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IDENTIFICATION OF EUPALITIN, EUPATOLITIN AND PATULETIN GLYCOSIDES IN *IPOMOPSIS AGGREGATA**

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Abstract—Eupalitin, eupatolitin and patuletin 3-*O*-galactosides as well as eupalitin and patuletin 3-*O*-diglucosides have been isolated and identified from *Ipomopsis aggregata* (Polemoniaceae). These 6-methoxylated flavonols are important taxonomic markers in the family, occurring as they do in nine of the eighteen known genera. Microscale methods of identifying these and other related highly substituted flavonols are discussed. Studies of the mass spectral fragmentation patterns have shown that quercetagetin derivatives methylated in the A-ring can be distinguished from similar compounds methylated in the B-ring and from the analogous gossypetin derivatives.

INTRODUCTION

THE CHEMOSYSTEMATICS of the Polemoniaceae has not yet been studied, although it is probably one of the most potentially rewarding plant groups for such examination. Thus, it is a family which has been presented as a model of evolution among higher plant groups.[1,2] It is large enough to include a great diversity of morphological and cytological conditions (18 genera and over 300 species) but small enough to allow a representative chemical survey. Again, its occurrence on 3 continents presents interesting phytogeographical situations and the presence of most of the members in western North America means they are reasonably accessible for study. Furthermore, the morphology, cytology, pollination biology, and breeding systems are sufficiently well-known for a confident exposition of evolutionary relationships of genera and species and trends of specialization within the family. Chemical data are also of interest in relation to the systematic position of the Polemoniaceae relative to other angiosperm families. It is usually placed close to the Convolvulaceae and/or the Hydrophyllaceae in most systems but its precise relationships with these and other Tubiflorae families are still a matter of debate.

In spite of the advanced state of our knowledge of the systematic biology of the Polemoniaceae, very little is known of its chemistry. According to Hegnauer,[3] only phenolic compounds, saponins and cyclitols have been examined in the family and these in only a few taxa. Our attention has been directed in the first instance to the flavonoid and other phenolic constituents, since these are among the most promising of taxonomic markers in

* Part I in the projected series "Chemosystematics of the Polemoniaceae".

[1] V. GRANT, *Natural History of the Phlox Family, Systematic Botany*. M. Nijhoff, The Hague (1959).

[2] V. GRANT and K. GRANT, *Flower Pollination in the Phlox Family*, Columbia Univ. Press, New York and London (1965).

[3] R. HEGNAUER, *Chemotaxonomie der Pflanzen*, Vol. V. Birkhauser Verlag, Basle (1969).

higher plants (cf.[4]). The only flavonoids to have been fully characterized in these plants are the anthocyanin, monardein, in flowers of *Ipomopsis rubra*[5] and two *C*-xylosylglycoflavones in *Phlox drummondii*.[6] In the course of a general survey of the family, three rare flavonols were detected in a number of species, including *I. aggregata*, and it is the purpose of this first paper in the series to record the discovery of quercetagetin and 6-hydroxykaempferol methyl ethers in this family.

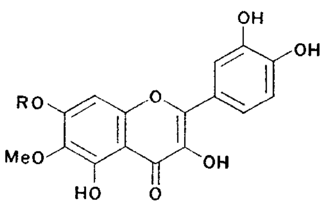
RESULTS

Eupalitin, Eupatolitin and Patuletin in Ipomopsis

In the course of a survey of the flavonoid aglycones in the leaves of the Polemoniaceae, three compounds were found with the same colour reactions as the common flavonols, myricetin, quercetin and kampferol but of different R_f. Their presence/absence was therefore easily determined and they were found in largest amount in four species of *Ipomopsis*, where they occurred to the exclusion of the common flavonols. They were also found in eight other genera of the family, in *Bonplandia*, *Cantua*, *Huthia*, *Collomia*, *Gilia*, *Eriastrum*, *Langloisia* and *Navarretia;* in all these plants except *Eriastrum* they occurred in conjunction with the common flavonols.

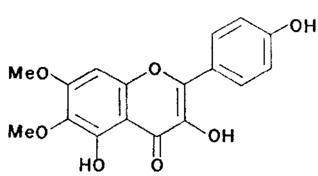
The aglycones were, therefore, isolated in quantity from the hydrolysed extracts of the dried leaves and stems of an *Ipomopsis* species, namely *I. aggregata* subspecies *bridgesii*. Their molecular formulae were determined by exact mass spectral measurements and subsequent UV spectral, R_f and demethylation studies showed that they were the 6-mono-

(I)



(I, R = H) Patuletin
(I, R = Me) Eupatolitin

(II)



Eupalitin

methyl ether (I, R = H) and 6,7-dimethyl ether (I, R = Me) of quercetagetin and the 6,7-dimethyl ether (II) of 6-hydroxykaempferol. The 6-monomethyl ether (I) is a well-known compound, patuletin, and its identity was readily confirmed by comparison with authentic material isolated from *Tagetes patula*.[7] The two 6,7-dimethyl ethers are, however, rare compounds and they have only recently been detected for the first time in plants in *Eupatorium ligustrinum* (Compositae) by Quijano *et al.*[8] These authors isolated them as the 3-rhamnosides, eupalin and eupatolin, calling the respective aglycones eupalitin (II) and eupatolitin (I, R = Me). Direct comparison of the *Ipomopsis* flavonols with authentic aglycones from *Eupatorium* confirmed these identifications.

[4] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, Academic Press, London (1967).
[5] J. B. HARBORNE, *Phytochem.* **2,** 85 (1963).
[6] T. J. MABRY, H. YOSHIOKA, S. SUTHERLAND, S. WOODLAND, W. RAHMAN, M. ILYAS, J. N. USMANI, N. HAMEED, J. CHOPIN and M. L. BOUILLANT, *Phytochemistry* **10,** in press (1971).
[7] J. GRIPENBERG, in *The Chemistry of Flavonoid Compounds* (edited by T. A. GEISSMAN) p. 427, Pergamon Press, Oxford (1962).
[8] L. QUIJANO, F. MALANCO and T. RIOS, *Tetrahedron* **26,** 2851 (1970).

TABLE 1. R_fS AND SPECTRAL CHARACTERISTICS OF FLAVONOL AGLYCONES AND GLYCOSIDES OF IPOMOPSIS

Flavonoid	R_f (X100) in* BAW	PhOH	15% HOAc	H_2O	Colour in UV light
Glycosides					
Eupalitin 3-galactoside	60	93	68	34	V. dark brown (changing to brown + NH_3)
Eupatolitin 3-galactoside	43	81	60	23	
Patuletin 3-galactoside	43	68	45	12	
Eupalitin 3-diglucoside	27	83	72	54	
Patuletin 3-diglucoside	18	52	68	42	
Aglycones			50% HOAc	Forestal	
Eupalitin	83	94	64	79	bright yellow-green
Eupatolitin	68	77	47	63	
Patuletin	61	53	35	50	
6-Hydroxykaempferol†	52	36	29	40	dark black

	λ_{max} (nm) in MeOH		$\Delta\lambda$ +NaOAc	+$AlCl_3$	+NaOMe	+H_3BO_3
Glycosides‡	Band I	Band II	Band I	Band II	Band II	Band II
Eupalitin 3-galactoside	270	337	0	15	60	0
Eupatolitin 3-galactoside	259	355	7	59	51	22
Patuletin 3-galactoside	258, 270*	348	15	57	56	25
Aglycones						
Eupalitin	270	364	0	60	74	0
Eupatolitin	259, 270	370	4	66	69	18
Patuletin	258, 270	367	15	66	73	19
6-Hydroxykaempferol	273	360	7	40	40	—

* Solvents: BAW, *n*-BuOH–HOAc–H_2O (4:1:5); PhOH, water satd. phenol; Forestal, HOAc–HCl–H_2O (30:3:10).

† Formed by demethylation of Eupalitin; authentic sample obtained by demethylation of 3,6,7-trimethoxy-5,4′-dihydroxyflavone.

‡ The 3-*O*-diglucosides were spectrally identical with the 3-*O*-galactosides. They were formulated as such on the basis of R_f values and their giving glucose on hydrolysis. H_2O_2 oxidation failed to give the expected disaccharide (yielding only glucose) but this is probably due to the instability of the linkage in this moiety. The compounds cannot be 3-monoglucosides because of their different R_f values relative to the 3-monogalactosides.

§ The 3-glycosides were stable in alkali; the aglycones were unstable, showing 20% decomposition after 10 min, except for 6-hydroxykaempferol which showed 50% decomposition after 2 min.

Studies of the unhydrolysed leaf extracts of *Ipomopsis aggregata* then revealed the presence of five flavonoid glycosides based on the above three aglycones. These were isolated and characterized by standard procedures (see Table 1 and Experimental) and found to be the 3-*O*-galactosides of eupalitin, eupatolitin and patuletin and the 3-*O*-diglucosides of eupalitin and patuletin. Patuletin 3-glucoside has been recorded before, e.g. in *Lasthenia*,[9] but the 3-galactoside has not, and nor have the other four glycosides.

The spectral and chromatographic properties of all the compounds mentioned above are recorded in Tables 1 and 2. Data are included for the two rare flavonol 6,7-dimethyl ethers (I, R = Me) and (II), since Quijano *et al.* did not give these properties in detail in their paper. The properties are also given for 6-hydroxykaempferol, obtained by demethylation of eupalitin. This agreed in every way with a specimen prepared by demethylation of 3,6,7-trimethoxy-5,4′-dihydroxyflavone, kindly provided by Professor L. Farkas. Incidentally, this compound, the kaempferol analogue of quercetagetin, has yet to be recorded as a

[9] N. A. M. SALEH, B. A. BOHM and R. ORNDUFF, *Phytochem.* **10**, 611 (1971).

TABLE 2. MASS SPECTRAL FRAGMENTATION PATTERNS OF QUERCETAGETIN AND GOSSYPETIN METHYL ETHERS

Flavonol	M	Molecular weights of Ions formed (intensities as % of parent ions) M-15	M-18	M-29	M-43
Quercetagetin derivatives					
6-Methyl ether (patuletin)*	332	—	314(37)	303(12)	289(100)
6,7-Dimethyl ether (Eupatolitin)	346	—	328(48)	318(30)	303(100)
3′-Methyl ether†	332	317(7)	—	303(8)	289(4)
3,6,3′-Trimethyl ether‡	360	345(64)	342(15)	—	317(21)
6-Hydroxykaempferol derivatives					
6,7-Dimethyl ether (Eupalitin)	330	—	312(52)	301(10)	287(120)
Gossypetin derivatives					
7-Methyl Ether§	332	317(172)	—	303(127)	289(18)
8-Methyl Ether‖	332	317(154)	—	303(8)	?

* Nielsen *et al.*[12] report on M-15 ion for synthetic patuletin, but we can find no evidence for this in several natural specimens isolated from different plant sources.

† Isolated from *Chrysanthemum* (see Harborne *et al.*[19])

‡ Data taken from Bowie and Cameron.[10]

§ Isolated from *Lathyrus pratensis* flowers (see Harborne[18]) mass found 332·0530, $C_{16}H_{12}O_8$ requires 332·0532.

‖ Data kindly supplied by Dr. G. J. NIELSEN (unpublished results).

natural plant constituent. Although its 6,7-dimethyl ether (II) is present in several Polemoniaceae, no evidence could be found during this survey for the 6-methyl ether, which might be expected by analogy with the presence in these plants of patuletin, or for the parent compound.

Mass Spectral Fragmentation Patterns

While elucidating the structures of the three *Ipomopsis* flavonols, some evidence of their identity was obtained by comparing mass spectral fragmentation patterns with those of known methylated flavonols. These data are collected, together with measurements made on several related compounds recently available in this laboratory, in Table 2. Although the mass spectra of eleven methylated quercetagetin derivatives have been analysed by Bowie and Cameron,[10] and of some gossypetin derivatives by Nielsen and Moller,[11] the technique has not been widely used as yet for structural elucidation. The first authors, however, suggested that 6-methoxylated flavonols could be distinguished by their giving a prominent M-15 ion,[10] while the latter authors have found differences in the A-ring fragments which allow distinction between 6- and 8-methoxy substitution in these compounds.[11,12]

In our experience (Table 2), four readily detectable large fragments are produced from simple methyl ethers of quercetagetin and gossypetin; M-15 representing demethylation, M-18 representing loss of water, M-29 representing loss of CHO and M-43 representing loss of CH_3CO. Not all these ions are produced in every case and their intensities vary enormously. The differences are clearly sufficient for them to be used in structural assignments.

[10] J. H. BOWIE and D. W. CAMERON, *Austral J. Chem.* **19**, 1627 (1966)

[11] J. G. NIELSEN and J. MØLLER, *Acta Chem. Scand.* **24**, 2665 (1970).

[12] J. G. NIELSEN, P. NORGAARD and H. HJEDS, *Acta. Chem. Scand.* **24**, 724 (1970).

One unexpected result, contradicting the earlier prediction,[10] is that the 6-monomethyl and 6,7-dimethyl ethers of quercetagetin and 6-hydroxykaempferol apparently fail to undergo demethylation; there are no M-15 ions. According to Bowie and Cameron, the ease of demethylation of the 6-methoxyl group is striking and we have also found this to be true in the flavone series, with the corresponding methyl ethers of scutellarein and 6-hydroxyluteolin.[13] The significant point, however, is that all the compounds studied by Bowie and Cameron contain a 3-hydroxyl masked by *O*-methylation; not surprisingly, the corresponding flavones behave similarly. It is clear, from our results, that *when the 3-hydroxyl of the flavonol is free*, demethylation at the 6-methoxyl position does not occur. Concomitant with this lack of demethylation is the appearance of a strong peak (of similar intensity to the parent ion) at M-43. On the other hand, B-ring *O*-methyl groups are apparently cleaved, since quercetagetin 3′-methyl ether gives a small but significant M-15 ion. The quercetagetin 6-mono or 6,7-dimethyl ethers also behave very differently from the related gossypetin 7- and 8-methyl ethers, which fragment to give a very intense M-15 peak and a relatively low M-43 peak. Mass spectral measurements thus clearly distinguish between the two series of related flavonols.

Bowie and Cameron[10] have also suggested that the M-43 ion represents the loss of CH_3CO from the 3-position of 3-methoxylated flavonols and that it is thus diagnostic for 3-methoxyl substitution. However, since it is clear from our data (Table 2) that several quercetagetin and gossypetin derivatives lacking a 3-methoxyl group also give rise to M-43 fragments, it appears that this interpretation requires some modification.

DISCUSSION

The discovery of three *O*-methylated 6-hydroxyflavonols in *Ipomopsis* (and eight related genera in the family) is of systematic interest from the point of view of the position of the Polemoniaceae in angiosperm classification, since quercetagetin derivatives are relatively rare and ‘biosynthetically advanced’ chemical characters. 6-Hydroxyflavonols with the particular 6,7-dimethylation pattern as in eupalitin and eupatolitin are very rare and they have only been reported once before, in *Eupatorium ligustrinum* (Compositae).[8] Related flavonols, however, are also known, e.g. penduletin (3,6,7-trimethoxy-5,4′-dihydroxyflavone) from *Brickellia* and artimetin (5-hydroxy-3,6,7,3′,4′-pentamethoxyflavone) from *Kuhnia*, both these genera incidentally being in the same tribe of the Compositae as *Eupatorium*, namely the Eupatorieae. Related flavones with 6,7-dimethylation occur not only in *Eupatorium* (Compositae) but also in the Scrophulariaceae and Labiatae (*Scrophularia* and *Salvia*).[4,13]

The 6-methoxyflavonol, patuletin, found in *Ipomopsis*, is a little more widespread, having been recorded in *Spinacia* (Chenopodiaceae) and *Prosopis* (Leguminosae) as well as in several genera of the Compositae.

The most significant systematic point about the presence of 6-hydroxyflavonols such as patuletin in the Polemoniaceae is not the obvious link with the Compositae, but, instead, the implied link with those families in the Tubiflorae which contain 6-hydroxyflavones (notably the Bignoniaceae, Buddleiaceae, Globulariaceae, Labiatae and Scrophulariaceae).[13] Although recent treatments[14,15] separate the Polemoniaceae into its own order the Polemoniales, according to Engler[16] it should be included in the Tubiflorae and the chemical

[13] J. B. Harborne and C. A. Williams, *Phytochem.* **10**, 367 (1971).
[14] A Takhtajan, *Flowering Plants, Origin and Dispersal*, Oliver & Boyd, Edinburgh (1969).
[15] A Cronquist, *The Evolution and Classification of Flowering Plants*, Nelson, London (1968).
[16] H. Melchior (editor), *Engler and Prantl's Syllabus der Pflanzenfamilien*, Vol. II, Borntraeger, Berlin (1964).

evidence is now more in favour of this traditional system. Certainly, neither a 6-hydroxyflavonol nor a 6-hydroxyflavone has been found so far in any of the other families constituting this new order.

Since highly substituted flavonols are presumably biosynthetically more complex than simple flavonols, they must represent advanced characters, which suggests that *Ipomopsis* (and other genera containing these rare flavonols) have evolved further chemically than most other members of the Polemoniaceae family. Indeed, this correlates beautifully with the biological evidence. Thus, the *Ipomopsis aggregata* complex are annual or biennial herbs seemingly derived from perennial woody ancestors such as *I. gloriosa* from Baja California.[1] Furthermore, in its adaptations to bird and moth pollination, *I. aggregata* shows further evidence of evolutionary advancement over other members of the group.[17]

The taxonomic and evolutionary significance of these three interesting flavonoids will be further discussed in a later paper which will present the results of a flavonoid survey of the whole family.

EXPERIMENTAL

Plant material. Ipomopsis aggregata (Pursh) V. Grant subsp. *bridgesii* (Gray) V. & A. Grant was collected at Kaiser Pass, Sierra Nevada Range, California, and dried. Voucher specimens have been deposited in the herbarium of the University of California at Santa Barbara.

Flavonoid Identification. Flavonoids were extracted, separated, purified and identified by standard procedures.[4] R_f, UV spectral and mass spectral data are given in Tables 1 and 2. The three aglycones were obtained as pale yellow needles and their molecular formulae were determined by high resolution mass spectroscopy as follows: patuletin measured mass 332·0533 $C_{16}H_{12}O_8$ requires 332·0531; eupalitin measured mass 346·0691, $C_{17}H_{14}O_8$ requires 346·0688; eupatolitin measured mass 330·0742, $C_{17}H_{14}O_7$ requires 330·0739. Demethylation of patuletin and eupatolitin with pyridinium chloride gave quercetagetin; eupatolitin gave two intermediates, one identified as patuletin and the other presumably being quercetagetin 7-methyl ether. Eupalitin also gave two intermediates during demethylation, eventually yielding 6-hydroxykaempferol.

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[17] V. GRANT, *El Aliso* **3**, 351 (1956).
[18] J. B. HARBORNE, *Phytochem.* **8**, 177 (1969).
[19] J. B. HARBORNE, V. H. HEYWOOD and N. A. M. SALEH, *Phytochem.* **9**, 2011 (1970).