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TWO GOSSYPETIN METHYL ETHERS AS ULTRAVIOLET PATTERNING GUIDES IN THE FLOWERS OF *CORONILLA VALENTINA*

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Key Word Index—*Coronilla valentina* subsp. *glauca*; Leguminosae; flowers; UV patterning; gossypetin 3′-methyl ether; gossypetin 8,3′-dimethyl ether; limocitrin.

Abstract—The 3′-monomethyl and 8,3′-dimethyl ethers of gossypetin have been identified in the flowers of *Coronilla valentina* where they occur as the 3-rutinosides. These two yellow flavonols occur specifically in the wings and thus provide both visible yellow colour and UV absorption to bees, which land on the wings and trigger the self-fertilization mechanism. These yellow pigments are absent from the flowers of the related *C. emerus*, where their role in UV patterning is taken over by colourless kaempferol and quercetin glycosides.

INTRODUCTION

While the chemical basis of UV patterning has been studied in the yellow-flowered species in a variety of angiosperms and especially in members of the Compositae [1–3], little attention has been given to this phenomenon in the Leguminosae. The presence of such patterning is recorded in several genera, including *Cercidium* [4] and *Genista* [5]. As part of a biochemical investigation of UV patterning in this family, attention was drawn to the flowers of *Coronilla valentina* subsp. *glauca* where all the flowering parts contain yellow carotenoid but only the wings contain UV-absorbing flavonoids. This paper records the identification of the two main flavonoids present as the 3-rutinosides of gossypetin 3′-methyl ether and gossypetin 8,3′-dimethyl ether.

RESULTS

Extraction of the yellow flowers of *Coronilla valentina* yielded two yellow flavonoids, the spectral properties of which indicated them to be derivatives of gossypetin (3,5,7,8,3′,4′-hexahydroxyflavone). On hydrolysis, two aglycones were obtained, one of which was readily identified as gossypetin 8,3′-dimethyl ether (limocitrin) by comparison with literature data. This dimethyl ether was first obtained from *Citrus limon* (Rutaceae) [6] but has more recently been detected in flowers of *Lotus corniculatus* (Leguminosae) [7]. Its present discovery in the flowers of another legume, in *Coronilla*, is therefore expectable.

The second aglycone was established as a gossypetin monomethyl ether from its molecular formula, its spectral properties and its ready demethylation to gossypetin. It was different in R_f and spectrum from the two known naturally occurring 7- and 8-monomethyl ethers [8] and also differed from the 4′-methyl ether, a synthetic sample of which was available for comparison [9]. Since the colour reactions and absorption spectrum indicated that it was not substituted in either the 3- or 5-positions, its structure as the 3′-methyl ether follows by a simple process of elimination. This was confirmed by the mass spectrum, which showed the presence of a B-ring fragment (m/z 151, 10.6%) characteristic of a flavonol with one *O*-methyl substituent in the B-ring.

After completion of the above identification, gossypetin 3′-methyl ether was reported for the first time to occur as the 7-(6-acetylglucoside) in aerial parts of *Haplophyllum perforatum* (Rutaceae) [10]. This therefore becomes the second report in nature of the 3′-methyl ether. Considering its common methylation pattern (i.e. it corresponds to isorhamnetin in the quercetin series), it is surprising that it has not been reported before. Its discovery brings the number of known monomethyl ethers of gossypetin to three. The chromatographic and spectral properties of these natural gossypetin derivatives together with those of the synthetic 4′-methyl ether are shown in Table 1. It will be observed that all four monomethyl ethers can be distinguished from each other chromatographically by a combination of paper and TLC systems.

As mentioned above, the two gossypetin methyl ethers of *Coronilla* flowers occur in glycosidic combination, and on hydrolysis, the two sugars glucose and rhamnose were obtained in equal proportions. Spectral shift studies on the glycosides and the isolation of rutinose from H_2O_2 oxidation showed that the two compounds are the 3-rutinosides. These are both new glycosides, since no glycoside of limocitrin has yet been described.

Separate two-dimensional chromatography of extracts of standard, keel and wings confirmed that the two gossypetin derivatives occurred exclusively in the wings. By contrast, carotenoids were present in all floral tissues. The presence of the UV-absorbing flavonols was correlated with the fact that the wings were UV-absorbing when examined in UV light whereas the other flower parts were UV-reflecting. The ecological significance of this UV patterning has yet to be fully determined but preliminary observations indicate that bumblebees visiting the flowers land on the wings and thus trigger the self-fertilization mechanism present in these blossoms.

The occurrence of visibly yellow, UV-absorbing

Table 1. Chromatographic and spectral properties of gossypetin and its methyl ethers

	R_f values (×100)*						
	Forestal	Paper 50% HOAc	BAW	CAW	Si gel BPF	Polyamide BEM	Colour in UV light
Gossypetin	26	16	22	03	06	09	brown-black
3′-methyl ether	34	22	50	19	22	28	brown-black
4′-methyl ether	43	31	50	23	18	31	brown-black
7-methyl ether	43	30	27	15	20	35	brown-black
8-methyl ether	46	34	54	39	26	26	brown-yellow
8,3′-dimethyl ether	61	46	74	62	48	60	brown-yellow

	λ_{max} (nm)†		MS (m/z)				
	in MeOH	MeOH–$AlCl_3$	M	M−15	M−43	A-ring	B-ring
Gossypetin	264, 277, 338, 386	370, 449	318 (100)	—	289 (14)	169 (25)	137 (28)
3′-methyl ether	260, 272, 338, 385	377, 484	332 (100)	317 (3.5)	289 (1.4)	169 (6.4)	151 (10.6)
4′-methyl ether	261, 272, 337, 384	374, 498	332 (100)	317 (20.3)	289 (5.2)	169 (7.3)	151 (8.5)
7-methyl ether	260, 275, 342‡, 392	388, 470	332 (100)	317 (20.4)	289 (13.4)	—	137 (19.2)
8-methyl ether	260, 271, 340‡, 381	367, 444	332 (48)	317 (100)	289 (13)	—	137 (12)
8, 3′-dimethyl ether	258, 272‡, 340, 380	370, 438	346 (30)	331 (100)	307 (20)	—	151 (12)

*Solvent key: Forestal = conc HCl–HOAc–H_2O (3:10:30); BAW = *n*-BuOH–HOAc–H_2O (4:1:5, top layer); CAW = $CHCl_3$–HOAc–H_2O (2:1:1, bottom layer); BPF = *n*-BuOH–pyridine–formic acid (36:9:5); BEM = C_6H_6–MeCOEt–MeOH (4:3:3).

†In the presence of NaOAc, all the methyl ethers, except the 7-, give a positive shift in band I; in alkali, there is a bathochromic shift but the spectra are unstable.

‡Shoulder.

flavonols in the wings of *Coronilla valentina* seems to be a distinctive feature of this species. Other yellow-flowered legume species so far examined appear to have different patterns. For example, *Lotus corniculatus*, which contains gossypetin and gossypetin 8-methyl ether as galactosides [8], has these UV-absorbing compounds in both wing and standard. Again, *Ulex* species, which contain UV-absorbing yellow chalcones [11], have these substances in all the floral parts. Finally, other patterns appear to be present even in the same genus. Thus, the yellow-flowered *Coronilla emerus* has UV-absorbing wings but the UV absorbance is due to the presence of colourless flavonols. Work on the flavonoids of these and other legume flowers is in progress and will be reported later.

EXPERIMENTAL

Plant material. Plants of *Coronilla valentina* L. subsp. *glauca* (L.) Batt. were grown from seed supplied by the Botanic Garden of the University of Lisbon and a voucher specimen is deposited in the herbarium of this university.

Isolations and identifications. These were carried out using standard procedures. The acid hydrolysis of the gossypetin derivatives was carried out in N_2 to avoid losses due to ready oxidation.

Gossypetin 3′-methyl ether 3-rutinoside. The 3-rutinoside was isolated as yellow crystals, λ_{max} in MeOH: 258, 279, 310, 340; +NaOAc, 286; +H_3BO_3, 323, inf. 345; +$AlCl_3$, 367, 420; +NaOH, 398 nm (stable). R_f values (rutin in parentheses) were 0.37 (0.46) in BAW, 0.27 (0.32) in BEW and 0.51 in 15% HOAc. On acid hydrolysis, it gave glucose and rhamnose in equal amounts. On H_2O_2 oxidation, it gave rutinose in good yield, the identity of the disaccharide being confirmed by co-chromatography in 4 solvents with authentic material. The 3′-methyl ether was obtained as deep yellow powder, mp 338° (decomp.). Molecular ion in MS at 332.0538 ($C_{16}H_{12}O_8$ requires 332.0532). R_f and spectral properties are given in Table 1. On demethylation with pyridinium chloride for 2 hr at 130°, it gave gossypetin.

Gossypetin 8,3′-dimethyl ether 3-rutinoside. The 3-rutinoside was a yellow powder, λ_{max} in MeOH: 257, 273, inf. 340, 358; +NaOAc, 279; +$AlCl_3$, 277, 368, 406; +NaOH, 426 nm (stable). R_f values were 0.45 (0.46) in BAW, 0.32 (0.32) in BEW, 0.30 (0.44) in H_2O and 0.54 (0.51) in 15% HOAc. On acid hydrolysis, it gave glucose and rhamnose in equal amounts and on H_2O_2 oxidation, it gave rutinose. The aglycone, limocitrin, was obtained as a yellow powder, MS, M^+ 346 ($C_{17}H_{14}O_8$ requires 346). The R_f and spectral data are shown in Table 1 and match exactly lit. data [7]. On demethylation with pyridinium chloride, it yielded a mixture of monomethyl ethers and gossypetin.

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