2. Barau, A. K., Chakraborti, P. and Sanal, P. K. (1969) *J. Indian Chem. Soc.* **46**, 271.
3. Nair, A. G. R., Ramach, P., Nagarajan, S. and Subramanian, S. S. (1973) *Indian J. Chem.* **12**, 1316.
4. Mathuram, S., Purushathaman, K. K., Sarada, A. and Connolly, J. D. (1976) *Phytochemistry* **15**, 838.
5. Harborne, J. B. and Williams, C. A. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H. eds) pp. 376–441. Chapman & Hall, London.
6. Hörhammer, L., Stich, L. and Wagner, H. (1961) *Arch. Pharm.* **294**, 685.
7. Spiteller, G. (1971) *Physical Methods in Heterocyclic Chemistry* (Katritzky, A. R. ed.) Vol. III, p. 267. Academic Press, New York.
8. Percheron, F. (1966) *Chromatography* (Heftmann, E. ed.) p. 578. Reinhold, New York.

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ON THE NATURAL OCCURRENCE OF GOSSYPETIN 7- AND 8-MONOMETHYL ETHERS

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Key Word Index—*Eriogonum nudum*; Polygonaceae; *Lotus corniculatus*; Leguminosae; *Geraea canescens*; Compositae; yellow flower pigments; gossypetin 7-methyl ether; gossypetin 8-methyl ether.

Abstract—The 7-methyl ether of gossypetin occurs, as a mixture of 4 glycosides, in the yellow inflorescence of *Eriogonum nudum*. In contrast to previous reports, however, it does not occur in *Lotus corniculatus* flowers, nor is it present in leaves of *Medicago sativa*. The 8-methyl ether, which is present in *Lotus* flowers, has been found for the first time in the Compositae, in flowers of *Geraea canescens*.

INTRODUCTION

Although gossypetin (8-hydroxyquercetin) is well known as a relatively common yellow pigment in plants [1], its monomethyl ethers have rarely been recorded [2]. The 8-methyl ether was first reported in flowers of *Lotus corniculatus* [3], while a yellow pigment which appeared to be different and hence was assigned the 7-methyl ether structure was also reported from the same source [4]. Gossypetin 7-methyl ether was also described as occurring in *Ranunculus* flowers [5] and in *Medicago sativa* leaves [6]. There were difficulties in identification since the 7-methyl ether had not been synthesized and spectral and chromatographic properties of authentic material were not known. However, this situation has recently been rectified by Wagner *et al.* [7] who have synthesized both isomers, together with the 4′-methyl ether, and at the same time have shown that the pigment from *Ranunculus repens* flowers is the 8- and not the 7-methyl ether.

In our continuing studies of the contribution of yellow flavonoids to flower colour in the angiosperms, we have discovered that the 7-methyl ether is the major yellow pigment of flowers of *Eriogonum nudum*. In reporting this, we wish to correct the earlier misidentification of the

RESULTS

Eriogonum is a Western North American genus of some 205 spp., the flowers of which are gathered in a showy involucre, but which are generally pink or white. Flowers of *Eriogonum nudum* ssp. *saxicola* are unusual in the genus in having a distinctive yellow colour, reminiscent in shade of the flower colour of those members of *Primula* and *Rhododendron* which are pigmented by gossypetin [1] rather than by the much more common carotenoids. Hydrolysis of the flower extract gave a gossypetin-like aglycone, which had slightly higher R_fs in all solvents when compared with gossypetin. It was clearly a monomethyl ether, and gave gossypetin on demethylation. It was readily identified as the 7-methyl ether by direct comparison (Table 1) with a synthetic specimen, being clearly distinguished in R_f and spectral properties from the 7-methyl ether.

Gossypetin 7-methyl ether is thus the major yellow colouring matter of these flowers, since carotenoids are essentially absent. It occurs in glycosidic form and four glycosides were identified: the 8-glucoside, the 3-arabinoside, the 3-galactoside and the 3-galactoside-8-glucoside. Small amounts of an isomeric aglycone were also detected during identification of these glycosides and this was identified as the 8-methyl ether by direct comparison with authentic material from *Lotus* (see below). *Eriogonum nudum* is thus the first plant in which both the 7- and 8-monomethyl ethers of gossypetin have been detected. Analyses of the other flavonoids in the flowers showed the presence of six common flavonol glycosides: the 3-galactoside, 3-glucuronide, 3-arabinoside and 3-rutinoside of quercetin and the 3-galactoside and 3-glucuronide of myricetin.

Table 1. Properties of gossypetin and its 7- and 8-methyl ethers.

Measurement	Gossypetin	Gossypetin 7-methyl ether*	Gossypetin 8-methyl ether†
λ_{max} (MeOH)	264, 277, 338, 386	260, 275, 342*i*, 392	260, 271, 340*i*, 381
MeOH–NaOAc	252, 370	263, 275*i*, 348*i* 402	283, 328, 405
MeOH–$AlCl_3$	370, 449	276, 388, 470	276, 367, 444
mp	313–4°	303–5°	273–5°
R_f (× 100)			
BAW	19	27	54
Forestal	26	43	46
PhOH	19	31	51
CAW	06	15	39
50%HOAc	18	30	34
TLC:BPF‡	08	19	28
Colour in UV	brown–black	brown–black	brown–yellow

* Synthetic material and natural material from *Eriogonum*.

† Data from synthetic material and natural material from *Lotus*.

‡ TLC on Si gel, BPF = C_6H_6–Py–HCO_2H (36:9:5).

Geraea canescens T. & G. (Compositae) is an important yellow-flowering desert annual in California, since valley floors up to 900 M may be covered with the blooms of this plant during the month of May. Although the flowers are pigmented by carotenoid, 2D chromatograms of the ray flower extract showed the presence of significant amounts of a yellow flavonol and this was identified as gossypetin 8-methyl ether, by direct comparison with material from *Lotus* (see Table 1). Identification was confirmed by MS, since the 8-methyl ether gives a very strong characteristic M-15 ion [3]. The 8-methyl ether was found to be present in *Geraea* flowers as the 3-galactoside.

The discovery of the 7- and 8-methyl ethers of gossypetin in the above sources suggested that a re-appraisal was necessary of the material earlier isolated from *Lotus corniculatus*, described as Lotus F1A, with mp 252–4°. It was thought to be the 7-methyl ether, since it showed some differences in properties from the 8-methyl ether, identified about the same time from this plant. On re-examination, the sample was found to be a mixture of gossypetin methyl ether with quercetin and when purified, the former was readily recognized as the 8-methyl ether, on the basis of identical properties with the synthetic material [7]. Furthermore, examination of the hydrolysate of fresh flowers of *Lotus corniculatus* showed that only a single gossypetin monomethyl ether was present, namely the 8-methyl ether reported earlier [3].

Gossypetin 7-methyl ether was described, on very limited evidence, as being present in *Medicago sativa* leaves [6]. Our own study of both leaves and flowers of *Medicago* failed to reveal any gossypetin-like pigments. Difficulties in identifying the flavonols of this plant probably arise from the fact that the leaves contain kaempferol 7-glycosides which are resistant to acid hydrolysis and appear as false 'aglycone' spots on chromatograms of hydrolyzed extracts. Our preliminary results on *Medicago* leaf flavonoids indicate the presence of the 7-glucuronide, 7-glucoside and 7-rhamnoside of kaempferol.

DISCUSSION

The discovery of gossypetin 7-methyl ether in *Eriogonum* (Polygonaceae) is the first report of this pigment in flowers. Remarkably enough, the same compound has just been reported (as the 3-rhamnoside) as a leaf constituent in *Atraphaxis purpurea*, also Polygonaceae [8]. The presence of a yellow gossypetin derivative in the family brings the number of families with these pigments to twelve [1]. The presence of the gossypetin rather than the isomeric quercetagetin (6-hydroxyquercetin) skeleton is that to be expected in a family which is relatively primitive in its morphological features (advancement index 58% [9]) and which is usually classified near the Caryophyllales and Plumbaginales. Gossypetin derivatives have, however, not yet been reported in either of these orders, and the nearest occurrence seems to be in the Primulaceae [10].

In conclusion, it may be noted that as a result of this work the 8-methyl ether appears to be more common as a flower pigment than gossypetin 7-methyl ether. Thus the 8-methyl ether contributes to yellow flower colour in *Geraea canescens* (Compositae), *Lotus corniculatus* (Leguminosae) and several *Ranunculus* species (Ranunculaceae). It has also been detected in yellow flowers of *Cowania* (Rosaceae) [11]. By contrast, the only plant with the 7-methyl ether in the flowers is apparently *Eriogonum nudum*. Surveys, however, are in progress to see whether the 7-methyl ether occurs elsewhere in this genus.

EXPERIMENTAL

Plant material. The *Eriogonum* and *Geraea* species were collected in S. California and voucher specimens are deposited in the UCSB herbarium. The *Eriogonum* species was identified as *E. nudum* Dougl. ex Benth. subsp. *saxicola* (Heller) Munz. *Lotus corniculatus* and *Medicago sativa* were analyzed from material in the University of Reading living collection.

Flavonoid identifications. The flavonol glycosides of *Eriogonum* flowers were separated and purified by repeated PC in the standard solvents. The glycosides of quercetin and myricetin were identified by standard procedures, and where possible by direct comparison with authentic specimens. The four glycosides of gossypetin 7-methyl ether appeared during purification as yellow bands in visible light, being dark brown–black in UV. Data on these four new glycosides follow; R_f values are given × 100 and comparative values for rutin are 37 (BAW), 43 (PhOH), 37 (H_2O) and 41 (15% HOAc). All spectral measurements are given in MeOH as solvent (*i* = inflection). The *3-arabinoside* had R_f 24 (BAW), 60 (PhOH), 03 (H_2O) and 14 (15% HOAc), λ_{max} 255, 275, 312, 350; + NaOAc, 271, 278*i*, 312*i*, 390; + $AlCl_3$, 400 nm. On hydrolysis, it gave gossypetin 7-methyl ether (see Table 1) and arabinose. H_2O_2 oxidation gave arabinose, confirming the attachment of sugar in the 3-position. The *3-galactoside* had R_f 23 (BAW), 56 (PhOH), 06 (H_2O) and 21 (15% HOAc), spectral properties as for the 3-arabinoside. On hydrolysis, it gave the 7-methyl ether and galactose; H_2O_2 oxidation confirmed the attachment of sugar in the 3-position. The *8-glucoside* had R_f 11 (BAW), 34 (PhOH), 02 (15% HOAc), λ_{max}260, 275*i*, 345, 392, + NaOAc, 260, 280*i*, 430; + $AlCl_3$, 477 nm. Hydrolysis gave glucose and the 7-methyl ether. The *3-galactoside-8-glucoside* had R_f 32 (BAW), 72 (PhOH), 09 (H_2O) and 30 (15% HOAc), λ_{max} 260, 273, 308, 350, + NaOAc, 258*i*, 272; + $AlCl_3$, 452; + NaOMe, 403; and + H_3BO_3, 292 nm. Acid hydrolysis gave the 7-methyl ether, galactose and glucose. Partial acid hydrolysis gave the 8-glucoside (see above), while H_2O_2 oxidation gave galactose and β-glucosidase hydrolysis gave the 3-galactoside as intermediate. The major yellow flavonol

glycoside of *Geraea canescens* was purified by PC and obtained as a crystalline solid, R_f 60 (BAW), 64 (PhOH) and 44 (15% HOAc), λ_{max} 263, 272, 366; +NaOEt, 420; +NaOAc, 283; + H_3BO_3, 386; and + $AlCl_3$ 366, 414 nm. On acid or β-glucosidase hydrolysis, it gave galactose (with some glucose) and gossypetin 8-methyl ether (see Table 1). The aglycone was further confirmed by MS: M 332 ($C_{16}H_{12}O_8$ requires 332) (48%), M-15 (100%), M-29 (3%), M-43 (13%) and B ring fragment at 137 (12%).

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REFERENCES

1. Harborne, J. B. (1972) *Recent Adv. Phytochem.* **4**, 107.
2. Gottlieb, O. R. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H. eds) 296–375. Chapman & Hall, London.
3. Nielsen, J. G. (1970) *Tetrahedron Letters* 803.
4. Harborne, J. B. (1969) *Phytochemistry* **8**, 177.
5. Urschler, J. (1968) *Phyton (Horn, Austria)* **13**, 15.
6. Bradner, N. R. and Brink, V. C. (1968) *Can. J. Plant Sci.* **48**, 97.
7. Wagner, H., Rüger, R., Maurer, G. and Farkas, L. (1977) *Chem. Ber.* **110**, 737.
8. Chumbalov, T. K. (1976) *Khim. Prir. Soedin.* 658.
9. Sporne, K. R. (1969) *New Phytologist* **68**, 555.
10. Harborne, J. B. (1968) *Phytochemistry* **7**, 1215.
11. Koehler, D. and Smith, D. M. (1977) *Madrono* in press.

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RARE METHYLATED FLAVONOLS FROM *ANGELONIA GRANDIFLORA*

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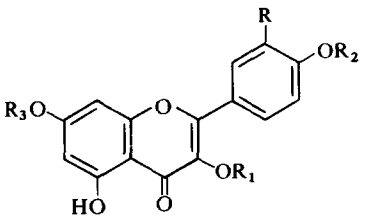
We previously identified scutellarein 7,4′-dimethyl ether and 5-hydroxy-6,7,4′-trimethoxyflavone (salvigenin) from leaf tissue of *Angelonia grandiflora* [1]. In continuation of our investigation, we now report the isolation and identification of five rare methylated flavonols: 3,4′-, 3,7- and 7,4′-dimethylquercetin and 3,4′ and 7,4′-dimethylkaempferol. All five compounds are known from other sources: quercetin 3,4′-dimethyl ether from *Baccharis sarothroides* [2], 3,7-dimethylquercetin from *Aeonium manriquiorum* [3] and *Larrea cuneifolia* [4], 7,4′-dimethylquercetin from *Phytolacca dioica* [5], 3,4′-dimethylkaempferol from propolis [6] and *Betula ermanii* [7] and 7,4′-dimethylkaempferol from *Cheilanthes farinosa* [8]. Their present isolation together with our previous finding [1] of 7,4′-dimethylscutellarein and salvigenin reveals an unusual co-occurrence of rare methylated flavonols and flavones in *A. grandiflora*.

EXPERIMENTAL

Plant material. A voucher specimen of *Angelonia grandiflora* C. Mor. has been deposited at JIPMER.

Flavonoid identification. A hot C_6H_6 extract of dried leaf material was subjected to column chromatography over silicic acid using petrol (60–80°), petrol–C_6H_6, C_6H_6–$CHCl_3$ and $CHCl_3$–MeOH as eluents. Petrol and petrol–C_6H_6 yielded carotenoids and triterpenes; other fractions gave flavone and flavonol derivatives including the five rare methylated flavonols, A–E.

Flavonol A (**1**). Major ($CHCl_3$–C_6H_6, 3.1), mp 238–39°, UV—purple UV/NH_3—dull yellow; λ_{max} (nm) . 220 *sh*, 253, 268 *sh*, 353 (MeOH); 273, 321, 367 (NaOAc); 210 *sh*, 235 *sh*, 265, 301, 363, 395 *sh* ($AlCl_3$); 210 *sh*, 237 *sh*, 264, 301, 360, 396 *sh* ($AlCl_3$/HCl) and 235 *sh* 273, 325 *sh*, 410 (NaOMe); PMR signals (CD_3SOCD_3, δ values, ppm) at 12.60 (broad *s*, 1H, disappeared on D_2O exchange, 5-O<u>H</u>) 7.73 (*d*, 2 Hz, 1H, 2′-<u>H</u>) 7.67 (*dd*, 2 Hz and 9 Hz, 1H, 6′-H) 7.05 (*d*, 9 Hz, 1H, 5′-<u>H</u>) 6.52 (unresolved *d*, 1H, 8-H) 6.28 (unresolved *d*, 1H, 6-H) 3.96 (*s*, 3H, 3-OC<u>H</u>$_3$) and 3.88 (*s*, 3H, 4′-OMe). Its MS exhibited the following ions: (*m/e*) 330 (M^+, 100%), 329 (M^+-H, 40), 315 (M^+-Me, 50), 299 (M^+-OMe, 18), 297 (315-H_2O, 18), 287 (M^+-MeCO, 90), 272 (287-Me, 18), 244 (M^+-2MeCO, 35), 153 (RDA fragment A_1 + H, 50)*, 151 (fragment C,24) and 147 (RDA fragment B_1 − OMe, 30). It had R_f (× 100, Whatman No. 1, ascending, 30°) 16 (15% HOAc), 47 (30% HOAc), 67 (50% HOAc), 94 (BAW), 93 (phenol), 84 (Forestal) and 90 (*t*BAW). On methylation, it gave quercetin pentamethyl ether and on demethylation quercetin. It was



1 R = OH, R_3 = H, R_1 = R_2 = Me
2 R = OH, R_2 = H, R_1 = R_3 = Me
3 R = OH, R_1 = H, R_2 = R_3 = Me
4 R = R_3 = H, R_1 = R_2 = Me
5 R = R_1 = H, R_2 = R_3 = Me

*For explanations of A_1, B_1, etc, see [9].