristic spectral properties and is unusually acid-labile. A similarly unstable compound in the petals of *Lamium album* appears to be the analogous 5-glucoside of quercetin. The natural distribution of flavone 5-glucosides and their value as taxonomic markers are discussed.

INTRODUCTION

5-GLYCOSYLATION of flavones is probably a thermodynamically unfavourable reaction in plants since it presumably involves the disruption of hydrogen bonding between the 5-hydroxyl and the 4-carbonyl. Certainly 5-glycosylation appears to be a very rare event in plant metabolism and only such derivatives of luteolin, apigenin, genkwanin and chrysin have been reported.[1] The best-known 5-glucoside, that of luteolin, was isolated from the seed of *Galega officinalis* (Leguminosae) in 1923 by Barger and White,[2] who named it galuteolin (I). It was also reported in horsetail, *Equisetum arvense* (Equisetaceae), by Nakamura and Hukuti in 1940,[3] and later it was identified by chromatographic and spectral methods in petals of the blue variety "Dandy" of *Dahlia variabilis* (Compositae).[4] In seeking a source of authentic galuteolin for chromatographic comparison with the isomeric 7-, 3'- and 4'-glucosides, the



(I)

* For Part IV of the series, see J. B. HARBORNE, *Phytochem.* 6, 1415 (1967).

1 S. HATTORI, In *Chemistry of the Flavonoid Compounds* (Edited by T. A. GEISSMAN), pp. 316–352. Pergamon Press, Oxford (1962).
2 G. BARGER and F. D. WHITE, *Biochem. J.* 17, 836 (1923).
3 H. NAKAMURA and G. HUKUTI, *J. Pharm. Soc. Japan* 60, 449 (1940).
4 C. G. NORDSTROM and T. SWAIN, *J. Chem. Soc.* 2764 (1953).

present author[5] used *Dahlia* petals and the identity seemed good since a compound with similar R_f values and colour reactions was obtained at the same time from *Galega* seed.

More recently the author, while studying the flavones of plants of the Umbelliferae as part of a chemotaxonomic survey of the family, found a luteolin derivative in the leaves of two *Torilis* species, the properties of which were very different from those of the *Dahlia* component but which nevertheless seemed to be the 5-glucoside. While a re-examination of the component isolated earlier from *Galega* showed it to be the free aglycone, a comparison of the *Torilis* glucoside with a second component overlooked during the first examination, confirmed that it was indeed the 5-*O*-β-D-glucoside (I). As a result of the earlier mistake in identification, the chromatographic and spectral properties of (I) have been given incorrectly in a review[5] and in a recent book[6] and it is the purpose of the present paper to report the identification and true properties of this compound and to comment on its natural distribution.

RESULTS

During a chromatographic survey of the phenolics of members of the tribe Caucalineae, family Umbelliferae, an unusual constituent was noted in the leaves of two of seven *Torilis* species. It had a bright blue fluorescence on paper in u.v. light changing to yellow–green with ammonia, colour reactions usually associated with caffeic acid esters such as chlorogenic acid. It differed from the caffeic esters, however, in the intensity of the colours and by the fact that it had low R_f values in both butanolic and aqueous solvents. On acid hydrolysis it yielded luteolin and glucose as the only components. Since it had an R_f very close to luteolin 7-glucoside (with which it co-occurs in *Torilis*) and was hydrolysed by β-glucosidase, it clearly had to be one of the three other isomeric monoglucosides of luteolin. It was quite different in its properties from the 4′-glucoside, an authentic specimen of which was available,[7] and the fact that the spectrum gave a boric acid shift (Table 1) ruled out the 3′-glucoside structure; it was therefore, by elimination, the 5-glucoside. This was confirmed by the fact that its spectrum failed to give the bathochromic shift in the presence of aluminium chloride shown by the other three isomers all of which have the 5-hydroxyl free. Additional evidence of 5-substitution rested in its striking blue fluorescence which is characteristic also of flavone 5-methyl ethers. The *Torilis* compound also differed from other flavone glycosides in being unusually acid-labile and in being very strongly adsorbed on cellulose.

Since the *Torilis* constituent was clearly different in its properties from the "5-glucoside" reported in *Dahlia*[4] and *Galega*,[5] which had a dull ochre colour in u.v. light and a high R_f in butnaol–acetic acid–water, the flavones in these plants were therefore re-examined. Chromatography of a *Galega* seed extract showed that there were two major components, one of high R_f similar to the *Dahlia* compound and a second blue-fluorescent substance (R_f 0·35), overlooked in the earlier analysis, which is completely identical with the *Torilis* compound and which is clearly the 5-glucoside (I) first isolated in crystalline form by Barger and White.[2] The component of higher R_f in *Galega* was readily identified as luteolin by direct comparison with authentic material. Reconsideration of the properties of the *Dahlia* petal "band 2"[4] suggests that it, too, must be free luteolin. Since no substance with the R_fs, colour reactions and spectrum of luteolin 5-glucoside as now reported (Table 1) appears to occur in *Dahlia*,[4] it can be assumed that this glycoside is in fact absent from this plant.

[5] J. B. HARBORNE, *J. Chromatog.* **2**, 581 (1959).
[6] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, p. 46. Academic Press, New York (1967).
[7] A. H. WILLIAMS, *Chem. Ind. (London)* 1318 (1964).

TABLE 1. PROPERTIES OF LUTEOLIN AND ITS FOUR MONOGLUCOSIDES

Flavone*	Colour reactions		R_f (×100) in				
	In u.v.	In u.v.+NH_3	BAW	H_2O	15% HOAc	PhOH	CEF
Luteolin	Dull ochre	Bright green	82	00	07	70	69
The 5-glucoside	Bright blue	Yellow-green	35	01	14	48	29
The 7-glucoside	Dull ochre	Bright yellow	44	01	18	56	14
The 3′-glucoside	—	—	40	—	21	—	—
The 4′-glucoside	Dull ochre	Dull ochre	68	01	34	60	18

Flavone*	λ_{max} in EtOH (nm)		$\Delta\lambda$ nm			
	Band I	Band II	NaOEt Band II	$AlCl_3$ Band II	NaOAc Band I	H_3BO_3 Band II
Luteolin	255, 264	352	+51	+40	+14	+25
The 5-glucoside	250, 260	345	+56	0	+13	+27
The 7-glucoside	255, 267	353	+54	+45	0	+22
The 3′-glucoside	256, 268	350	+52	—	—	—
The 4′-glucoside	270	337	+39	+45	+8	+1

* Sources: 5-glucoside from *Galega officinalis* and *Torilis nodosa;* 7-glucoside from *Solanum stoloniferum;* 3′-glucoside was not available for study, data given here from the abstract of the paper by Litvinenko and Sergienko,[8] who isolated it from *Dracocephalum thymiflorum;* 4′-glucoside from *Pyrus ussuriensis* leaf[7] and from *Turgenia latifolia* seed (J, B. Harborne, unpublished results).

Curiously, re-examination of both fertile and barren stems of the common horsetail, *Equisetum arvense*, has so far failed to yield luteolin 5-glucoside as a major constituent, as earlier reported.[3] Neither could this rare glucoside be detected by two-dimensional chromatography in extracts of two other common horsetails, *E. sylvaticum* and *E. telmateja.* The difficulty of detecting it in *Equisetum* may be a question of the plants' development or environment, particularly since experience with *Torilis* indicates that the synthesis of the 5-glucoside in vegetative organs fluctuates considerably with the age of the plant. Further studies of the flavonoids in horsetails are in progress.

It is abundantly clear from the present studies that luteolin 5-glycosides have very distinct properties when compared with the much more common 7-glycosides. They can be recognized by (1) their blue fluorescence in u.v. light, (2) their acid lability, (3) their spectral properties (the lack of an aluminium chloride shift is especially diagnostic) and (4) their strong adsorption on cellulose (they are eluted with difficulty from filter paper). Other flavone 5-glycosides are clearly as distinctive; thus Plouvier[9] reports that apigenin 5-rhamnosylglucoside in the leaves of the plum yew *Cephalotaxus drupacea* (Cephalotaxaceae) is completely hydrolysed by 3% H_2SO_4 at 100° in 10 min. Flavonol 5-glycosides are probably similar in properties and indeed a glycoside recently isolated in this laboratory from petals of the white dead-nettle, *Lamium album* (Labiatae), has properties which suggest it is the so far undescribed 5-glucoside of quercetin. In particular it has a bright yellow fluorescence on paper similar to that of azaleatin (quercetin 5-methyl ether) and it is very rapidly hydrolysed by acid to quercetin. Its instability and the fact that it becomes irreversibly adsorbed onto filter paper

[8] V. I. LITVINENKO and T. A. SERGIENKO, *Chem. Abstr.* 63, 10233 (1965).
[9] V. PLOUVIER, *Compt. Rend.* D263, 1529 (1966).

during chromatography have so far prevented isolation of sufficient material for confirming this structure.

DISCUSSION

Flavone (and flavonol) 5-glycosides may have been overlooked during earlier chromatographic surveys either because they are so easily hydrolysed by acid or else because they have colour reactions on paper different from other flavone glycosides. Nevertheless, present evidence is that luteolin 5-glucoside is very rare in the Umbelliferae. Thus a search of some 300 species of this large family, using both fresh and herbarium material, has indicated its occurrence in only one further species, in *Chaetosciadum trichospermum*, a monotypic genus in the same tribe as *Torilis*. Its occurrence in *Torilis* is limited to two of the seven species so far studies, *T. arvensis* and *T. nodosa*. It was also detected in herbarium material of *T. caerulescens* and *T. heterophylla* but according to a recent taxonomic revision,[10] these plants are probably best considered as sub-species of *T. arvensis*. It is too early to assess the taxonomic value of this rare glycoside character in the Umbelliferae, since only a third of the seventy-five odd species of the tribe Caucalineae have been investigated. However it is worth noting that *Torilis* and *Chaetosciadum* are the only genera in the group so far studied with a chromosome number of twelve and that the two genera have very similar seed anatomy. As soon as all seventy-five taxa have been surveyed, luteolin 5-glucoside will be used together with other chemical and with biological characters in a numerical analysis of what is a particularly "difficult" taxonomic group.[11]

There seems to be little general systematic significance in the natural occurrence of 5-glycosides since they have been found so infrequently and in such widely scattered groups. Whether they occur in horsetails remains to be seen, but they have been reported once in gymnosperms (*Cephalotaxus*), twice in the Leguminosae (*Amorpha* and *Galega*) and once in the Rosaceae (*Prunus*). The two reports in the Umbelliferae (*Chaetosciadum* and *Torilis*) can now be added to this list.

EXPERIMENTAL

Plant Material

Leaf of Umbelliferae was taken from plants grown mainly from spontaneous seed at Ness Gardens, Neston, Cheshire. Voucher specimens are being deposited in the University of Liverpool herbarium, under the accession numbers given below. Seed of *Galega officinalis* L. was obtained from the Botanic Garden, Johannes Gutenberg University, Mainz, Germany. Petal of *Dahlia variabilis* was taken from "Bonny Blue", a variety either synonymous with "Dandy" or very similar to it. *Equisetum arvense* L. was collected locally, *E. sylvestris* L. from near Strömstad, Sweden, and *E. telmateja* Ehrhart, from Glastonbury Tor, Somerset.

Chromatography and Spectroscopy

Solvents used for paper chromatography were: BAW, butanol–acetic acid–water (4:1:5); 15% HOAc, aqueous acetic acid; and PhOH, water-saturated phenol. Solvent for thin-layer chromatography on silica gel G was CEF, chloroform–ethyl acetate–formic acid (2:1:1). In general, TLC was not very useful for the flavones, the luteolin derivatives being particularly easily destroyed on silica gel. Two-dimensional chromatography was carried out on Whatman No. 1 paper in BAW and 5% HOAc. All spectra were measured on a Unicam SP800 spectrophotometer.

[10] J. F. M. CANNON, In *Flora Europea* (Edited by T. G. TUTIN, V. H. HEYWOOD *et al.*), Vol. 2. Cambridge University Press (1967). In press.

[11] A multivariate approach to the taxonomy of the Umbelliferae is being carried out at this University by the author in collaboration with V. H. Heywood, P. F. Parker and D. C. Stuart.

Isolation and Identification of Luteolin 5-Glucoside

This compound was isolated from fresh leaf of *Torilis nodosa* by extraction with hot EtOH and chromatography on No. 3 paper in BAW, H_2O and BAW. It only just separated under this treatment from the co-occurring 7-glucoside. It tended to hydrolyse during chromatography, particularly in acid solvents and was also strongly adsorbed on paper and was best eluted from fresh chromatograms. It separated during purification from a second blue-fluorescent band with higher R_f in aqueous solvents which may be the 5-rhamnosylglucoside (luteolin 7-rhamnosylglucoside also occurs in *Torilis* leaf). The 5-glucoside was isolated from *Galega* seed after 70% EtOH extraction at 100° for 30 min and chromatography in BAW.

The properties of luteolin 5-glucoside from both sources are shown in Table 1. Co-chromatography in nine solvent systems showed no separation. On hydrolysis with β-glucosidase or acid, the compound from both sources gave luteolin and glucose. The luteolin band in *Galega* had R_f 0·82 in BAW and 0·02 in water and was identified by direct comparison with authentic material.

Survey of the Tribe Caucalineae for Luteolin 5-Glucoside

Luteolin 5-glucoside was detected by paper chromatography in four samples of *Torilis arvensis* (Hudson) Link (accession nos. 1749, 1784, 1817, 2085) and three samples of *T. nodosa* (L.) Gaertner (nos. 2084, 3008, 3009) and one sample of *Chaetosciadum trichospermum* (L.) Boiss. It was absent from *T. japonica* (Houth) DC, *T. leptophylla* (L.) Reichenb. fil, *T. tenella* (Delile) Reichenb. fil. *T. triradiata* Boiss. & Heldr. and *T. ucranica* Sprengel. It was also absent from species of *Artedia* (1), *Astrodaucus* (2), *Caucalis* (1), *Daucus* (9), *Orlaya* (2), *Psammogeton* (2), *Pseudorlaya* (1) and *Turgenia* (1). All the above taxa were examined as fresh and herbarium material. Herbarium material of some 200 species representing genera in all the other tribes of the Umbelliferae was examined for the 5-glucoside with negative results.

Quercetin 5-Glucoside (?) *from* Lamium album

A flavonol glycoside was isolated from EtOH extracts of fresh petals by chromatography in BAW. The band at R_f 0·38 which had intense yellow fluorescence in u.v. light (exactly like azaleatin) was eluted and purified. It had λ_{max} at 256 and 373 nm and $\Delta\lambda^{AlCl_3}$ +57 nm, $\Delta\lambda^{H_3BO_3}$ +17 nm and $\Delta\lambda^{NaOAc}$ +2 nm. It decomposed in alkaline solution. R_fs were (quercetin given for comparison): 0·38 (0·78) in BAW, 0·25 (0·34) in PhOH and 0·62 (0·45) in 50% HOAc. It was readily hydrolysed by acid to quercetin. It was rather close in R_f to quercetin 7-glucoside (quercimeritrin) but differed in the colour reactions (the 7-glucoside is dull yellow in u.v.) and in ease of hydrolysis.

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