prepared from hexadecanol *via* the chloride, the Grignard, and ethyl orthoformate. A solution of 1.7 g. (0.0067 mole) of freshly prepared heptadecanal (m.p. 36°) in 25 ml. of 95% ethanol was added to a solution of 1.0 g. (0.0057 mole) of L-cysteine hydrochloride monohydrate[15] and 0.6 g. (0.007 mole) of potassium acetate in 25 ml. of water. Upon vigorous agitation for a few minutes, precipitation occurred. After standing at room temperature for an hour the mixture was refrigerated overnight. Following filtration by gentle suction, the precipitated thiazolidine carboxylic acid was thoroughly washed with water, cold 95% ethanol, ether, and air-dried. Upon recrystallization from boiling isopropyl alcohol, there were obtained 1.54 g. (76%) of product melting at 142–143°, dec.

(15) Purchased from Mann Fine Chemicals Inc., New York, N. Y.

Cysteinyl cinnamyl mercaptal. This was prepared similarly, using a 2:1 ration of water to ethanol. The product, for which no recrystallization solvent was found, is a white powder, melting at 179.3–180.5°, dec., obtained in 81% yield.

Anal. Calcd. for $C_{15}H_{20}O_4N_2S_2$: C, 50.54; H, 5.66; N, 7.86. Found: C, 50.45; H, 5.75; N, 7.55.

Acknowledgment. Thanks are due to Monsanto Chemical Co. for samples of *o*-vanillin and bourbonal, to Heyden Chemical Corp. for 2,6-dichlorobenzaldehyde, to Shell Chemical Corp. for 2-ethyl-2-hexenal, and to Rohm & Haas Co. for 2,2,4,8,10,10-hexamethylundecene-5-al-5.

BROOKLYN 1, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF CALIFORNIA AT LOS ANGELES]

Flavonoid Petal Constituents of *Chrysanthemum segetum* L.

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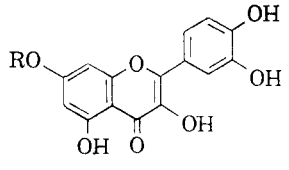
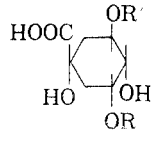
The petals of *Chrysanthemum segetum* L. contain gossypitrin (3,3′,4′,5,7,8-hexamethoxyflavone-7-glucoside), quercimeritrin, and chlorogenic and isochlorogenic acids. This is the first recorded occurrence of gossypitrin in a plant family other than the Malvaceae. The co-occurrence of gossypitrin and quercimeritrin is observed in both *C. segetum* and in *Gossypium* species, and appears to be of significance in the problem of the biogenesis of the flavonoid pigments.

Chromatographic examination on paper of the methanol extract of the bright yellow petals of *Chrysanthemum segetum* L. disclosed the presence of five distinct substances. Three of these were readily identified as quercimeritrin (quercetin-7-glucoside) (I), chlorogenic acid (II), and isochlorogenic acid (III). A fourth was isolated by methanol extraction of the fresh or dried petals and concentration of the extract, when it separated as a bright yellow crystalline substance in an amount constituting six per cent of the weight of the dried petals. Spectral and R_f data suggested that this compound was a derivative of a hexahydroxyflavone, and analyses of the glucoside, the aglucon, and their acetates were in agreement with the formulation of the pigment as a monoglucoside of a hexahydroxyflavone. The melting point of the aglucon hexaacetate was in agreement with that reported for gossypetin (IV, R = H) hexaacetate,[1] but since spectral data for gossypetin derivatives were lacking, and authentic samples were not at hand for direct comparison, the structure of the glycoside was established by degradative and synthetic procedures.

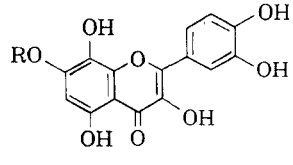
The presence of a free hydroxyl group in the 3-position of the glycoside was indicated by the characteristic shift of 60 mμ in the long wavelength absorption maximum (from 388 to 448 mμ) induced by the addition of aluminum chloride.[2] Methylation of the aglycon yielded gossypetin hexamethyl ether, identical with an authentic sample prepared by synthesis from quercetin 3,3′,4′,7-tetramethyl ether.[3]

Alkaline cleavage of the fully methylated aglycon yielded veratric acid and 2′-hydroxy-2,3′,4′,6′-tetramethoxyacetophenone.[4]





I, R = $C_6H_{11}O_5$ (glucosyl)
II, R = H, R′ = 3,4-dihydroxycinnamoyl
III, R′ = H, R = 3,4-dihydroxycinnamoyl



IV, R = H
V, R = $C_6H_{11}O_5$ (glucosyl)

The determination of the position of the sugar residue in the glycoside, and thus the establishment of the identity of the latter with gossypitrin (V),

(1) A. G. Perkin, *J. Chem. Soc.*, **95**, 2181 (1909).
(2) T. A. Geissman and L. Jurd, unpublished results.
(3) P. S. Rao and T. R. Seshadri, *Proc. Indian Acad. Sci.*, **25A**, 379 (1946).
(4) A. G. Perkin, *J. Chem. Soc.*, **103**, 653 (1913)

was accomplished by methylation of the glycoside and subsequent removal of the sugar residue. The product had the properties reported for 7-hydroxy-3,3′,4′,5,8-pentamethoxyflavone.[5] Identity of the sugar as glucose was established by paper chromatographic methods.

Quercimeritrin (I) was identified by direct comparison with an authentic sample on paper chromatograms, and by hydrolysis and chromatographic identification of quercetin and glucose. Quercimeritrin, a 7-glucoside, is readily distinguished from quercetin-3-glycosides on paper chromatograms: It shows a bright yellow fluorescence in ultraviolet light, changing to a vivid yellow-green when sprayed with aluminum chloride; 3-glucosides are, respectively, brown to purple, and yellow under these conditions.

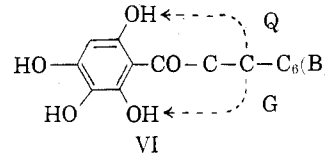
Chlorogenic and isochlorogenic acids were identified by their characteristic color (blue, changing to bright blue-green with ammonia vapor) on paper under ultraviolet light, by their absorption spectra, by the formation of caffeic acid (identified by comparison with authentic material on paper) upon alkaline hydrolysis, and by chromatographic comparison with authentic samples.

The fifth substance was neither strongly visible nor well differentiated on a paper chromatogram. Its spectral behavior was that of a flavonol, but it has not yet been identified.

The presence of gossypitrin, quercimeritrin, and the two caffeic acid esters in *C. segetum* emphasizes several biogenetic relationships that have been recognized in other cases. Their common possession of the 3,4-dihydroxyphenyl grouping parallels numerous observations already recorded,[6,7] and suggests the biogenetic importance of the 3,4-dihydroxyphenylpropane unit.[8,9]

The co-occurrence of gossypitrin (V) and quercimeritrin (I) may have a special significance. The members of this pair of glucosides differ only in the presence of the 8-hydroxyl group in gossypitrin. The occurrence in the same plant of these two compounds has heretofore been known only in two species of Malvaceae,[1,10,11] although the respective aglycons have been found in other species of this family.[12,13] The presence in a single species of pairs of flavonoid compounds differing only by a single hydroxyl group has been observed in other cases,[7,14] and has been offered by Seshadri[14] as evidence to support a theory of biogenesis that includes a direct hydroxylation step (*e.g.*, I → V).

It is of interest to note that flavones with the related 3,3′,4′,5,6,7-hydroxylation pattern (*i.e.*, that of quercetagetin) have been observed in other compositae.[15,16] This arrangement of hydroxyl groups is uniquely related to that present in IV and V by the common relationship of both quercetagetin (containing hydroxyl groups at 5,6,7) and gossypetin (containing hydroxyl groups at 5,7,8) to the hypothetical open-chain precursor VI, in which ring closure according to VI-G would lead to the gossypetin arrangement (5,7,8), and according to VI-Q to the quercetagetin arrangement (5,6,7). Moreover, the rutaceous species *Melicope ternata* contains (*O*-alkylated) flavones belonging to *both* the quercetagetin and the



VI

gossypetin classes.[17] It is clear that much remains to be learned of the biogenetic origin of flavonoid hydroxyl groups, for while the examples of quercimeritrin and gossypitrin, herbacitrin and populnin, and sulfurein and maritimein support the hypothesis of hydroxylation of the fully constituted flavone or aurone, the presence of both gossypetin and quercetagetin derivatives in *Melicope* species can better be accounted for by supposing that the hydroxylation occurs before the heterocyclic ring is formed (VI).

EXPERIMENTAL

Isolation of gossypitrin. Chrysanthemum segetum L. flowers were collected in August near Caspar, Calif. Rays and disks were separated and immersed in methanol. The methanol and ray flowers were slurried in a Waring Blendor, filtered, and the marc extracted further with hot methanol. The total filtered extract (2.5 l.) was evaporated under reduced pressure to 175 ml. A fine, yellow crystalline solid separated. The filtrate was washed with petroleum ether and with ether, concentrated further to 50 ml. and retained for chromatographic study.

The yellow solid, crystallized from dilute acetic acid, melted at 237–241°. It proved to be identical with the most prominent component observed on paper chromatograms.

The plant material remaining after the extraction weighed 65 g. (dry), and the total extractives amounted to 25 g.

(5) T. R. Seshadri, *Proc. Indian Acad. Sci.*, **24A**, 375 (1946).

(6) P. S. Rao and T. R. Seshadri, *Proc. Indian Acad. Sci.*, **18A**, 222 (1943).

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(13) A. G. Perkin, *J. Chem. Soc.*, **95**, 1855 (1909).

(14) T. R. Seshadri, *Proc. Indian Acad. Sci.*, **30A**, 333 (1939).

(15) P. S. Rao and T. R. Seshadri, *Proc. Indian Acad. Sci.*, **14A**, 289 (1941).

(16) Z. Cěkan and V. Herout, *Chem. Listy*, **49**, 1053 (1955).

(17) L. H. Briggs and R. H. Locker, *J. Chem. Soc.*, 3131 (1951) and earlier papers cited therein.

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA OF GOSSYPETIN AND MYRICETIN DERIVATIVES

Compound	95% Ethanol		Ethanol-$AlCl_3$	Ethanol-NaOEt
	λ_{max}	log ϵ	λ_{max}	λ_{max}
Gossypitrin	262, 278, 350,[a] 388	4.33, —, —, 4.20	272, 377, 448	—
Gossypetin	262, 278, 341,[a] 386	4.26, 4.23, —, 4.15	272, 370, 446	—
Gossypetin hexaacetate	255, 295, 320[a]	—	—	—
Gossypetin hexamethyl ether	252, 273, 351	4.34, 4.33, 4.34	—	—
5-OH-3,3′,4′,7,8-penta OMe[b]	255, 272, 360	4.34, 4.33, 4.18	282, 360, 420	285, 395
7-OH-3,3′,4′,5,8-penta OMe[b]	251, 270,[a] 351	—	No shift	280, 368
5,8-diOH-3,3′,4′,7-tetra OMe[b]	255, 280, 330	—	290, 365	—
Myricetin	255, 378	4.21, 4.29	270, 435	—
Myricetin hexamethyl ethyl	262, 332	—	—	—
5-OH-3,3′,4′,5′,7-penta OMe[b]	265, 305,[a] 345	—	280, 345, 395	285, 380

[a] Inflection. [b] Flavone.

The crude gossypitrin weighed 6.0 g., thus constituting about 6% of the dry weight of the petal material.

Anal. Calcd. for a hexahydroxyflavone monoglucoside, $C_{21}H_{20}O_{13}$: C, 50.60; H, 4.40. Found: C, 50.40; H, 4.53.

Gossypitrin acetate was prepared by heating a mixture of 300 mg. of the glucoside, 150 mg. of dry sodium acetate, and 2.5 ml. of acetic anhydride for 90 min. on the steam bath. The white solid that separated when the solution was poured onto ice was recrystallized from aqueous ethanol. The colorless needles melted at 232–236°.

Anal. Calcd. for the nona-acetate of gossypitrin, $C_{39}H_{37}O_{22}$: C, 54.50; H, 4.40. Found: C, 54.51; H, 4.36.

Gossypetin. Hydrolysis of the glucoside with boiling 2*N* sulfuric acid afforded the aglucon. Recrystallized from aqueous acetic acid, it had m.p. 299–304°. Gossypetin has been reported to melt at 304°, 305°, and 310–14° with composition.[13,18,19] The sugar released in the hydrolysis was identified as glucose by means of paper chromatography.

Gossypetin hexaacetate formed colorless needles, m.p. 226–28°, in agreement with the value reported by Perkin.[1]

Anal. Calcd. for $C_{27}H_{22}O_{14}$: C, 56.87; H, 4.11. Found: C, 57.15; H, 4.09.

Gossypetin hexamethyl ether was prepared by the methylation of gossypetin with dimethyl sulfate in acetone in the presence of potassium carbonate. The product melted at 171–172.5°, in agreement with the reported value.[2] There was no depression in the melting point when it was mixed with an authentic sample.

Alkaline cleavage of gossypetin hexamethyl ether with 10% methanolic potassium hydroxide yielded veratric acid and 2′-hydroxy-2,3′,4′,6′-tetramethoxyacetophenone, m.p. 114.5–115.5°, in agreement with the value reported by Perkin.[4]

Position of the sugar residue. Methylation of the glucoside according to Murti and Seshadri,[20] and hydrolysis of the product with dilute sulfuric acid yielded 7-hydroxy-3,3′,4′,-5,8-pentamethoxyflavone,[20] m.p. 250–251°, the *acetate* of which melted at 164–168°.

Gossypetin hexamethyl ether was prepared by the nuclear oxidation of quercetin 3,3′,4′,7-tetramethyl ether and methylation of the resulting 5,8-diol.[2]

Identification of quercimeritrin, chlorogenic acid, and isochlorogenic acid. Paper chromatograms of the solution from which gossypitrin had crystallized showed, in addition to gossypitrin, four clearly defined substances. Three of these were identified as follows:

1. Quercimeritrin. The component having R_f 0.50 in butanol–27% acetic acid (1:1) was separated by chromatography on Whatman No. 1 paper, purified by rechromatography on paper, and eluted. Its ultraviolet spectrum corresponded with that of quercimeritrin, and the expected shift of the long wave maximum (377 to 427 mμ) was observed when aluminum chloride was added to the alcoholic solution. Hydrolysis yielded quercetin, identified by chromatographic methods. Finally, chromatographic comparison with an authentic sample of quercimeritrin[21] in three solvent systems showed complete agreement in appearance, reaction to spray reagents, and R_f values.

2. Chlorogenic and isochlorogenic acids. Two components visible on the chromatograms of extracts of *C. segetum* petals showed the characteristic behavior of chlorogenic and isochlorogenic acids; they had R_f of 0.72 and 0.89, respectively; they showed the typical color reactions under ultraviolet light (blue, changing to bright greenish blue on fuming with ammonia); and they reacted quickly to an ammoniacal silver nitrate spray, giving dark brown spots. They were separated and isolated by chromatography on paper, and the eluted bands were hydrolyzed (under nitrogen) with alkali. Caffeic acid was formed in both cases; it was identified by its behavior on paper (R_f and reaction to Tollen's reagent and ferric chloride), in comparison with that of a synthetic specimen of the authentic substance. Confirmation of the identities of these bands was carried out by chromatographic comparison with authentic specimens of chlorogenic and isochlorogenic acids,[22] with which they agreed in all respects.

Absorption spectra. The ultraviolet absorption spectra of many of the compounds prepared in the course of this study have not been recorded. In Table I are gathered together the spectral data for several gossypetin derivatives and several model substances of related structures.

LOS ANGELES 24, CALIF.

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(21) We are indebted to Professor T. R. Seshadri for a specimen of this glucoside.

(22) These were kindly supplied by Dr. H. M. Barnes, who isolated them from coffee beans. [See H. M. Barnes, J. R. Feldman, and W. V. White, *J. Am. Chem. Soc.*, 72, 4178 (1950).]