Acknowledgment.—The authors are indebted to C. H. Van Etten and Mrs. Mary Wiele of the Analytical and Physical Chemical Division of this Laboratory for the phosphorus and chlorine analyses.

Summary

1. Starch granules show increased chemical reactivity after heating in pyridine.

2. The reactivity of various species of starch decreases with increasing granule size.

3. Insoluble non-swelling phosphates may be obtained in the granular form by treating activated starch granules with phosphorus oxychloride in pyridine.

4. Characterization of the phosphate linkages in starch phosphates by electrometric titration shows varying amounts of singly-, doubly- and triply-linked phosphate, depending on the reaction conditions.

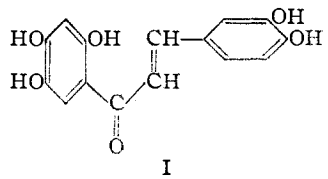
PEORIA, ILLINOIS RECEIVED APRIL 20, 1950

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA]

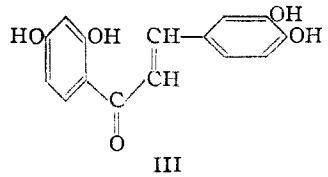
Anthochlor Pigments. VI. The Pigments of *Coreopsis Stillmanii*

BY MARGARET K. SEIKEL[1] AND T. A. GEISSMAN

The anthochlor pigment of *Coreopsis Stillmanii*[2] has been isolated in the form of its octaacetate and has been shown to be a glycoside of 3,4,2′,4′,5′-pentahydroxychalcone (I). It will be called stillopsin (II).

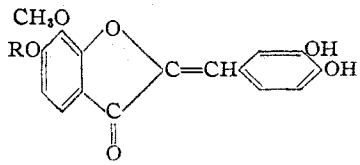


I



III

This isolation represents the first time that the polyhydroxychalcone (I) has been discovered among plant pigments. Anthochlor pigments previously identified include the chalcone, butein (III), found in yellow *Dahlia variabilis*,[3] *Coreopsis Douglasii*,[4] and in *Coreopsis gigantea*,[5] a hexoside of butein, coreopsin, found in *Cosmos sulphureus*[6] and in *Coreopsis gigantea*,[5] and the benzalcoumaranone glucoside leptosin (V), and its aglucone, leptosidin (IV),



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

found in *Coreopsis grandiflora*, Nutt.[7] Recent work[8] has shown that the anthochlor pigment of yellow *Antirrhinum majus* is a benzalcoumaranone derivative. The chalcone nucleus is present in isocarthamin (isolated from safflower[9,10]), pedicin and its ethers (from the leaves of *Didymocarpus pedicellata*[10,11]), and isosalipurposide (from *Salix purpurea L.*[12]).

Hitherto the method of distinguishing between the two types of anthochlor pigments required a study of the products obtained on acidic hydrolysis of glycosides or acetylated pigments. Under these conditions pigments with a chalcone nucleus have yielded chalcones or flavanones, readily identified by color tests, while those with a benzalcoumaranone nucleus retained their initial structure. Absorption spectra have now been found to differentiate the two types readily. The differentiation is best with the acetates, either of the aglycones or the glycosides (Figs. 1 and 2 and Table I), and since the acetates of the pigments are commonly prepared for the purpose of identification, purification or isolation, this method is experimentally the most suitable. With chalcone itself and with the acetyl derivatives of polyhydroxychalcones and of their glycosides, Band I, the longer wave length band,[13] has one maximum. With the corresponding compounds of the benzalcoumaranone series, it has two maxima. With the free polyhydroxy aglycones the differentiation is not as clear-cut because the second maximum has become merely a shoulder,[14] but Band I is at longer wave lengths for the benzalcoumar-

(1) Wellesley College, Wellesley, Mass. Sarah Berliner Research Fellow, 1948–1949, of the American Association of University Women.

(2) Gertz, *Kgl. Fysiograf. Sällskap. Lund, Förh.*, **8**, 65 (1938).

(3) Price, *J. Chem. Soc.*, 1017 (1939). It probably occurs also in *Butea frondosa*, see Perkin and Hummel, *ibid.*, **85**, 1459 (1904), and Lal and Dutt, *J. Indian Chem. Soc.*, **12**, 262 (1935) [*C. A.*, **29**, 6602 (1935)].

(4) Geissman, THIS JOURNAL, **63**, 656 (1941).

(5) Geissman, *ibid.*, **63**, 2689 (1941).

(6) Geissman, *ibid.*, **64**, 1704 (1942).

(7) Geissman and Heaton, *ibid.*, **65**, 677 (1943); **66**, 486 (1944).

(8) Seikel and Geissman, *ibid.*, **72**, 5725 (1950).

(9) Kuroda, *J. Chem. Soc.*, 757 (1930).

(10) Seshadri, *Proc. Indian Acad. Sci.*, **28**, 6 (1948).

(11) Sharma and Siddiqui, *J. Indian Chem. Soc.*, **16**, 1 (1939) [*C. A.*, **33**, 5824 (1939)]; Rao and Seshadri, *Proc. Indian Acad. Sci.*, **27**, 375 (1948).

(12) Zemplén, Bognar and Székeley, *Ber.*, **76**, 387 (1943).

(13) This designation follows that used by Skarzynski for flavones; *Biochem. Z.*, **301**, 150 (1939).

(14) In the one free glycoside studied, leptosin (see footnote 15), this maximum is just barely retained as a distinct entity.

TABLE I

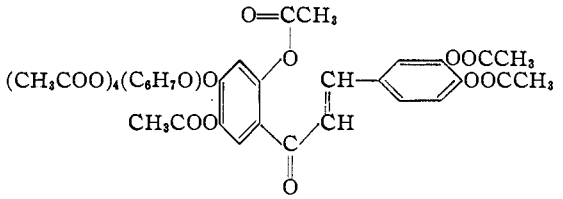
DATA ON ABSORPTION SPECTRA[a]

Compound	Band I λmax, mμ	log ε	λmin, mμ	log ε	Band II λmax, mμ	log ε	λmin, mμ	log ε	Ref.
Chalcone[b]	303 312.5		300		288		250		d,e
	309.5	4.35	243.5	3.61	228	3.91			f,g
Butein tetraacetate[c]	306.5	4.26	251	3.95					f
Coreopsin acetate[c]	304	4.27	252	4.01					f
Stillopsin octaacetate (VI)[c]	313	4.41	255	3.71	228	4.26	219	2.23	f
Butein (III)[c]	384				(1)290				e
					(2)267				
	382	4.44	283	3.77	263	3.99	249	3.90	f
3′,4′,6,7-Tetramethoxyflavanone (from stillopsin) (VII)[b]	337	3.86	300	3.40	(1)276	4.17	253.5	3.75	f
					(2)237	4.45	?		
3′,4′,6,7-Tetramethoxyflavanone (syn.)[b] (VII)	337	3.84	300	3.35	(1)276	4.16	254	3.75	f
					(2)237	4.44	222	4.23	

[a] Determined using a Beckman quartz spectrophotometer, model DU. [b] Present work in 95% alcohol. [c] Present work in absolute alcohol. [d] Shibata and Nagai, *J. Chem. Soc. Japan*, 43, 101 (1922); *C. A.*, 16, 2513 (1922). The abstract gives 3300 Å., but since all other Japanese papers use frequency, the Å. may be an error. [e] Russell, Todd and Wilson, *J. Chem. Soc.*, 1943 (1934). [f] Present work. [g] In addition to the two main bands, two well-defined bands and a shoulder appeared superimposed on Band I; $\lambda_{max.}$ = 246.7, 252.5 and *ca.* 258.5 mμ at log ε 3.64, 3.73 and 3.83, respectively. Russell, Todd and Wilson (footnote *e*) do not show these, but instead reported only the weak band at 288 mμ. This discrepancy will be rechecked. However, the two new bands may correspond to the weak, sharp bands which they reported at *ca.* 269 and 289 mμ for polyhydroxychalcones.

anones and Band II has two weak but distinct maxima[15] (Fig. 3).

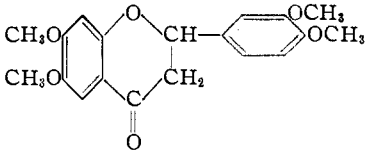
Stillopsin was easily isolated in the form of its crystalline octaacetate (VI) from dried *Coreopsis Stillmani* petals. When the rays and disk florets were continuously extracted for a month with



VI (position of glycosyl uncertain)

ether, the impure pigment separated from the extract as a gum. Acetylation of this residue and extraction with ether left VI as an insoluble residue. Subsequent methanol extractions of the residual petal meal for a few days yielded pigments, which, after purification by fractional precipitation with lead acetate, could be similarly acetylated and purified.

The chalcone nature of the acetylated pigment was evidenced by: (a) the deep purplish-red color which slowly appeared when VI was treated with alkali; (b) the deep blue color characteristic of a flavanone obtained in the magnesium–hydrochloric acid test after hydrolysis of VI; (c) the similarity of its absorption spectrum with those of coreopsin and butein tetraacetate (Figs. 1 and 2). Its glycosidic nature was evidenced by the positive Molisch test obtained after hydrolysis of VI and by the formation of an osazone. Because of insufficient material the sugar could not be positively identified, but it is probably glucose. The position of the hydroxyl groups was proven by hydrolyzing VI to a flavanone whose tetramethyl ether was shown to be 3′,4′,6,7-tetramethoxyflavanone (VII).[16]



VII

Substitution of hydroxyl and methoxyl groups in the 3′,4′,6,7-positions of the flavanone nucleus seems to confer certain distinctive properties on the compounds so that they are easily distinguished from many other polyhydroxy- and polymethoxyflavanones. The absorption spectrum of 3′,4′,6,7-tetramethoxyflavanone (Fig. 4) shows three well-separated bands, $\lambda_{max.}$ = 337, 276 and 237 mμ. In all the polymethoxyflavanones studied by Skarzynski,[13] except butin trimethyl ether (3′,4′,7-), the long wave length band of flavanone at 320 mμ has been subordinated to a shoulder or less as the two short wave-length bands of flavanone have both shifted toward the visible, giving maxima at 275–87 mμ and 228–234 mμ. The spectrum resembles somewhat that of butin trimethyl ether, which shows a poorly separated band at about 310 mμ as well as the other maxima at 275 and 234 mμ, but the distinction of the long wave length band is outstanding because of its great shift toward the visible. In the color test for flavanones in alcoholic solution with magnesium and hydro-

(15) The complete data and discussion of the absorption spectra of the benzalcoumaranone derivatives will appear in a separate paper (see footnote 8), in which curves for both the leptosin and the new aureusin series are given. The main characteristics of the two families are in accord with the generalizations discussed above, so only some curves of the leptosin family are reproduced in this paper for purposes of comparison with the chalcones.

(16) The acetyl derivative of the flavanone was also prepared, but its melting point did not check with that of any known tri- or tetraacetoxyflavanone.

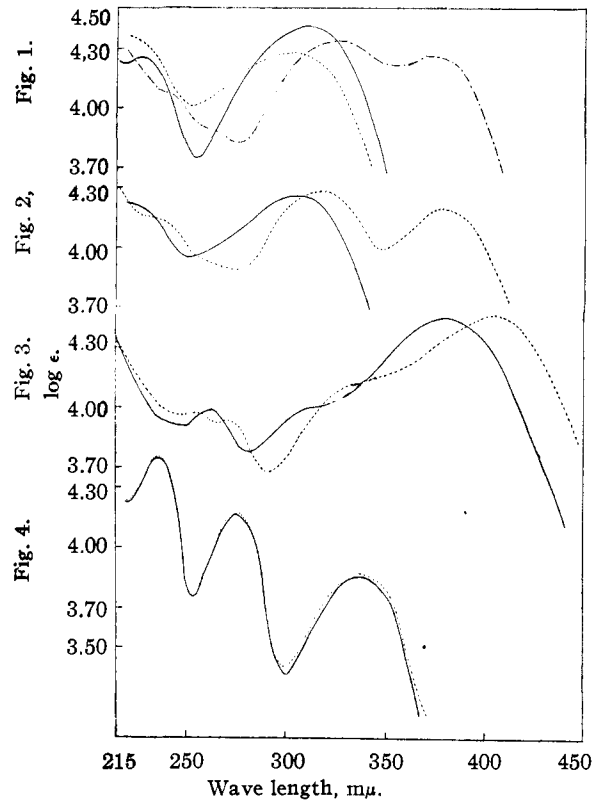


Fig. 1.—Absorption spectra: ——, stillopsin octaacetate (VI); - - - -, coreopsin acetate; — - — -, leptosin hexaacetate.

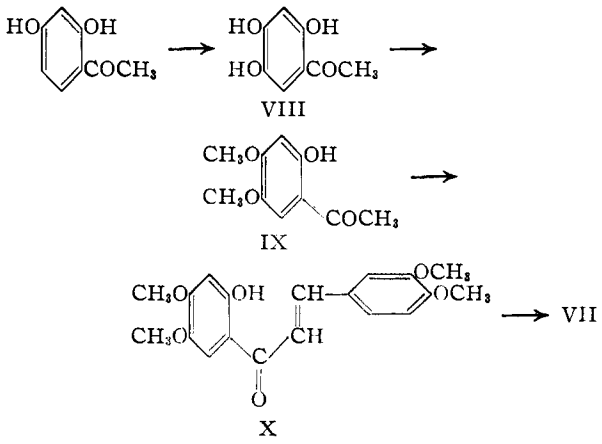
Fig. 2.—Absorption spectra: ——, butein tetraacetate; - - - -, leptosidin triacetate.

Fig. 3.—Absorption spectra: ——, butein(III); - - - -, leptosidin(IV).

Fig. 4.—Absorption spectra: 3′,4′,6,7-tetramethoxyflavanone(VII), ——, synthesized; - - - -, from stillopsin octaacetate.

chloric acid, 3′,4′,6,7-tetrahydroxy-, tetraacetoxy- and tetramethoxyflavanone yield a blue color peculiarly stable to excess acid. With small amounts of acid the blues were of an aqua shade; with more and up to an equal volume they became truly blue (from medium to royal) with no purple tints. Comparative tests on 3′,4′,7-, 3′,4′,5-, 4′,7,8- and 2′,7,8-trihydroxy- and 3′,4′,7,8- and 3′,4′,5,7-tetrahydroxyflavanones, recorded as yielding blue colors, showed many were purplish initially and all gave colors ranging from blue-purple to vivid purple, wine-red to blue-pink with excess acid. The close correspondence in these distinctive properties between the flavanone derived from stillopsin and the synthetic sample of 3′,4′,6,7-tetramethoxyflavanone serve to identify the latter.

For the synthesis of VII the nuclear oxidation of resacetophenone by potassium persulfate to the known 2,4,5-trihydroxyacetophenone (VIII),[17,18,19] was employed as the means of introducing the 6-hydroxyl group of the flavanone nucleus. Preferential methylation of VIII in the 4- and 5-positions using Seshadri's method[18] gave the resulting 2-hydroxy compound (IX). This was condensed with veratraldehyde to form the chalcone (X).[20] The chalcone (X) melted at 172° in contrast to 152°, the melting point reported by Bargellini[21] for 2′-hydroxy-3,4,4′,5′-tetramethoxychalcone, but it gave the correct analysis, and ring closure yielded the desired flavanone (VII), which melted at 164.5°, only 3° higher than the melting point reported by Bargellini[21] (161°).



Experimental[22]

Plant Material.—A sample of *Coreopsis Stillmanii* blossoms picked about five years previously and stored in the dry state was used. The disks and ray-florets were separated manually as well as possible from the non-pigmented portions of the dried whole flower heads.

Isolation of the Crude Pigment (II).—Dried plant material (67 g.), moistened with petroleum ether, was beaten to a pulp in a Waring blendor. It was then extracted continuously in a Soxhlet extractor with 800 ml. of the same solvent until no further yellow color[23] was removed (*ca.* one day). The residual plant material was then extracted continuously with three 800-ml. portions of ether during the course of a month, the third extract yielding far less product than the second. From the bright yellow, slightly cloudy extracts, which gave deep red colors with 10% sodium hydroxide, small amounts of orange gum precipitated. The solutions were decanted from these, but no crystalline material could be isolated from the solutions by the methods employed. The gums weighed, respectively, 0.7, *X* and 0.56 g. and were acetylated as described below.

The residual petal meal was next extracted for one day with 800 ml. of methanol. The red-brown methanol extract, which gave a purple-tinged red color with base, was

(17) Elbs, *M. prakt. Chem.*, **48**, 179 (1893).

(18) Seshadri and co-workers, *Proc. Indian Acad. Sci.*, **23**, 23, 262, 273 (1946); **24**, 233, 238 (1946); **25**, 417, 427, 432, 444 (1947); **26**, 13, 18, 182 (1947); **27**, 37, 85, 91, 209, 217, 375 (1948); **28**, 1, 31, 98, 189 (1948).

(19) Bargellini, *Gazz. chim. ital.*, **43I**, 164 (1913); *Chem. Zentr.*, **84**, II 1596 (1913).

(20) Geissman and Clinton, THIS JOURNAL, **66**, 697 (1946).

(21) Bargellini and Marini-Bettolo, *Gazz. chim. ital.*, **70**, (1940); *C. A.*, **34**, 4736 (1940).

(22) All melting points are uncorrected and were taken by the method described by Mulliken in "Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1904, Vol. 1, p. 218, on a 360° thermometer immersed in Dow-Corning Silicone to the −10° point and are uncorrected.

(23) Probably caused by carotenoid pigments; see refs. 4 and 8.

evaporated to one-half *in vacuo*. From the concentrated solution a brown amorphous solid separated, and more was forced out by cooling with Dry Ice and, after filtering, by re-evaporation. From these precipitates no crystalline products would be isolated by the methods employed at the time. The residual filtrate was evaporated *in vacuo;* 8.76 g. of a brown glass resulted. Since no crystalline material could be obtained when this glass was acetylated, it was dissolved in 75 ml. of water and purified by fractional precipitation of the lead salt as follows. Ten portions of saturated lead acetate solution were added in successively larger volumes (0.2 to 14 ml.) to the well-stirred, brown solution. Each of the resulting yellow to bright rust-colored, pasty precipitates was mixed with a filter-aid (Super-cel) and filtered onto a mat of the same material. While still damp the precipitates were separately suspended in methanol and treated with hydrogen sulfide. Clear yellow to orange solutions of the partially purified pigments were obtained by filtering immediately from the lead sulfide and Super-cel and washing exhaustively with methanol. These methanol solutions all gave similar red shades with 10% sodium hydroxide, but the color tests with ferric chloride and with magnesium in alcoholic hydrochloric acid solution gave evidence of a flavanone in the later fractions. That is, the colors with ferric chloride changed from brown to black to khaki to green with successive fractions and the colors with magnesium-hydrochloric acid from grayish-pink through green to ink-blue. This flavanone, which may be formed by isomerization of the chalcone during the extraction procedure, could not be isolated; its color tests were the ones characteristic of VII. Fractions of partially purified pigment (II) were obtained in the form of brown-orange glasses after evaporation of the methanol *in vacuo;* their combined weight was 2.49 g.

A second and final extraction of the plant material was made with 800 ml. of methanol. This extraction was continued until, after four days, no pigment producing a color with base was being extracted. When this extract was worked up in the manner described above, the 6.79 g. of crude brown gum yielded a total of only 0.70 g. of partially purified pigment after fractionation with lead acetate. The tests for a flavanone were even stronger in this run.

Stillopsin Octaacetate (VI).—The samples of gummy, crude II which separated during the course of the ether extractions and those of significant size of the partially purified II from the methanol extractions were separately acetylated by boiling for a minute or two with about twice their weight of anhydrous sodium acetate and a volume of acetic anhydride equal to about twenty times this weight. The orange to brown reaction mixtures were generally decomposed at once with ice, yielding tan gummy solids or amorphous powders. These were extracted once with ether. Crude VI remained as flocculent residues, gray-tan on drying. From the original ether extractions of the plant material were obtained 0.133 g., m. p. 161.5–163.5°, 0.144 g., m. p. 150–160°, 0.056 g., m. p. 170–171.5°, a total of 0.333 g. From the two methanol extractions were obtained, respectively, 0.772 g. (sum of seven precipitates) and 0.076 g. (sum of two precipitates), all melting between 150–160°, a total of 0.848 g. Very little of VI, therefore, was isolated from the second methanol extraction. From the first methanol extraction VI was isolated in the largest amount from the fourth to the seventh fractions of lead precipitate, comprising here 24–39% of the crude acetylated material. No further crystalline material could be obtained from the ether-soluble portion of the crude acetylated material.

The acetate VI derived from the original ether extractions was purified readily by two or three recrystallizations from methanol (20–25 ml. per 0.1 g.) with over-all recoveries of 25–40%. The samples of VI derived from the original methanol extractions required, after three recrystallizations from methanol, one recrystallization from ethyl acetate–petroleum ether in order to remove a waxy material; the over-all recovery was only 14%.

Pure VI, of which 0.21 g. was obtained, crystallized in tiny, delicately yellow-tinted needles from methanol. The melting point varied with the source: from the ether extractions 191.5–192.5°, from the methanol extractions, 196.5–197.5°. A mixed melting point was 192–194°, and both samples gave the correct analysis.

Anal. Calcd. for $C_{37}H_{38}O_{19}$: C, 56.49; H, 4.87. Found: from ether extractions, C, 56.60, 56.59; H, 5.00, 5.01; from methanol extractions, C, 56.84, 56.74; H, 5.27, 4.90.

The compound gives the following color tests: 10% sodium hydroxide, slowly deep purplish-red; concentrated sulfuric acid, bright red-orange; alcoholic magnesium-hydrochloric acid (after boiling for two or three minutes with concentrated acid), clear blue fading to gray with excess acid.

Hydrolysis of Stillopsin Octaacetate.—A sample of VI (0.100 g.) was hydrolyzed by refluxing for one day with a mixture of 4 ml. of methanol and 20 ml. of 0.6 *N* hydrochloric acid. The resulting yellow solution was extracted with ether until the extracts and residual solution no longer gave a color test for flavanone. The ether solution was washed with a saturated ammonium sulfate solution, dried over sodium sulfate and evaporated to dryness. The residue, dried in a vacuum desiccator, was a yellow glass which did not crystallize. The following tests showed its flavanone nature: sodium hydroxide, dirty yellow; concentrated sulfuric acid, orange; ferric chloride, aqueous or alcoholic, green; saturated lead acetate, orange; alcoholic magnesium-hydrochloric acid, green-blue growing dull blue with excess acid.

An 18-mg. sample of this oil, obtained from a preliminary hydrolysis of 50 mg. of VI, was acetylated in the usual way. After three recrystallizations from 50% alcohol and two from methanol, 3 mg. of a delicate yellow powder was obtained, m. p. 146.5–148.5°. This is undoubtedly 3′,4′,6,7-tetraacetoxyflavanone, but too little was obtained for analysis and no further plant material was available. It gave the following color tests: sodium hydroxide, yellow growing orange; sulfuric acid, vivid red-orange; magnesium-alcoholic hydrochloric acid (after hydrolysis with concentrated acid), deep ink-blue.

The aqueous solution from which the flavanone had been removed was freed from hydrochloric acid by treatment with freshly precipitated silver carbonate and filtration through a Super-cel–Norite mat. The colorless filtrate, which gave a positive Molisch test, was evaporated *in vacuo* to a few milliliters and treated with 40 mg. of phenylhydrazine hydrochloride and 60 mg. of anhydrous sodium acetate. After heating the mixture on the steam-bath for 37 minutes, a yellow osazone precipitated. A control run with an estimated equivalent quantity of glucose deposited glucosazone in thirty minutes. The crystalline forms of the two crude products were not quite identical, and too little of the unknown osazone was obtained for purification or a melting point.

3,′4′,6,7-Tetramethoxyflavanone (VII) from Stillopsin Octaacetate.—The oily flavanone obtained from the hydrolysis of 100 mg. of VI was dissolved in 5 ml. of methanol, cooled to 0° and treated with a similarly cooled ether solution containing approximately a tenfold excess of freshly prepared diazomethane.[24] After the solution had stood 2.5 days at 2–5°, the excess diazomethane was destroyed with acetic acid, the ether was evaporated, and water was added to the residue. Cooling produced 25 mg. (57%) of the crude methyl ether (VII), m. p. 151.5–156.5°.

After three recrystallizations from 50% methanol and from methanol, 2 mg. of the pure, delicate yellow flavanone methyl ether (VII) was obtained, m. p. 160.5–162°. It gave a vivid red-orange color with concentrated sulfuric acid. When hydrochloric acid was added slowly to its alcoholic solution containing a bit of magnesium turning, the color changed from aqua to robin's-egg-blue to a medium blue, unchanged even after an equal volume of acid had been added but fading to a dull blue on standing. Too little material was available for analysis, but the compound was identified as VII[25] by comparison with a synthetic sample. The two samples gave exactly the same changes of color in the magnesium-hydrochloric acid test, the absorption spectra were identical (see Fig. 4 and Table I), and a mixed melting point was as follows: natural (still

(24) Geissman and Heaton, THIS JOURNAL, **65**, 682 (1943).

(25) M. p. 161°, footnote 21.

yellow), 159.5–160°; mixed, 161–162.5°; synthetic (white), 165°.

Synthesis of 3′,4′,6,7-Tetramethoxyflavanone (VII)

2,4,5-Trihydroxyacetophenone (VIII).[26]—A solution of 20 g. of resacetophenone[27] in 400 ml. of water containing 40 g. of potassium hydroxide was cooled to 15° and to it was added a solution of 5 g. of ferrous ammonium sulfate. The mixture was stirred and cooled for five hours while a solution of 40 g. of potassium persulfate in 900 ml. of water was added. After the brown reaction mixture had stood for five days at room temperature, it was acidified to *p*H 3 and extracted with ether; from this extract 25% of unreacted resacetophenone was recovered. The residual solution was treated with 40 g. of sodium sulfite and 400 ml. of concentrated hydrochloric acid, heated for five hours on the steam-bath, and filtered while hot and then after cooling through Super-cel in order to remove a black by-product. The product was isolated by exhaustive extraction with ether and readily purified by extracting the ether solution with 10% sodium acetate until significant amounts of the phenol, which could be recognized by the green color it gave with ferric chloride, began to appear in the acetate solution. Evaporation of the ether yielded 3.63 g. (16.5%) of crude VIII, m. p. 180–190°. It was purified by recrystallization from absolute alcohol, then from water, then by solution in ether and extraction with water, and finally by recrystallization from water. Salmon-colored material was obtained, 1.97 g. (8.9% over-all yield), m. p. 200–200.5° (recorded, 200–202°,[19] 206–207°[28]).

2-Hydroxy-4,5-dimethoxyacetophenone (IX).[29]—A mixture of 1.9 g. of VIII, 75 ml. of anhydrous acetone, 10 g. of potassium carbonate and 2.2 ml. of freshly vacuum-distilled dimethyl sulfate was refluxed for seven hours. After cooling, the inorganic salts were filtered off and washed with boiling acetone. The product was isolated by evaporating the combined acetone solutions nearly to dryness and adding water. The crude material, 1.83 g. (83%), m. p. 95–100°, was contaminated not with the expected alkali-insoluble trimethyl ether but with the monomethyl ether which was removed with difficulty. After two recrystallizations from water had yielded a mixture of crystals, the material was recrystallized from absolute alcohol with a limited time allowed for crystallization; in this way 0.477 g. of stout, grayish prisms were obtained, m. p. 111.5–112° (recorded, 114–115°,[30] 111–112°[31]).

2′-Hydroxy-3,4,4′,5′-tetramethoxychalcone (X).—Forty-five hundredths of a gram of IX was condensed with 0.34 g. of veratraldehyde in the presence of strong alkali by a hot condensation method.[32] After one recrystallization from methanol the yield of chalcone was 0.377 g. (61%), m. p. 172–172.5°. After two further recrystallizations from alcohol the melting point of the shining orange plates was unchanged 171.5–172.5° (recorded, 152°[21]). The chalcone gives the following color tests: vivid orange-red with concentrated sulfuric acid and alcoholic sodium hydroxide, warm brown with ferric chloride, and a green-blue (which became medium blue and did not fade with excess acid) with magnesium and hydrochloric acid after a preliminary heating in acidified alcohol solution to isomerize the chalcone to flavanone.

Anal. Calcd. for $C_{19}H_{20}O_6$: C, 66.27; H, 5.85. Found: C, 66.29; H, 6.00.

3′,4′,6,7-Tetramethoxyflavanone (VII).—The isomerization of X (0.25 g.) to the flavanone was carried out by the acidic isomerization method.[20] From the crude yellow product, 0.246 g., m. p. 157–160°, the contaminating chalcone was removed by recrystallizing rapidly several times from methanol with much decolorizing carbon. The pure white product, 0.040 g. (16% yield), melted at 164–164.5° (recorded 161°[21]). With sulfuric acid it gave a yellow-orange color becoming at once red-orange. With magnesium and hydrochloric acid it produced exactly the same color changes as described for the material derived from stillopsin octaacetate.

Anal. Calcd. for $C_{19}H_{20}O_6$: C, 66.27; H, 5.85. Found: C, 66.12; H, 6.06.

Summary

1. The anthochlor pigment of *Coreopsis Stillmanii* has been identified as a chalcone derivative.

2. The pigment, stillopsin, is a hexoside of the hitherto unreported 3,4,2′,4′,5′-pentahydroxychalcone.

3. Ultraviolet absorption spectra of the acetylated pigments have been found to differentiate clearly between the two types of anthochlor pigments: polyhydroxychalcones and polyhydroxybenzalcoumaranones.

LOS ANGELES, CALIFORNIA RECEIVED JULY 10, 1950

(26) The method was adapted from that used by Seshadri (footnote 18) for the nuclear oxidation of chalcones, flavones, flavonols and acetophenones and from that of Bargellini (footnote 19) for resacetophenone.

(27) Cooper, *Org. Syntheses*, **21**, 103 (1941).

(28) Chadha and Venkataraman, *J. Chem. Soc.*, 1074 (1933).

(29) The method of preparation follows that of Seshadri for selective methylation of chalcones, flavones, flavonols and acetophenones; see footnote 18.

(30) Bargellini and Aureli, *Atti accad. Lincei*, **20 II**, 118; *C. A.*, **5**, 3683 (1911).

(31) Mauthner, *J. prakt. Chem.*, **136**, 211 (1933).

(32) Footnote 20. The cold condensation method yielded only 16% of the chalcone, most of the acetophenone being recovered unchanged.