Identification of two Gossypetin Monomethyl Ethers as Yellow Flower Pigments in the Rutaceae

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Z. Naturforsch. **38 c**, 148–150 (1983);
received November 9, 1982

Rutaceae, *Haplophyllum*, *Ruta*, Gossypetin Monomethyl Ether, Chemotaxonomy

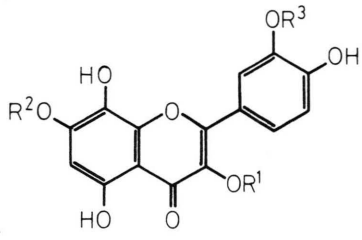
Gossypetin 7-methyl ether 3-rutinoside has been characterized as a yellow flower pigment in *Ruta graveolens* and in five other *Ruta* species. By contrast, in four *Haplophyllum* species, the 7-glucoside of gossypetin 3′-methyl ether provides the yellow coloration in the flower. Although carotenoid accompanies the yellow flavonol in *R. graveolens*, there is no evidence of UV patterning and the petals are uniformly UV absorbing.

Introduction

In the course of a chemotaxonomic survey of flavonoids in the plant family Rutaceae [1], yellow flavonols were unexpectedly detected in the flowers of two related genera, *Haplophyllum* and *Ruta*. One of these plants was the well known medicinal plant, the common rue *Ruta graveolens*, the leaves of which are a familiar source of the flavonol glycoside rutin [2]. In view of the relative rarity of yellow flavonoids as flower pigments in the angiosperms [3], a more detailed investigation of these pigments was undertaken and the present paper records the identification of two rare gossypetin monomethyl ethers in these plants.

Results

The major water-soluble yellow pigment present in flowers of *Ruta graveolens* was separated and purified by paper chromatography. It was identified by standard procedures as gossypetin 7-methyl ether 3-rutinoside (**1**). This is the first report of gossypetin 7-methyl ether (**2**) in the Rutaceae, although it has been detected as a yellow floral pigment in *Eriogonum nudum* (Polygonaceae) [4] and in *Ranunculus* spp. [5, 6]. Gossypetin 7-methyl ether has also been reported in leaves or stems of *Atraphaxis purpurea* (Polygonaceae) [7] and of several members of the Restionaceae [8]. Although six glycosides of gossypetin 7-methyl ether have variously been characterised in the above plants [9], the 3-rutinoside has not been reported before and is therefore a new plant glycoside.



1: R^1 = rutinose, R^2 = Me, R^3 = H
2: $R^1 = R^3$ = H, R^2 = Me
3: R^2 = glucose, R^1 = H, R^3 = Me
4: $R^1 = R^2$ = H, R^3 = Me

A 2-dimensional chromatographic survey of five of the six other known *Ruta* species indicated that the 3-rutinoside (**1**) occurs in the yellow flowers of all five, namely *R. montana* L., *R. angustifolia* L., *R. chalepensis* L., *R. oreojasme* Webb and *R. pinnata* L. fil. It is generally absent as a leaf constituent in these plants but does occur exceptionally in the leaves of *R. montana*.

The new gossypetin glycoside **1** is accompanied in flowers and leaves variously by the common flavonols, quercetin, kaempferol and isorhamnetin, which appear to be present also as the respective 3-rutinosides. In the flowers of *R. graveolens*, this yellow pigment is accompanied by considerable amounts of carotenoid so that the two classes of pigment together contribute to visible yellow colour. Under ultraviolet light, the freshly picked flowers of rue are uniformly absorbing, indicating that the yellow flavonol occurs throughout the corolla, effectively quenching the normal reflectance of the carotenoid present. Ultraviolet patterning, which has been observed in other flowers with both yellow flavonol and yellow carotenoid [*e.g.* 10], is absent in this instance.

In *Haplophyllum*, yellow flower colour is also common and a detailed examination of petals of *H. linifolium* (L.) G. Don fil. led to the isolation and characterisation of gossypetin 3′-methyl ether 7-glucoside (**3**); an acylated 7-glucoside of the same aglycone was also present. Gossypetin 3′-methyl ether (**4**) is newly reported in flowers of *Haplophyllum*,

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0341-0382/83/0100-0148 $ 01.30/0

but it has been found before in the leaves of *H. perforatum* as the 7-glucoside [11] and 7-(6″-acetylglucoside) [12]. It is possible that the acylated glucoside in the flowers is also the 6″-acetate but this could not be confirmed, since insufficient material was available for ^{13}C NMR spectral analysis. The 3′-methyl ether of gossypetin is very rare in plants, the only other record of its occurrence being as a flower pigment in *Coronilla valentina* (Leguminosae) where it occurs as the 3-rutinoside [10].

A two-dimensional chromatographic survey of four other *Haplophyllum* taxa showed that this gossypetin derivative **3** is probably a characteristic floral constituent. Thus it was detected in flowers of *H. suaveolens* (DC.) G. Don f., *H. tuberculatum* A. Juss., *H. buxbaumii* (Poir.) G. Don. f. subsp. *buxbaumii* and *H. buxbaumii* subsp. *mesopotamicum* (Boiss.) C. C. Townsend; **3** was accompanied by glycosides of kaempferol, quercetin and isorhamnetin in flowers and leaves.

Discussion

Very little work has been done previously on yellow flower pigmentation in Rutaceae and this is the first report of these two gossypetin monomethyl ethers as floral constituents. Gossypetin and its derivatives are known to contribute to yellow flower colour in some ten angiosperm families, but they are not recorded in any family closely related to the Rutaceae [1]. It may be noted that gossypetin 7-methyl ether appears to characterise the genus *Ruta* (in all six spp. examined), while the isomeric 3′-methyl ether similarly characterised the genus *Haplophyllum* (in all of four spp. examined). The presence of yellow flavonols in both these genera is not really surprising, since they are closely related morphologically and indeed *Haplophyllum* (with some 70 spp. in all) has been included by some taxonomists in the same genus as *Ruta* (with 7 spp.). The differences in the methylation pattern of the floral pigments would seem to support their separation at the generic level and this is in accord with most modern taxonomic treatments.

While gossypetin 3′-methyl ether has been reported before [11, 12], the 7-methyl ether is new as a Rutaceous constituent. However, it may be observed that other related gossypetin methyl ethers are known elsewhere in the family. Thus the 7,4′-dimethyl ether occurs in *Xanthoxylum* fruit, the 8,3′-dimethyl and 3,7,3′,4′-tetramethyl ethers in *Citrus* peel and the 3,7,8,3′-tetramethyl ether in *Melicope* bark (for references, see 1). Curiously, the parent flavonol gossypetin has yet to be recorded anywhere in the family.

Experimental

Plant Material. Fresh flowers of *Ruta graveolens* were collected from plants growing in the University botanic garden. Recent collections of *Haplophyllum* and *Ruta* spp. have been made in the Mediterranean area and in the Canary Islands by Reading University taxonomists and all other plant material was taken from sheets of taxonomically verified specimens deposited in the Reading University herbarium.

Identification of gossypetin aglycones. Gossypetin 7- and 3′-methyl ethers were identified in hydrolysed flower or leaf tissues by colour and TLC comparison in at least 5 solvents with authentic markers. When obtained as the aglycones of the major glycosides of *Ruta* and *Haplophyllum* respectively, the identifications were confirmed by detailed UV spectral measurements and by characteristic MS fragmentation patterns [10]. MS of gossypetin 7-methyl ether ex *Ruta:* M 332 (100), M-15 317 (13.2), M-43 289 (6.6) and B-ring fragment 137 (12.6%). MS of gossypetin 3′-methyl ether ex *Haplophyllum:* M 332 (100), M-15 317 (3.1), M-43 289 (1.2), A ring fragment 169 (8.3) and B-ring fragment 151 (16.0%).

Identification of gossypetin glycosides. The major yellow pigment of *Ruta graveolens* flowers was isolated and purified by paper chromatography and identified as *Gossypetin 7-methyl ether 3-rutinoside* on the following evidence. On paper chromatograms, it appears yellow in vis. light, dark absorbing in UV light; R_f values are 0.25 in H_2O, 0.42 in *n*-BuOH-HOAc-H_2O (4:1:5) and 0.55 in 15% HOAc. Spectral maxima are at 262 sh, 277, 305 sh, 345 nm in MeOH and spectral shifts in the long wavelength band were observed with borate (+35 nm), $AlCl_3$ (+100 nm) and alkali (+40 nm, rapidly decomposing). There was no shift in band I with NaOAc. The large hypsochromic shift in the long wavelength band (47 nm) in the neutral spectrum when compared with its aglycone indicated 3-substitution. Acid hydrolysis under N_2 (2 M HCl, 15 min, 100°) gave gossypetin 7-methyl ether (identified as above), glucose and rhamnose. R_f data indicated the presence of a diglycoside. Confirmation of the 3-rutinoside

structure was achieved by H_2O_2 oxidation, which gave rutinose in good yield, identified by direct comparison with authentic disaccharide obtained by similar oxidation of rutin. Paper electrophoresis was carried out on the disaccharide to distinguish it clearly from the isomeric neohesperidose.

A second glycoside of gossypetin 7-methyl ether was also present in *Ruta graveolens* flowers. The spectral maxima in MeOH (272, 275, 310 sh, 340, 392 nm) indicated the presence of an 8-glycoside. However, it was still contaminated with accompanying rutin, even after several purifications on paper. Attempts to purify it further led to its decomposition.

The major yellow glycoside of *Haplophyllum linifolium* flowers was isolated by extraction with 80% MeOH and purified by paper chromatography. It was identified as gossypetin 3′-methyl ether 7-glucoside from spectral and R_f properties and from acid hydrolysis. The spectral maxima were at 261, 271, 345 and 390 nm in MeOH and positive shifts in the long wavelength bands were recorded with $AlCl_3$ ($\Delta\lambda$ 60 nm) and with alkali (rapid decomp.). No spectral shifts occurred in the presence of NaOAc or NaOAc-H_3BO_3. R_f values were 0.35 in *n*-BuOH-HOAc-H_2O (4:1:5), 0.04 in 15% HOAc and 0.60 in PhOH-H_2O (3:1). On acid hydrolysis under N_2, it gave gossypetin 3′-methyl ether (identified as indicated above) and glucose. The position of glucosylation at the 7-position was confirmed by complete methylation and hydrolysis to give 7-hydroxy-3,5,8,3′,4′-pentamethoxyflavone, identified by chromatographic and spectral comparison with authentic material prepared similarly from gossypitrin. A second glycoside of gossypetin 3′-methyl ether was also obtained from the same floral extract. It had similar spectral properties to the 7-glucoside, but slightly different R_f values (0.39 in *n*-BuOH-HOAc-H_2O, 0.56 in PhOH-H_2O). It was assumed to be acylated since it gave glucose on hydrolysis but was unaffected by treatment with β-glucosidase. Like the 7-glucoside, it was very unstable in solution and rapidly decomposed in the presence of acid or alkali.

Acknowledgement

The authors thank R. W. Butters (Tate & Lyle Research, Whiteknights, Reading) for MS analyses.

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