Phytochemistry, 1969, Vol. 8, pp. 419 to 423. Pergamon Press. Printed in England

OCCURRENCE OF FLAVONOL 5-METHYL ETHERS IN HIGHER PLANTS AND THEIR SYSTEMATIC SIGNIFICANCE*

J. B. HARBORNE

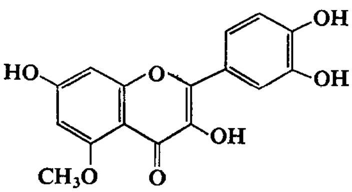
Phytochemical Unit, Hartley Botanical Labs., The University, P.O. Box 147, Liverpool

(*Received* 20 *September* 1968)

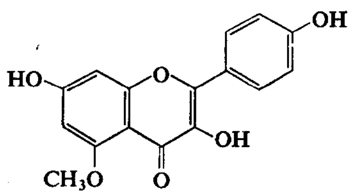
Abstract—Kaempferol 5-methyl ether has been identified for the first time in plants. It was found in leaves or flowers of *Rhododendron* spp., of *Erica, Kalmiopsis* and *Rhodothamnus* (all Ericaceae) and in *Tetracera* (Dilleniaceae). Surveys of the Ericaceae have also revealed that quercetin 5-methyl ether (azaleatin) is common as a leaf as well as a flower constituent; it is widespread in *Rhododendron* and also occurs in seven other genera. It is accompanied by the 3,5-dimethyl ether, caryatin, in leaves of some of these plants; in *Rhododendron*, caryatin is confined to species of the subgenus *Eurhododendron*. The presence of azaleatin in *Tetracera* (Dilleniaceae) has been confirmed. Together with earlier results, these findings show that flavonol 5-methyl ethers occur in fifteen genera from six plant families. Their systematic value is mainly confined to their distribution within these families but their presence in only woody families may have phyletic significance.

INTRODUCTION

5-*O*-METHYLATION of the commonly occurring flavonols of higher plants is a relatively rare process. It not only interferes with the normal hydrogen bonding between the 5-hydroxyl and 4-carbonyl in flavonols but also produces compounds which are intensely fluorescent in u.v. light and hence are readily detected in phytochemical surveys. 5-*O*-Methylquercetin (I) (azaleatin) was first identified in petals of *Rhododendron mucronatum* in 1956 by Wada,[1] and has been subsequently found in several other families, including the Plumbaginaceae[2] and Eucryphiaceae.[3] The related myricetin 5-*O*-methyl ether was found in *Rhododendron* by Egger[4] in 1962, but no report of the analogous derivative (II) of the other common flavonol, i.e. kaempferol, has appeared.



(I) Azaleatin



(II) Kaempferol 5-methyl ether

A deliberate search for this compound (II) in *Rhododendron* has been carried out and its discovery and characterization is the main subject of this paper. The same substance was

* Part X in the series "Comparative Biochemistry of the Flavonoids"; for Part IX, see H. T. CLIFFORD and J. B. HARBORNE, *Phytochem.*, in press.

[1] E. WADA, *J. Am. Chem. Soc.* **78**, 4725 (1956).

[2] J. B. HARBORNE, *Phytochem.* **6**, 1415 (1967).

[3] E. C. BATE-SMITH, S. M. DAVENPORT and J. B. HARBORNE, *Phytochem.* **6**, 1407 (1967).

[4] K. EGGER, *Z. Naturf.* **17b**, 489 (1962).

tentatively identified in *Tetracera* by Kubitzki and Reznik,[5] a result which has been confirmed in this laboratory. The same authors' report of azaleatin in *Tetracera* has also been confirmed and the opportunity is taken in the present paper to reassess the taxonomic significance of the occurrence of 5-*O*-methylated flavonols in plants.

RESULTS

Kaempferol 5-Methyl Ether

Chromatographic examination of acid hydrolysed extracts of leaves and flowers of the 100 *Rhododendron* species available for examination at this University showed the presence, in petals of about a third of these species, of a compound with the characteristic yellow fluorescence of azaleatin and myricetin 5-methyl ether but of higher R_f (see Table 1) than

TABLE 1. CHROMATOGRAPHIC PROPERTIES OF FLAVONOL 5-METHYL ETHERS

Flavonol	R_f (× 100)			
	Forestal	50% HOAc	PhOH	BAW
Kaempferol	62	44	58	91
Quercetin	45	31	28	76
Myricetin	29	21	10	41
Kaempferol 5-methyl ether*				
Actual value	70	43	78	82
Value predicted from ΔR_M	70	41	76	89
Quercetin 5-methyl ether (azaleatin)	53	29	42	55
Myricetin 5-methyl ether	37	21	23	27
Kaempferol 3,5-dimethyl ether†	85	72	95	91
Quercetin 3,5-dimethyl ether (caryatin)	78	56	88	72

* Colours in u.v. light: kaempferol derivative, fluorescent yellow green; azaleatin, fluorescent yellow; myricetin derivative, fluorescent yellow brown.

† Colours in u.v. light: both 3,5-dimethyl ethers, light blue fluorescence changing to yellow green with ammonia.

either of these two compounds. Its intense fluorescence masked the fact that it occurred in all these sources in very small amount and attempts to isolate it in sufficient amount for a full chemical study failed. However, sufficient was present for micro-scale analysis and the results of spectral, chromatographic (Table 1), demethylation and degradative studies showed that it is the so-far undescribed kaempferol 5-methyl ether. This identification was finally confirmed by comparison with synthetic material.

Azaleatin and Caryatin

The survey of *Rhododendron* species, which revealed the presence of kaempferol 5-methyl ether (see above) also showed that azaleatin, previously reported only as a flower constituent,[1,6] is also widespread in leaves, being found in 44 of 100 species. It is also accompanied

[5] K. KUBITZKI and H. REZNIK, *Beitr. Biol. Pflanzen* **42**, 445 (1966); and unpublished results.
[6] J. B. HARBORNE, *Arch. Biochem. Biophys.* **96**, 171 (1962).

by quercetin 3,5-dimethyl ether, caryatin, in twelve species, all of which significantly belong to the subgenus *Eurhododendron*. Caryatin was also detected in other members of the

TABLE 2. NATURAL DISTRIBUTION OF FLAVONOL 5-METHYL ETHERS IN HIGHER PLANTS

Plant family, genus and species	Organ	Flavonol 5-methyl ether present
Eucryphiaceae		
Eucryphia glutinosa (Poepp. et Endl) Baill. *E. cordifolia* Cav.	Leaf	Azaleatin (as 3-galactoside and as diglycoside) and caryatin
Juglandiaceae		
Carya pecan (Marsh.) Engl. et Graebn.	Heartwood	Azaleatin (in free state) and caryatin
Lauraceae		
Bielschmiedia miersii (Gay) Kosterm.	Leaf	Azaleatin (as diglycoside)*
Dilleniaceae		
Tetracera akara (Burmf.) Merr deVriese & Teysman *T. portobellensis* Beurl.	Leaf	Azaleatin and kaempferol 5-methyl ethers (as glycosides)
Ericaceae		
Cassiope cv. "Edinburgh" *Daboecia azorica* Tutin & Warb. *D. cantabrica* (Huds.) Koch	Leaf and Petal	Azaleatin and myricetin 5-methyl ethers
Erica vagans L.	Leaf	Azaleatin and kaempferol 5-methyl ethers
Gaultheria veitchiana Craib	Leaf	Kaempferol, quercetin and myricetin 5-methyl ethers
Kalmiopsis leachiana Rehd.	Leaf	Azaleatin and kaempferol 5-methyl ethers
Phyllodoce empetriformis D. Don × *Phyllothamnus erectus* Scheid	Leaf	Azaleatin
Rhodothamnus chamaecistus Reichenb.	Leaf	Kaempferol 5-methyl ether
Rhododendron ca. 50 spp.	Leaf and Petal	Azaleatin (as 3-rhamnoside and 3-galactoside), kaempferol and myricetin 5-methyl ethers, caryatin
Plumbaginaceae		
Plumbago capensis Thunb. *P. pulchella* Boiss. *P. scandens* L. *P. zeylanica* L.	Petal	Azaleatin (as 3-rhamnoside)
P. europea L.	Leaf	Myricetin 5-methyl ether (as glycoside)
Ceratostigma plumbaginoides Bunge	Leaf	Azaleatin and myricetin 5-methyl ethers (as 3-galactosides)
C. willmottianum Stapf.	Leaf	Azaleatin (as 3-galactoside)
Dyerophytum africanum (Lam.) Kuntze	Leaf	Azaleatin

* J. B. HARBORNE and J. MENDEZ, unpublished results.

Ericaceae, in *Phyllodoce* and *Cassiope* species (see Table 2). Caryatin, previously reported only in *Carya pecan*,[7] and *Eucryphia*,[3] was identified in these members of the Ericaceae by

[7] T. SASAKI, *Yakugaku Zasshi* **84**, 195 (1964).

direct spectral and chromatographic comparison with authentic material. Azaleatin was detected, but not fully identified, by Kubitzki and Reznik,[5] during a survey of leaf of species of the Polycarpicae for flavonoids. These authors found a yellow fluorescent substance of the right R_f value in *Bielschmiedia miersii* (Lauraceae) and in several *Tetracera* species. Confirmation of their findings in *Bielschmiedia* is described in a separate paper. Confirmation of their experiments with *Tetracera* are now given. Examination of leaf of both *T. portobellensis* and *T. akana* (Dilleniaceae) showed the presence of azaleatin and kaempferol 5-methyl ether, a result which was confirmed by co-chromatography and spectral analysis.

DISCUSSION

The natural occurrence of flavonol 5-methyl ethers, as at present known, is listed in Table 2. In view of the striking fluorescent properties of these compounds and the relatively large number of leaf surveys for flavonoids that have already been carried out in higher plants, it is doubtful whether more than a few other family records are likely to come to light in future surveys.

5-*O*-Methylation of the common flavonols is clearly a rare phenomenon in higher plants. It is also known among other flavonols, but again it is exceptional. Most polymethylated flavonols retain a free 5-hydroxyl group, as, for example, in combretol, the 3,7,3′,4′,5′-pentamethyl ether of myricetin of *Combretum* seed.[8] The only comparable example to azaleatin is vogeletin, the 5-methyl ether of 6-hydroxykaempferol, reported to occur in the seed of *Tephrosia vogelii* (Leguminosae).[9] Whether methylation of the 5-hydroxyl in compounds such as calycopterin, the 3,5,7,8-tetramethyl ether of 6,8-dihydroxykaempferol, found in *Calycopteris floribunda* leaf,[10] follows a similar process is more debatable. Whereas 5-methylation of quercetin, for example, to give azaleatin is probably controlled by a specific enzyme, polymethylated flavonols are more likely to be produced by means of a general methylating system and control is by protection of one or more hydroxyl groups.

Other modifications in the pattern of flavonoid synthesis may be analogous to 5-*O*-methylation, particularly if the protection of the 5-hydroxyl has some physiological significance. Among these, one may consider 5-*O*-glycosylation, which is extremely rare again and is only known at present in a very few plants (e.g. *Lamium*, Labiatae),[11] and the removal of the 5-hydroxyl to give 5-deoxyflavonols, compounds which are a characteristic feature of certain members of the Leguminosae.[12]

The taxonomic significance of 5-*O*-methylation, when considered in relation to the common flavonols, is difficult to discern, since the families reported to have these compounds (Table 2) are usually, as a whole, considerably separated from each other in most systems. It is, however, remarkable that they are, without exception, woody groups.[13] Even in the Plumbaginaceae, generally regarded as a herbaceous family, azaleatin is confined to a few woody members. Furthermore, the flavonoid patterns in other respects (e.g. leucoanthocyanin content, glycosidic pattern) are very similar. No less than three of the families, i.e. the Eucryphiaceae, Dilleniaceae and Ericaceae, have fairly close affinities morphologically. Indeed, although the position of the Eucryphiaceae has been the subject of much speculation

[8] S. MONGKOLSUK and F. M. DEAN, *J. Chem. Soc.* 4654 (1964).
[9] S. RANGASWAMI and K. H. RAO, *Proc. Indian Acad. Sci.* **49A**, 241 (1959).
[10] R. C. SHAH, V. V. VIRKAR and K. VENKATARAMAN, *J. Indian Acad Sci.* **19**, 135 (1942).
[11] J. B. HARBORNE, *Phytochem.* **6**, 1569 (1967).
[12] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, Academic Press, N.Y. (1967).
[13] E. C. BATE-SMITH, *Bull. Soc. Botan. Fr.* 16 (1965).

in the past, it is placed in the latest edition of Engler's Syllabus[14] next to the Dilleniaceae in the suborder Dilleniaceae of the order Guttiferales. Presence of azaleatin in both families thus provides useful chemical confirmation for this arrangement.

The main taxonomic interest in flavonol 5-methyl ethers remains, however, in their distribution patterns within the families in which they occur. Earlier, it has been demonstrated that the distribution of these compounds in the Eucryphiaceae is closely correlated with plant geography,[3] while the disjunct distribution in the Plumbaginaceae is correlated with pollen morphology and tribal divisions.[2] It is clear from the more recent surveys in a third family, the Ericaceae (Table 2), that the distribution of flavonol 5-methyl ethers is systematically significant at the generic and sub-family level. A more detailed account of the chemotaxonomy of the Ericaceae is reserved for a later paper.

EXPERIMENTAL

Plant Material

Material of the Ericaceae was obtained from the University of Liverpool Botanic Garden and was identified by K. Hulme and Dr. J. Cullen. Voucher specimens of all plants are preserved in the Botanic Garden Herbarium. Herbarium leaf material of *Tetracera* was provided by Dr. Kubitzki.

Authentic Pigments

A crystalline sample of azaleatin was available from earlier studies.[2,6] A sample of myricetin 5-methyl ether, isolated from *Rhododendron* petals, was kindly supplied by Dr. K. Egger. Synthetic samples of kaempferol and quercetin 3,5-dimethyl ethers were kindly supplied by Professor Chopin and Mlle. Grouiller. The former specimen contained kaempferol 5-methyl ether as a contaminant.

Isolation and Identification of Kaempferol 5-Methyl Ether

This pigment was isolated by preparative paper chromatography from hydrolysed extracts of petals of *Rhododendron racemosum* and *R. russatum.* It occurred in direct petal extracts as a glycoside, λ_{max} 262 and 335 nm, R_f 0·66 (BAW) and 0·35 (5% HOAc) tentatively identified as the 3-galactoside.

The pigment aglycone had λ_{max}^{EtOH} 265, 293 and 352; $\lambda_{max}^{EtOH-NaOAc}$ 265, 287 inf, 357; $\lambda_{max}^{EtOH-AlCl_3}$ 267, 300 inf, 340, 406; and $\lambda_{max}^{EtOH-H_3BO_3}$ 264, 355 nm. Its R_f values and colour reactions are shown in Table 1. On co-chromatography with synthetic material (see above) on paper and on TLC it did not separate. On demethylation with pyridinium chloride at 140° for 6 hr, it gave kaempferol. On reductive cleavage with Na–Hg for 1 hr, it yielded phloroglucinol monomethyl ether, *p*-hydroxyphenylpropanol and *p*-hydroxyphenylpropionic acid.

Other Identifications

The three flavonol 5-methyl ethers were identified variously in leaf and petal of the Ericaceae and in leaf of Dilleniaceae by chromatography and co-chromatography in four solvents and by spectral analysis. Quercetin 3,5-dimethyl ether (caryatin) was identified in *Rhododendron monosematum* leaf by co-chromatography with authentic material on paper (five solvents) and on TLC (three solvents), by full spectral analysis and by demethylation to give quercetin.

Acknowledgements—The author is grateful to Dr. Kubitzki and Dr. E. C. Bate-Smith for their interest, and for supplying leaf material. He acknowledges the experimental assistance of Miss C. A. Williams and thanks the Science Research Council for financial support.

[14] A. Engler, in *Syllabus der Pflanzenfamilien*, 12th ed. (edited by H. Melchior), Vol. 2, Springer-Verlag, Basle (1964).