Methylated Flavonols in the Genus *Gossypium*

Flavonol fractions of cotton flowers (*Gossypium hirsutum* var. Deltapine Smoothleaf) were obtained by ethanol extraction of flower petals, acid hydrolysis of the extract, and purification of the resulting aglycones by crystallization, sublimation, and thin-layer chromatographic separation. Thin-layer chromatography and ultraviolet and mass spectral analyses served to identify for the first time methylated flavonols in the genus *Gossypium*.

The flower petals of *Gossypium* species have been analyzed by many investigators for their flavonoid content. To date, only four flavonols, all without methyl substituents, have been identified in the genus. Thus, *G. herbaceum* (Neelakantam *et al.*, 1935, 1937; Perkin, 1899, 1909; Rao and Seshadri, 1943), *G. arboreum* (Parks, 1965; Perkin, 1916), *G. neglectum* (Perkin, 1916), *G. sanguineum* (Perkin, 1916), *G. indicum* (Neelakantam and Seshadri, 1936), and *G. hirsutum* (Denliev *et al.*, 1962; Neelakantam *et al.*, 1935; Parks, 1965; Sadykov *et al.*, 1960; Viehoever *et al.*, 1918) were shown to contain one or more of the four flavonols (quercetin, gossypetin, herbacetin, and kaempferol).

Using *G. hirsutum* var. Deltapine Smoothleaf, a white flower variety, we have identified for the first time methylated flavonols in ethanolic extracts of flower petals. Two monomethylated flavonols, tamarixetin (I) and kaempferide (II), have been unequivocally identified, and a dimethylated quercetin (III) has been partially characterized.

EXPERIMENTAL

Apparatus. Ultraviolet spectral studies were performed with a Cary Model 14 spectrophotometer. Thin-layer chromatographic separations and analysis were performed with Baker-flex polyamide 11 and Brinkmann MN-Polygram polyamide-11 sheets. Mass spectral measurements were obtained with a Hitachi high resolution double-focusing mass spectrometer, RMU-6D-3, equipped with peak-matching device and mass marker.

Isolation of Flavonols. Cotton flower petals (300 g) were extracted with 3 l. of absolute ethanol with occasional stirring for 3 days. Evaporation of the extract gave a residue, which was washed extensively with petroleum ether and refluxed 2 hr in a solution of 10 ml of concd. sulfuric acid and 500 ml. of water. After cooling to room temperature and adjusting the pH to 4 to 5, filtration served to collect flavonol aglycones (yield 1.5 g). After washing with petroleum ether, the flavonol fraction was crystallized from hot water, dried, and heated in a vacuum sublimator at 260° C and 10 μ for 3 days. The yellow sublimate was then fractionally sublimed, as described by Sim (1967), by heating at 200° C and 20 μ overnight; this step served to concentrate the more volatile methylated flavonols by leaving the bulk of the total fraction (quercetin and kaempferol) unsublimed. Separation of this second sublimate by thin-layer chromatography on polyamide sheets using chloroform-ethanol-methylethylketone (12:2:1), a modified system of Egger and Keil's (1965), gave intense bands for quercetin (R_f 0.18), kaempferol (R_f 0.37), and a third flavonol (I) (R_f 0.58), a slightly weaker band for a fourth flavonol (II) (R_f 0.75), and a very weak band for a fifth flavonol (III) near the solvent front. Preconditioning of the polyamide sheets, by first developing in the solvent before application of the flavonol fraction, was necessary to eliminate significant distortion of the front. The three bands corresponding to the unidentified flavonols were eluted with chloroform-ethanol (9:1), filtered, and the filtrates evaporated. The residues, which were obtained in trace amounts, were examined by thin-layer chromatography and by ultraviolet and mass spectral analysis.

Table I. Tlc of Cotton Flavonol Fractions and Standards

	R_f on Polyamide		
Flavonol	$CHCl_3$-EtOH-MEK (12:2:1)	BuOH-AcOH-H_2O (5:2:3)	BuOH-H_2O (86:14)
Quercetin	0.08	0.13	0.11
Kaempferol	0.38	0.18	0.23
I	0.46	0.23	0.19
Rhamnetin	0.55	0.25	0.19
II	0.59	0.29	0.25
III	0.96	0.39	0.37
Ombuin	0.96	0.42	0.37
Rhamnazin	0.96	0.40	0.40

RESULTS AND DISCUSSION

Thin-Layer Chromatographic Analysis. Thin-layer chromatography of I, II, and III on polyamide along with available standards gave the results given in Table I. These data, coupled with reported (Egger and Keil, 1965) R_f values of related flavonols, suggested that the unknowns were perhaps methylated derivatives of quercetin and kaempferol.

Ultraviolet Spectral Analysis. Table II records the results of ultraviolet spectral studies designed to locate the positions of the methyl groups (Jurd, 1962). The large bathochromic shift of the long wavelength band of I–III (58 to 65 mμ) in ethanolic aluminum chloride showed the presence of a free 3-hydroxyl group. The failure of sodium acetate-boric acid

Table II. UV Spectra of Cotton Flavonol Fractions

Flavonol	UV maxima (mμ) EtOH	$AlCl_3$	H_3BO_3-NaOAc	NaOEt
I	254, 265 (sh), 369	263, 430	253, 372	276, 420 (stable)
II	266, 365	268, 430	266, 366	279, 417 (stable)
III	253, 265 (sh), 367	257, 425	...	...

to cause a shift in the spectrum of I and II indicated no *o*-dihydroxyl groups, and the large shift of the long wavelength band (51 to 52 mμ) in sodium ethoxide solution with no reduction in intensity clearly demonstrated substitution in the 4′-position. Stability of III in sodium ethoxide solution was observed, as indicated by persistence of yellow coloration, although its spectrum in this solvent was not recorded because of lack of material.

Mass Spectrometric Analysis. Confirmation for methyl substitution in I and II was obtained by mass spectral analysis. I gave a strong molecular ion peak at *m/e* 316 (base peak) and a prominent peak at *m/e* 301, corresponding to loss of methyl. II similarly gave a base peak corresponding to the molecular ion at *m/e* 300 and a prominent peak at *m/e* 285 ($M^+ - CH_3$). III showed prominent peaks at *m/e* 330, 315, and 300, corresponding to a molecular ion of a dimethylquercetin and loss of one and two methyls, respectively. Mass spectra of related, partially methylated flavonols have been discussed by other investigators (Bowie and Cameron, 1966; Pelter *et al.*, 1965).

The data clearly identify I as tamarixetin (4′-*O*-methylquercetin) and II as kaempferide (4′-*O*-methylkaempferol). The limited data available for III identify it as a dimethylquercetin but do not allow for unequivocal location of the methyl groups. Comparison of UV maxima of III with that of known dimethylquercetins suggest ombuin (4′,7-di-*O*-methylquercetin) or 3′,4′-di-*O*-methylquercetin as the structure of III, with the latter being favored. If III is indeed 3′,4′-di-*O*-methylquercetin, it would represent a new naturally-occurring flavonol.

Both flavonols heretofore unreported in *Gossypium* species are methylated in the 4′-position, and the relative abundances of the two are of the same order as their dominant, unmethylated prototypes, quercetin and kaempferol. The existence of small amounts of I and II in comparison with the massive amounts of quercetin and kaempferol suggests the presence of a suppressed "methylating gene" in flower petals for methylation of flavonols, or their chalcone precursors, in 4′-position (Grisebach, 1965; Harborne, 1962).

The presence of a dimethylquercetin led to the speculation that other methylated flavonols may be present in the genus, although in very limited amounts. To investigate this possibility, the flavonol sublimate, which was used as the starting material for the tlc separation of I–III, was examined by mass spectrometry. The mass spectrum did indeed contain peaks corresponding to tri- (*m/e* 344) and tetra-methylquercetin (*m/e* 358) as well as di- (*m/e* 314) and trimethylkaempferol (*m/e* 328). The intensity of the peaks, however, was low and suggested that only trace amounts of these types, as well as III, were present relative to quercetin, kaempferol, tamarixetin, and kaempferide.

The results of this study raise the question as to whether or not the yellow flower varieties of *Gossypium* species contain monomethylated gossypetin and herbacetin, as well as tamarixetin and kaempferide, in concentrations comparable to those of tamarixetin and kaempferide found to be present in the white-flowered variety. Possibly the trace components observed by Parks (1965) upon paper chromatography of *G. hirsutum*, *G. barbadense*, and *G. arboreum* extracts correspond to some of these methylated flavonols.

LITERATURE CITED

Bowie, J. H., Cameron, D. W., *Aust. J. Chem.* **19,** 1627 (1966).
Denliev, P. K., Pakudina, Z. P., Sadykov, A. S., *Dokl. Akad. Nauk. Uzb. SSR* **19,** 34 (1962).
Egger, K., Keil, M., *Z. Anal. Chem.* **210,** 201 (1965).
Grisebach, H., in "Chemistry and Biochemistry of Plant Pigments" (Edited by T. W. Goodwin), Chap. 10. Academic Press, New York, 1965, p. 279.
Harborne, J. B., in "The Chemistry of Flavonoid Compounds," (Edited by T. A. Geissman), Chap. 18, MacMillan, New York, 1962, p. 608.
Jurd, L., in "The Chemistry of Flavonoid Compounds," (Edited by T. A. Geissman), Chap. 5, MacMillan, New York, 1962, p. 119.
Neelakantam, K., Ramachandra Rao, R. H., Seshadri, T. R., *Proc. Indian Inst. Sci.* **1A,** 887 (1935).
Neelakantam, K., Seshadri, T. R., Ramachandra Rao, R. H., *Proc. Indian Inst. Sci.* **2A,** 490 (1935).
Neelakantam, K., Seshadri, T. R., *Proc. Indian Inst. Sci.* **4A,** 54 (1936).
Neelakantam, K., Ramachandra Rao, R. H., Seshadri, T. R., *Proc. Indian Inst. Sci.* **5A,** 357 (1937).
Parks, C. R., *Amer. J. Bot.* **52,** 309 (1965).
Pelter, A., Stainton, P., Barber, M., *J. Heterocycl. Chem.* **2,** 262 (1965).
Perkin, A. G., *J. Chem. Soc.* **75,** 825 (1899).
Perkin, A. G., *J. Chem. Soc.* **95,** 2181 (1909).
Perkin, A. G., *J. Chem. Soc.* **109,** 145 (1916).
Rao, P. S., Seshadri, T. R., *Proc. Indian Acad. Sci.* **18A,** 204 (1943).
Sadykov, A. S., Pakudina, Z. P., Denliev, P. K., *Dokl. Akad. Nauk Uzb. SSR* **17,** 23 (1960).
Sim, K. Y., *J. Chem. Soc.* (*C*) 976 (1967).
Viehoever, A., Chernoff, L. H., Johns, C. O., *J. Agr. Res.* **13,** 345 (1918).

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