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

Anthochlor Pigments. VII. The Pigments of Yellow *Antirrhinum Majus*

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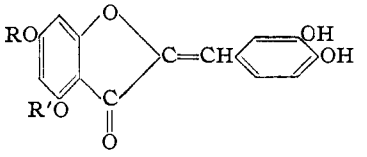
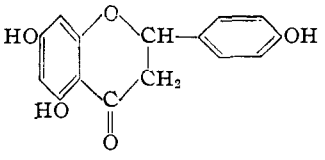
As the first step in a problem designed to study the relationship between the genetics and the flower pigments of *Antirrhinum majus*,[2] an examination of the petal pigments of yellow *Antirrhinum majus* was undertaken. Previously Wheldale and Bassett[3,4] had concluded that the yellow pigment was luteolin (3′,4′,5,7-tetrahydroxyflavone) and that the closely related apigenin (4′,5,7-trihydroxyflavone) was present in the cream flowers[5] and accompanied luteolin in the yellow blossoms. A publication reporting the results of later studies[6] contains the statement "recent work by Price (unpublished) indicates that the yellow pigment in *Antirrhinum* is not a flavone at all but a chalkone." The anthochlor pigment[7] of these flowers, whose presence in this species had been mentioned much earlier by botanists,[8,9,10] has now been isolated in the form of its heptaacetate (I) and shown to be a glucoside of 3′,4′,4,6-tetrahydroxybenzalcoumaranone (II). The pigment will be called aureusin (III), its aglucone (II) aureusidin.



II, R and R′ = H: aureusidin
III, R or R′ = glucosyl: aureusin

The anthochlor pigment of *Antirrhinum majus* belongs to the less common benzalcoumaranone type of which only one previous example has been positively identified: namely, leptosin and its algucone leptosidin (the 7-methyl ether of 3′,4′,6,7-tetrahydroxybenzalcoumaranone), recently characterized as the anthochlor pigments of *Coreopsis grandiflora*, Nutt., by Geissman and Heaton.[11] All other anthochlor pigments so far identified have been shown to be polyhydroxychalcones or their glycosides,[12] and Price's suggestion probably was made as a result of the similarity between many of the qualitative color reactions of polyhydroxychalcones and the (at that time) still-unrecognized benzalcoumaranone pigments.

In addition to the benzalcoumaranone pigment, a flavanone glycoside has also been isolated from yellow *Antirrhinum majus*. This was obtained in the form of its hexaacetate (IV) and was shown to be a hexoside of naringenin (V), 4′,5,7-trihydroxyflavanone. Whether or not it occurs in the flower



V

as the flavanone or as the isomeric chalcone is not yet known.

From genetically miscellaneous yellow blossoms no pure samples of acetylated flavone glycosides could be isolated by the methods used. Current work on genetically pure strains is producing far more promising results in this direction.

The phloroglucinol nucleus is present in all of the pigments so far isolated from all color-varieties of *Antirrhinum majus*, whatever the state of oxidation or cyclization of the heterocyclic ring. These include the benzalcoumaranone (II) and the flavanone (V) herein reported, the flavones, luteolin and apigenin, previously described,[3,4] and the anthocyanins studied by Scott-Moncrieff:[13] namely, the "magenta" pigment, antirrhinin, the 3-rhamnoglucoside of cyanidin chloride, and the "red" pigment, a 3-pentoseglycoside of pelargonidin. This observation suggests that all four types arise from a common precursor.

All of the pigments of *Antirrhinum majus* apparently occur in a glycosidic state. In the present work the only means found for crystallizing and separating them without hydrolysis of the glycosidic linkage involved acetylation of crude gums and isolation of the polyacetates of the pigments.

Aureusin (III) was isolated in the form of its colorless heptaacetate (I). It gave with concentrated sulfuric acid and (slowly) with alkali the deep red colors typical of anthochlor pigments. That it is a benzalcoumaranone pigment was shown both by its absorption spectrum and by the absorp-

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(2) In collaboration with Dr. B. L. Johnson of the Ornamental Horticulture Department of the University. The plant material used in the present work was grown under his supervision.

(3) Wheldale, *Biochem. J.*, **7**, 87 (1913); *J. Genetics*, **4**, 109 (1914).

(4) Wheldale and Bassett, *Biochem. J.*, **7**, 441 (1913); *Proc. Roy. Soc.* (*London*), **87B**, 300 (1914).

(5) The whites which are not true albinos.

(6) Beale, Price and Scott-Moncrieff, *J. Genetics*, **41**, 73 (1940).

(7) See Geissman, THIS JOURNAL, **63**, 656 (1941), for definition.

(8) Klein, *Sitzb. Akad. Wiss., Wien.*, **129**, 341 (1920); *C. A.*, **16**, 3110 (1922).

(9) Molisch, "Microchemie der Pflanze," Gustav Fischer, Jena, 1923, p. 271.

(10) Gertz, *Botan. Notiser*, **I**, 199 (1939); see also *Biol. Abstracts*, **13**, 12101 (1939), and *C. A.*, **24**, 7976 (1940).

(11) Geissman and Heaton, THIS JOURNAL, **65**, 677 (1943); **66**, 486 (1944).

(12) Anthochor pigments with the chalcone structure include butein (3,4,2′,4′-tetrahydroxychalcone) isolated from *Dahlia variabilis* (Price, *J. Chem. Soc.*, 1017 (1939)) and from *Coreopsis Douglasii* and *Coreopsis gigantea* (Geissman, THIS JOURNAL, **63**, 656, 2689 (1941)) its hexoside, coreopsin, isolated from *Coreopsis gigantea* and *Coreopsis sulfureus* (Geissman, *ibid.*, **63**, 2689 (1941); **64**, 1704 (1942)), and the hexoside of 3,4,2′,4′,5′-pentahydroxychalcone, stillopsin, isolated from *Coreopsis Stillmanii* (Seikel and Geissman, *ibid.*, **72**, 5720 (1950)).

(13) Scott-Moncrieff, *Biochem. J.*, **24**, 753 (1930); *J. Genetics*, **32**, 157 (1936).

tion spectrum of its hydrolysis product, II.[14] These spectra are shown in Figs. 1, 2 and 3 and are to be compared with those of leptosin, leptosidin, their acetates and the parent benzalcoumaranone. The glycosidic nature of III was proven by the isolation of the products of hydrolysis: namely, the aglucone (II) which gave a new acetate and a sugar convertible to glucosazone. The sugar is probably glucose; it is attached either at position 4- or 6- since the precipitation of the lead salt of the pigment by neutral lead acetate implies that the vicinal 3'- and 4'-hydroxyl groups are free. The positions of hydroxylation of the benzalcoumaranone nucleus in III were shown by the preparation of aureusidin tetramethyl ether (VI), which was identified by a mixed melting point with an authentic sample and by a comparison of absorption spectra (Fig. 4) as 3',4',4,6-tetramethoxybenzalcoumaranone.

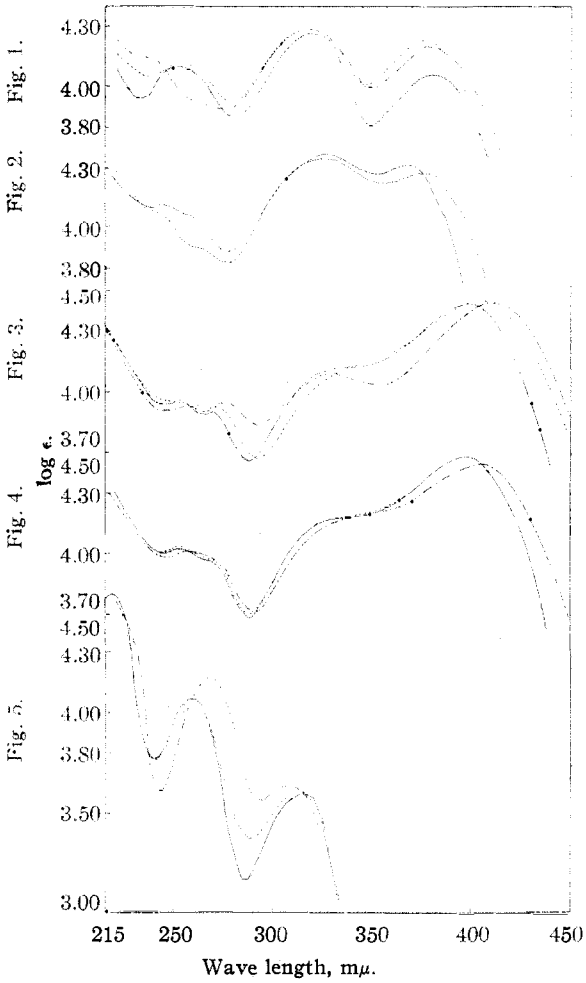


Fig. 1.—Absorption spectra: ——, benzalcoumaranone; – – – – –, aureusidin tetraacetate (VIII); — — — —, leptosidin triacetate.

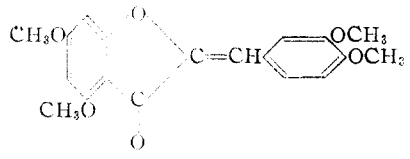
Fig. 2.—Absorption spectra: – – –, aureusin heptaacetate (I); – – – –, leptosin hexaacetate.

Fig. 3.—Absorption spectra: ——, aureusidin (II); – – – –, leptosidin; – – – –; leptosin.

Fig. 4.—Absorption spectra: ——, 3',4',4,6-tetramethoxybenzalcoumaranone; – · – · –, aureusidin tetramethyl ether (VI); — — — —, 3',4',6,7-tetramethoxybenzalcoumaranone.

Fig. 5.—Absorption spectra: — — — —, naringenin glycoside acetate (IV); ——, naringenin triacetate (VII), synthetic; – – – –, naringenin triacetate, from *A. majus*.

(14) Seikel and Geissman, THIS JOURNAL, **72**, 5720 (1950).



VI

The flavanone glycoside was isolated in the form of its colorless acetate (IV). This gave vivid yellow colors with concentrated sulfuric acid and (slowly) with base, in contrast to the red colors given by the benzalcoumaranone glucoside. The flavanone nature of IV was revealed at once by its behavior with magnesium and hydrochloric acid in alcohol solution:[15] the color developed was a blue-pink reminiscent of that given by eriodictyol. The spectrum of IV (Fig. 5) had the sharp bands characteristic of a flavanone.[16] Its glycosidic nature was shown in general by its properties and analysis and by the fact that on hydrolysis a flavanone was obtained which formed a new acetate. The positions of hydroxylation of the flavanone nucleus were shown to be 4',5,7- by identification of this aglucone acetate as naringenin triacetate (VII) by a mixed melting point determination with an authentic sample[17] and by a comparison of absorption spectra (Fig. 5).

A comparison of the absorption spectra of the two naturally-occurring benzalcoumaranone pigments (aureusin (III) and leptosin) and their derivatives reveals such a close similarity in the type, number and position of bands that actual comparison of curves would be required for absolute differentiation of the aureusin series from the leptosin series. Only one consistent difference exists: the position of the maximum at the longest wave length (designated maximum *a* of Band I) is 4.5–8.5 mμ nearer the visible in all members of the leptosin series. This and other slight diver-

(15) Without preliminary hydrolysis; see Experimental section.

(16) Skarzynski, *Biochem. Z.*, **301**, 150–169 (1939).

(17) Authentic VII, prepared by acetylation of naringenin (V) with acetic anhydride in pyridine, melted at 125.5–126.5°, a range checking with neither of those reported by Asahina and Inubuse, *Ber.*, **61**, 1515 (1928): namely, 53–55° for the flavanone acetate and 133–136° for the chalcone tetraacetate. The melting point does not check with other work in this Laboratory: VII prepared by the acetic anhydride-sulfuric acid method, m. p. 82–84° (Friess, Ph.D. Thesis, University of California at Los Angeles, 1948). It did check that of the unknown flavanone acetate, m. p. 122.5–124.5°, prepared by the acetic anhydride–sodium acetate procedure. Further study of these acetates is contemplated.

gencies are probably caused by the presence of the 4-hydroxyl group in aureusidin and to a smaller extent by the presence of the free 7-methoxyl in the acetylated compounds of the leptosin series.

The aglucone acetates most closely resemble the parent (unsubstituted) benzalcoumaranone in their spectra (Fig. 1), in agreement with earlier work on flavanols and flavones[16,18,19] and on chalcones,[20] since acetylation largely removes the contribution of the hydroxyl groups to the resonance of the molecule. They differ (1) in the strength of maximum *a* of Band I and (2) for leptosidin triacetate in the position of Band II. The latter difference may be caused by the free 7-methoxyl group. The spectra of the glucosidic acetates diverge considerably from that of the parent (Fig. 2): the maxima of Band I, in addition to being still stronger, have shifted toward each other. These effects must be caused by the contribution of the glucosidated hydroxyl group to the resonance, and since it is in contrast to the similarity in spectra given by the chalcone butein tetraacetate and its glycosidic acetate, coreopsin acetate,[14] it may indicate a different point of glycosidation with respect to the resonating system.

The spectra (Fig. 3) of the compounds containing the free hydroxyl groups (leptosin and the aglucones II and leptosidin) show (1) that the absorption has shifted to the visible, as expected, and (2) that benzalcoumaranones absorb at longer wave lengths than chalcones and than flavones.[21] The latter effect is probably the combined result of the greatly strained condition of the heterocyclic ring[22] and the presence of the exocyclic double bond.[23] In addition, in comparison with the spectra of the acetylated compounds there has been (1) a great change in the relative intensity of the two maxima of Band I, maximum *a* becoming far more prominent, maximum *b* almost or completely disappearing, and (2) the emergence of a second weak maximum in Band II at a somewhat longer wave length than the still remaining benzalcoumaranone band near λ 251 mμ. As expected[16,18-20] the spectra (Fig. 4) of the completely methylated aglucones are practically identical with those of the aglucones, differing mainly in the loss of the new maximum as a distinct peak.

As yet no theoretical interpretation of the spectra of the benzalcoumaranones has been made, but further work on the spectra of polyhydroxybenzalcoumaranones and their acetates is planned in order to elucidate the changes in the spectra caused by acetylation, glycosidation and etherification.

(18) Hattori, *Acta Phytochim.*, **4**, 41, 63 (1928), **5**, 219 (1931), **6**, 131 (1932); *C. A.*, **22**, 3661 (1928); **26**, 1282, 4816 (1932).

(19) Shibata and Kimotsuki, *Acta Phytochim.*, **1**, 91 (1923); *C. A.*, **17**, 3451 (1923); *Chem. Zentr.*, **94**, III, 244 (1923).

(20) Shibata and Nagai, *Acta Phytochim.*, **2**, 25 (1924); *C. A.*, **19**, 3064 (1925); *Chem. Zentr.*, **95**, II, 1688 (1924).

(21) *Cf.* II, λ_{max} 398.5 mμ; butein, λ_{max} 382 mμ[14]; 3′,4′,5,7-tetrahydroxyflavone, λ_{max} 355 mμ.[16]

(22) Lewis and Calvin, *Chem. Revs.*, **25**, 273 (1939).

(23) Woodward, THIS JOURNAL, **64**, 76 (1942).

Experimental[24]

Plant Material.—Miscellaneous yellow *Antirrhinum majus* blossoms from plants in the F_2 and F_3 plantings of the original cross between the yellow "Ball Gold" (*ppMMyy*) and the pink "Cheviot 33" (*PPmmYY*) were used for this work. The fresh, mature blossoms were dried for two to four days in wire trays in a forced-draft oven kept at 45°[2] and then temporarily stored over calcium chloride. For the preliminary runs, the dried petals were moistened with petroleum ether and ground in a Waring Blendor. The larger amounts were ground while dry in a Wiley Mill, stored in bottles and used directly. For one run on fresh material the blossoms were suspended in 95% alcohol immediately after picking.

Extraction Procedures.—Dried petals, either about 80 g. or 1 lb., were extracted continuously in a Soxhlet extractor with 800 ml. or 3.25 l., respectively, of petroleum ether for approximately two days, a time sufficient to remove the waxes. An absorption spectrum showed that the yellow color of the resulting extract was caused by carotenoid pigments.[25] The petroleum-ether extracted materials were then extracted for about two days with ether; the beautiful green color of the extracts was shown to be due to the presence of chlorophyll.[26] Since prolonged extraction with ether failed to remove more than traces of the glycosidic pigments, the residual plant materials were dried and extracted continuously with methanol for about five days, a time sufficient to remove all of the yellow color from the petal meal. The brown extracts deposited yellow to mustard-brown, amorphous precipitates representing 6–9% of the weight of the petals. In several cases these were worked up separately from the material in the filtrate, but they proved to contain somewhat less aureusin than the filtrates. The latter were evaporated *in vacuo* to thick brown gums.

One run was carried out on fresh petals, 6 lb.[27] of which were allowed to stand for four days in 9 l. of 95% alcohol, then ground up in a Waring Blendor and allowed to stand for five days more. The resultant yellow extract was concentrated in small portions to 1100 ml. The aqueous residual solution was filtered free of waxes, extracted with petroleum ether and finally with ether to remove chlorophyll; it was then treated with lead acetate as described below.

Lead Acetate Treatment.—Since no crystalline pigments or acetyl derivatives of the pigments could be isolated from the crude precipitates and gums, the latter were purified by precipitation of the lead salts. As a result, not only could crystalline acetyl derivatives of the benzalcoumaranone and flavone pigments be obtained, but the flavanone pigment was separated from the others. First, the crude amorphous or gummy materials were dissolved in several hundred milliliters of water. To the well-stirred, cloudy, brown solutions saturated lead acetate solution was added dropwise until small amounts of brown precipitates were obtained. These were removed as described below and dis-

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

(25) Substance	Solvent	λ_{max}, mμ A.	B	Ref.
Unknown	Petrol. ether	467	440	[a]
α-Carotene	Ligroin, 30–35°	479.5	445	[b]

[a] Present work. [b] Miller, *Plant Physiol.*, **9**, 179 (1934).

(26) Substance	Solvent	λ_{max}, mμ A	A	B[a]	Ref.
Unknown	Ether	658	612	428	[b]
Chlorophyll A	Ether	660	610	427	[c]

[a] The relative heights of maxima A and B were correct. [b] Present work. [c] Zscheile, *J. Phys. Chem.*, **38**, 96 (1934); values estimated from curve.

(27) Equivalent to 325 g. of dried petals.

carded since they carried down many suspended impurities. To the somewhat clarified filtrates excess lead acetate solution was then added slowly. Isolation of the pasty, brick-red or orange-red lead salts was effected efficiently by adding large amounts of a filter-aid (Super-cel) and filtering the solution through a mat of the same material. The filtrates were found to contain the flavanone pigments and were treated as described below. The damp precipitates, admixed with Super-cel, were suspended at once in methanol and treated with hydrogen sulfide. The resultant mixtures of lead sulfide and filter-aid were removed immediately and washed exhaustively with methanol; the filtrates were clear yellow methanol solutions of the pigments. Color tests on these solutions were generally as follows: 10% sodium hydroxide, orange-red; concentrated sulfuric acid, orange-red; dilute ferric chloride, greenish-black; saturated lead acetate, orange-red; magnesium and alcoholic hydrochloric acid, dull orange or pink-orange. These colors did not differ significantly from those given by the crude materials. The methanol solutions were evaporated *in vacuo* to orange or red-brown glasses or gums which comprised about half of the weight of the crude precipitates and 10% of the weight of the dried petals.

Aureusin Heptaacetate (I).—In order to isolate crystalline material from the partially purified gums resulting from the lead acetate treatment, described above, these residues were acetylated by boiling for one to five minutes with about twice their weight of anhydrous sodium acetate and a volume of acetic anhydride equal to about twenty times their weight. After standing for about an hour, the cooled, black solutions were poured onto ice; gray or tan amorphous products were obtained by vigorous stirring and frequent changes of water. Crystalline acetates were isolated from these crude materials in two ways.

(a) Small amounts (0.2–6.0 g.) of the amorphous precipitates were extracted by boiling several times with large volumes (20–200 ml.) of ether. The ether extracts were evaporated to dryness and the residual yellow gums dissolved in warm methanol, sufficient being used to dissolve the gums while hot and to prevent clouding as the solution cooled. Colorless crystals of I appeared either as the ether evaporated or when the methanol was added or after the solutions had stood several hours. The products were washed exhaustively with methanol in order to remove a gelatinous impurity.[28]

(b) Large amounts (8–34 g.) of amorphous, crude acetylation products were dissolved in 50–150 ml. of acetone, five to ten volumes of ether were added, and the resultant black amorphous precipitates were removed by filtration of the solution through Super-cel. The latter were re-treated successively with acetone and ether until most of the material was in solution. The ethereal filtrates were washed free of acetone with large volumes of water and this washing continued until the ether solutions were light yellow in color. Much dark gum precipitated during these washings; it was also re-treated with acetone and ether. The purified ether solutions were dried over sodium sulfate, evaporated and the residues treated with methanol as described above.

The samples of crude I obtained by these manipulations varied widely in purity. All samples melting below 250° and showing orange or yellow tints when tested with alkali were purified by extraction with hot methanol or by solution in acetone and reprecipitation with methanol. The final yields of crude I, melting around 250–255° and slowly giving pure wine colors with alkali, were 3.5–7% of the crude acetylated materials and 0.4–0.5% of the weight of the dried petals.

Aureusin heptaacetate (I) was best purified by treating it with excess boiling ethyl acetate (50–120 ml. per gram) and evaporating the resultant solution *in vacuo* at room temperature to one-third or less of the original volume; this method prevented the appearance of a yellow color in the solution. Recoveries averaged only 60%. The dead-white, fibrous solid appeared under the microscope as long thin needles arising from a central core. Its melting point varied somewhat with the rate of heating; samples melting at 264.5–265.5° and 260–260.5° gave correct analyses. With 10% sodium hydroxide it slowly dissolved, giving a rose-red to cardinal-red solution; with concentrated sulfuric acid the color was a vivid orange-red.

Anal. Calcd. for $C_{35}H_{34}O_{18}$: C, 56.60; H, 4.61. Found: C, 56.55, 56.98; H, 4.70, 4.89.

Aureusidin (II).—Aureusin heptaacetate (I) was hydrolyzed to its aglucone (II) by refluxing with a mixture of 1 *N* hydrochloric acid (17 ml. per 0.1 g.) and methyl alcohol (3 ml. per 0.1 g.) for approximately two days. From the hot solution, which grew orange-red in color as the hydrolysis proceeded, orange-brown crystals separated. More product was obtained both by cooling and by extracting with ether so that the yield of aglucone was 80–100%.

The other product of the hydrolysis, glucose, was identified as follows in the aqueous yellow filtrate from which II had been removed. The filtrate was freed of hydrogen ion and chloride ion by treatment with freshly prepared silver carbonate. It then gave a strong Molisch test. After evaporation to a few milliliters the solution was treated with phenylhydrazine hydrochloride and sodium acetate in the calculated amounts and heated on the steam-bath simultaneously with controls prepared from glucose. In two experiments the precipitates of yellow osazone appeared in the same (11 min.) or approximately the same (38 *vs.* 30 min.) time for unknown and control. After recrystallization from 60% alcohol the unknown osazone decomposed immediately at 225° when dropped onto a hot block, glucosazone at 227°, and a mixture at 225°. The two osazones had exactly the same form when viewed with the microscope, forming perfect sheaves of hair-fine needles.

Aureusidin (II) was purified by dissolving it in the least volume (*ca.* 5 ml. per 0.1 g.) of cold methanol, treating it with decolorizing carbon and reprecipitating it by adding five to ten volumes of hot water. Since individual recoveries averaged 90%, 50% of the original material was obtained after six recrystallizations. Its purity was judged by the decrease in color of the crystals to a shining, deep yellow, metallic-gold shade and by the decrease in color of the filtrates. Under the microscope the purified compound appeared as plate-like needles. The melting point was very indefinite. When plunged into a preheated bath it distinctly melted and resolidified at 270 ± 5°. If the tube was removed and then replunged into the bath at higher temperatures, it melted rapidly followed at once by decomposition at 295 ± 5°. With 10% sodium hydroxide and with concentrated sulfuric acid II gave deep orange-red colors; in alcohol solution it gave a brown color with ferric chloride and a dull orange-red precipitate with lead acetate. The analysis indicated a monohydrate, but the molecule of water was not removed by drying at 100° *in vacuo*.

Anal. Calcd. for $C_{15}H_{10}O_6 \cdot 1H_2O$: C, 59.21; H, 3.98. Found: C, 59.20, 58.98; H, 4.31, 4.05.

Aureusidin Tetraacetate (VIII).—Aureusidin (II) was readily acetylated by the acetic anhydride–sodium acetate method described under I. Crude VIII crystallized at once when the reaction mixture was poured onto ice, giving 82–97% yields of delicately tinted material which melted at about 178–181°. It could be recrystallized from methanol (8–13 ml. per 0.1 g., 46–73% recoveries) or dissolved in hot ethyl acetate (5–6 ml. per 0.1 g.) and reprecipitated with petroleum ether (1.5–3 volumes, 77–97% recoveries). The last traces of color could not be removed even after four recrystallizations by the latter method with the use of decolorizing carbon. When pure, VIII forms very delicately tinted yellow, hair-fine needles, m. p. 184–185° (put into

(28) The methanol filtrates seldom contained further significant amounts of I. Instead, on long standing (during which they evaporated and were rediluted several times) they slowly deposited in large amounts crystalline acetates of the glycosidic flavone pigments, as evidenced by the yellow colors given in tests with alkali and with concentrated sulfuric acid. Despite repeated attempts to purify these, no products could be obtained which had a permanent crystalline form, gave satisfactory melting points, analyzed correctly, or on hydrolysis produced a pure flavone which could be acetylated to a crystalline acetate in high yield. It is believed that the genetically miscellaneous material used contained a mixture of flavones since current work on a pure genotype has, by the same methods, given pure material.

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
Benzalcoumaranone[b,c]	(a)	379	4.06	349	3.81	251	4.10	232.5	3.94
	(b)	316.5	4.27	279	3.85				
Aureusidin tetraacetate (VIII)[b]	(a)	374.5	4.23	346.5	4.01	251	4.12	237.5	4.05
	(b)	317	4.29	279.5	3.93				
Leptosidin triacetate[d]	(a)	379	4.20	350	4.00				
	(b)	319	4.29	276	3.89				
Aureusin heptaacetate (I)[b]	(a)	368	4.32	353	4.28				
	(b)	326	4.37	276	3.88	244	4.11	239	4.11
Leptosin hexaacetate[b,e]	(a)	374	4.27	354	4.22				
	(b)	325.5	4.35	278	3.83				
Aureusidin (II)[b]	(a)	398.5	4.44	288	3.66	269	3.90	263	3.89
	(b)					254?	3.95	Indefinite	
Leptosidin[d]	(a)	405.5	4.45	292	3.67	272	3.92	266	3.91
	(b)					257	3.95	251	3.93
Leptosin[d]	(a)	411	4.44	352.5	4.03	276.5	3.95	266	3.90
	(b)	328.5	4.09	293.5	3.83	257	3.93	*ca.* 246	3.91
Aureusidin tetramethyl ether (VI)[b]		397	4.48	288	3.71	254	4.03	243	4.01
3′,4′,4,6-Tetramethoxybenzalcoumaranone (*syn.*) (VI)[b]		397	4.49	288	3.69	253	4.02	243	4.00
3′,4′,6,7-Tetramethoxybenzalcoumaranone[d,f]		405.5	4.44	290	3.71	256.5	4.02	247	3.98
Naringenin glycoside acetate (IV)[b]		309	3.64	293.5	3.57	268	4.18	242.5	3.61
Naringenin triacetate from *A. maius* (VII)[b]		313	3.61	289	3.38	260	4.03	241	3.76
Naringenin triacetate (*syn.*) (VII)[b]	(a)	314	3.59	285	3.17	260	4.07	240	3.77
	(b)					218[g]	4.60		

[a] Determined with a Beckman quartz spectrophotometer, model DU. [b] In 95% alcohol. [c] Shibata and Nagai gave the maxima at 378, 318 and 250 mμ, *Acta Phytochim.*, **2**, 25; *C. A.*, **19**, 3064 (1925); *Chem. Zentr.*, **95**, II, 1688. Units not given in abstracts; assumed to be frequency and converted. [d] In absolute alcohol. [e] Done also in absolute alcohol. Curves checked within limits of experimental error. [f] Compound identical with leptosidin trimethyl ether. [g] This low wave length was not studied with the natural material.

bath preheated to 180°). With concentrated sulfuric acid and (slowly) with 10% sodium hydroxide it gives red-orange colors.

Anal. Calcd. for $C_{25}H_{18}O_{10}$: C, 60.79; H, 3.99. Found: C, 60.72; H, 3.97.

Aureusidin Tetramethyl Ether (VI).[29]—The ether (VI) was prepared by a deacetylation-methylation of the acetate (VIII).[30,31] Seventy milligrams of VIII was dissolved in 5 ml. of warm methanol and treated alternately with 0.5 ml. of dimethyl sulfate and 0.2 ml. of 50% potassium hydroxide (added dropwise). The mixture was stirred mechanically and the additions repeated four times. Next 0.2 ml. of the dimethyl sulfate and 0.5 ml. of the alkali were used, and additions continued until the alkali no longer produced a red color. After a 1-ml. excess of the alkali had been added and the mixture allowed to stand for a few minutes, the solution was poured onto ice and extracted with 500 ml. of ether. An 86% yield (45 mg.) of the crude ether was obtained, m. p. 155–160°.

The crude material was purified by recrystallization from 75% alcohol (recovery, 53–63%) but despite mechanical separation from a gum and frequent use of decolorizing carbon, the melting point of VI could not be sharpened or raised above 169–172°. It crystallized in vivid, almost greenish-yellow, feathery bunches of needles which gave a dark red color with concentrated sulfuric acid.

Anal. Calcd. for $C_{19}H_{18}O_6$: C, 66.65; H, 5.89. Found: C, 66.96; H, 5.33.

It was identified as 3′,4′,4,6-tetramethoxybenzalcoumaranone, m. p. 173–174°,[32] by comparison with an authentic sample. The mixed melting point was 172–174° (VI, 169.5–171.5°, authentic 175.5–176.5°); in Fig. 4 and Table I are given the spectral data.

Naringenin Glycoside Acetate (IV).—The aqueous filtrates from which lead acetate had precipitated the lead salts of the glycosidic benzalcoumaranone and flavone pigments were shown to contain a flavanone pigment by extracting them with ether, evaporating the ether and testing the residues. With 10% sodium hydroxide these gave a bright yellow color; with concentrated sulfuric acid, a dirty yellow or brown; and in alcoholic solution with magnesium and hydrochloric acid a vivid blue-pink or fuchsia color reminiscent of eriodictyol.

The flavanone pigment was isolated by first precipitating the excess lead ion with ammonium sulfate, saturating the filtrates with ammonium sulfate and extracting them with ethyl acetate until the magnesium-hydrochloric acid test on the extract became negative (about five extractions). The brownish-yellow ethyl acetate extracts were purified by washing with 2 *M* ammonium carbonate which removed impurities without isomerizing the flavanone to chalcone, as sodium carbonate tended to do. After fifteen extractions the extracts had decreased in color from red-orange to deep yellow, the ethyl acetate layer from yellow-orange to delicate yellow, and color tests on test residues of the ethyl acetate solutions grew brighter and clearer. The ethyl acetate layers were evaporated *in vacuo*, leaving yellow to brown gums. One of these was further purified[33] by dissolving the water-soluble portion in hot water, filtering the solution, and treating the filtrate with a basic lead ace-

(29) Direct methylation of II with diazomethane at 0° produced uncrystallizable oils.

(30) Freudenberg, *Ann.*, **433**, 234 (1923).

(31) Geissman and Heaton, THIS JOURNAL, **65**, 678, 682 (1943).

(32) Geissman and Fukushima, *ibid.*, **70**, 1688 (1948).

(33) Preliminary experiments showed that acetylation of this material would not yield a crystalline product.

tate solution. A lead derivative of the flavanone appeared with the first drop of lead acetate solution and was yellow in color. It was collected by filtration and decomposed with hydrogen sulfide as before. The resultant brown oil was subjected to a second basic lead acetate precipitation. The final product was a yellow gum; it still gave slightly dirty-yellow colors with concentrated sulfuric acid. However, an ethyl acetate extract of the first aqueous filtrate of the lead derivative left a residue on evaporation which yielded a brown color in this test, while similar treatment of the second aqueous filtrate gave only a dirty yellow, so that most of this impurity must have been removed.

The partially purified, gummy pigment (about 1 g.) was acetylated with acetic anhydride and sodium acetate as previously described. The light yellow reaction mixture was decomposed with ice and the amorphous product (0.8 g.) was purified by dissolving it in a minimal volume of methanol, adding a large volume of ether and washing out the methanol with large volumes of water. This treatment precipitated dark gums which, since they gave only faint color tests for the flavanone, were discarded. After drying and evaporating the light yellow ether solution, the residual gum was dissolved in methanol and allowed to stand. After it evaporated to a gum in the course of two days and was redissolved, crystals commenced to form. These were removed after five days; the 0.123 g. of dead-white powder represented 15% by weight of crude IV and a 0.029 wt. % yield from the dried petals. It melted at 181.5–189°.

Crude IV was purified by five recrystallizations from methanol (12 ml. per 0.1 g.; 74–87% recovery). It crystallized in dead-white, interlaced needles growing from a denser center, m. p. 195.5–197°. With concentrated sulfuric acid and (slowly) with 10% sodium hydroxide IV gave vivid yellow solutions. In alcoholic solution with magnesium and hydrochloric acid it yielded a vivid blue-pink color similar to that given by eriodictyol, but if it was heated first with strong hydrochloric acid to hydrolyze the acetyl groups, the color was rose-pink.

Anal. Calcd. for $C_{33}H_{34}O_{16}$: C, 57.72; H, 4.99. Found: C, 57.57; H, 5.01.

Its identification as a naringenin derivative is based upon its analysis and the properties of its hydrolysis product, which is described below.

Naringenin Triacetate (VII).—When early attempts failed to crystallize the samples of IV which had been prepared from insufficiently purified gums containing the flavanone pigment, a combined weight of about 2 g. of the gummy acetates was collected and hydrolyzed to the corresponding aglucone. After six hours of refluxing in a mixture of 50 ml. of methanol and 250 ml. of 0.6 *N* hydrochloric acid, practically all of the gum had dissolved. The solution was cooled, filtered several times through a Super-cel mat to remove traces of black gum (which did not contain flavanone), and allowed to stand overnight at 0°. The semi-solid yellow material which separated dried out to an amorphous yellow powder; it weighed 0.25 g. The filtrate gave a positive Molisch test.

This crude flavanone (V) was acetylated in the usual way with acetic anhydride and sodium acetate. The amorphous product (0.319 g.) was induced to crystallize by dissolving it in 5 ml. of methanol and allowing the solution to stand for a few hours. The white granular powder obtained after several days weighed 0.126 g. and melted at 118–123°.

Crude VII was purified first by recrystallization from methanol (2 ml. per 0.1 g., recovery 70–83%). Crystal growth was very slow, with a tendency toward the formation of an oil, and often one large rosette grew. VII was also precipitated from ethyl acetate by petroleum ether (87% recovery) with a similar tendency to oil formation. After three further recrystallizations from methanol the heavy, granular, transparent crystals melted at 122–124°.

Anal. Calcd. for $C_{21}H_{18}O_8$: C, 63.31; H, 4.55. Found: C, 63.42; H, 4.80.

Samples of VII gave a vivid yellow solution with concentrated sulfuric acid and (slowly) became yellow with 10% sodium hydroxide; when its alcoholic solution was treated with magnesium and hydrochloric acid (with or without previous heating with the concentrated acid) a vivid rose color resulted. It was identified as naringenin triacetate by comparison with the authentic sample described below; the two samples gave practically identical absorption spectra, had the same peculiar habits of crystallizing, yielded the same color tests and gave a mixed melting point of 124.5–126.5° (unknown 124.5–126.5°, authentic, 126.5–127.5°).

Naringenin Triacetate (VII) (Synthetic).—Naringenin (0.2 g.) was acetylated by dissolving it in 2 ml. of dry pyridine and 2 ml. of acetic anhydride and allowing the mixture to stand overnight. The amorphous product obtained when the reaction mixture was poured onto ice was dissolved in ether and the ether solution washed with 0.3 *N* and then 3 *N* sodium carbonate solution. The residual oil obtained on evaporation of the ether crystallized when a few drops of alcohol were added; a 77% yield was obtained and the product melted at 123.5–124.5°.

After four recrystallizations from methanol (*ca.* 3 ml. per 0.1 g., recovery 80–95%), VII was obtained as colorless, granular crystals, m. p. 125.5–126.5°.[14] Its color tests were the same as those of the natural material.

Anal. Calcd. for $C_{21}H_{18}O_8$: C, 63.31; H, 4.55. Found: C, 63.55; H, 4.68.

Summary

1. The pigment mainly responsible for the yellow colors in *Antirrhinum majus* is not luteolin as previously reported but is a glucoside of 3′,4′,4,6-tetrahydroxybenzalcoumaranone.

2. The identification of this pigment, aureusin, represents the second anthochlor pigment found to have a benzalcoumaranone structure.

3. A flavanone pigment was also isolated from yellow *Antirrhinum majus* and shown to be a glycoside of naringenin.

4. The absorption spectra of two series of benzalcoumaranone pigments (leptosin and aureusin) and their derivatives have been determined and compared.

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