ground). The material from the mother liquor likewise showed no detectable radioactivity.

2,4- and 2,5-Dibromobenzoic Acids-carboxyl-C¹⁴.—A mixture of 2.0 g. (0.00583 mole) of 2,4,7-tribromotropone-1-C¹⁴, 0.82 g. of sodium hydroxide and 10 ml. each of water and ethanol was swirled for 15 min., heated 30 min. on the steam-bath and then concentrated to half its volume on the steam-bath by blowing with a stream of nitrogen. The mixture was cooled, acidified with 6 N hydrochloric acid and filtered. The product was washed with water, dried *in vacuo* over calcium chloride and sublimed at 130° and 1 mm. to yield 1.46 g. (89.6% of theory) of the mixed dibromobenzoic acids, m.p. 110–125° (1.12₅ mμc./mg. C; 7.88 mμc./mg. in Cα).

Benzoic Acid-carboxyl-C¹⁴.—A mixture of 1.41 g. (0.00504 mole) of the mixed dibromobenzoic acids, 0.82 g. (0.01 mole) of sodium acetate, 0.10 g. of 10% palladium-on-charcoal catalyst and 10 ml. of ethanol was hydrogenated at atmospheric pressure, the reaction being complete in 3 hr. and 279 ml. of hydrogen being absorbed. The filtered reaction mixture was concentrated, treated with 3 ml. of 2 N hydrochloric acid and extracted with two 10-ml. portions of ether. Evaporation of the ether left a residue which was dried *in vacuo* and crystallized from cyclohexane giving 0.35 g. (57% of theory) of benzoic acid-carboxyl-C¹⁴, m.p. 121–122° (1.13₅ mμc./mg. C; 7.94 mμc./mg. in Cα).

Degradation of Benzoic Acid-carboxyl-C¹⁴.—To a solution of 0.122 g. (0.00100 mole) of benzoic acid-carboxyl-C¹⁴ (1.13 mμc./mg. C) in 1 ml. of concentrated sulfuric acid cooled to 0°, there was added 0.08 g. (0.00123 mole) of sodium azide. The flask was immediately attached to a Phares apparatus,³² which contained 10 ml. of 1 N sodium hydroxide solution. The mixture was heated for 2 hr. at 40°, and then nitrogen was bubbled through for 30 min. The sodium hydroxide solution was then poured into a solution of 0.50 g. of barium chloride in 10 ml. of water. The barium carbonate was filtered under nitrogen, washed successively with water, 1:1 water–methanol and then dried at 100°, 0.176 g. (89.3% of theory) (8.08 mμc./mg. C).

The acidic solution was cooled to 0°, diluted to 7 ml. with water, made alkaline with concentrated aqueous sodium hydroxide solution and shaken for 10 min. with 0.16 g. (0.00114 mole) of benzoyl chloride in 5 ml. of chloroform. Separation and concentration *in vacuo* of the chloroform solution left a crystalline mass which was washed with water and dried *in vacuo* over calcium chloride. Two crystallizations from ethanol–water afforded 0.13 g. (66% of theory) of benzanilide, m.p. 161.5–162.5° (radioactivity indistinguishable from background).

Katonah, N. Y.
Upton, L. I., N. Y.

Anthochlor Pigments. X. Aureusin and Cernuoside

By T. A. Geissman and J. B. Harborne

Aureusin and cernuoside, glucosides of 3′,4′,4,6-trihydroxyaurone (aureusidin) have been shown to be the 6- and 4-glucoside, respectively.

The orange-yellow pigment aureusidin (I or II, R = R′ = H) was first isolated in the form of the heptaacetate of its glucoside, aureusin, from a yellow variety of the garden snapdragon, *Antirrhinum majus*.¹ It was subsequently found to be present in the flowers of all of the non-albino color forms of the same plant, in amounts controlled by a single genetic factor.² Aureusin and other aureusidin glycosides have also been found to occur in a number of other flowers.³ In particular, *Oxalis cernua* L. contains aureusin and, in larger amounts, another aureusidin glucoside, cernuoside.³⁻⁵ Cernuoside is clearly distinguished from aureusin by its different rate of movement on paper chromatograms. Since both pigments are monoglucosides of aureusidin¹,⁴ the establishment of the point of attachment of the sugar residue in each of the two pigments would determine their complete structures.

Complete methylation of the two glucosides, followed by acid hydrolysis, yielded the two monohydroxy-trimethoxyaurones. The glucosides, their methyl ethers and the partially methylated aglucones were examined by spectrophotometric and chromatographic methods. Particular use was made of the fact that the spectra only of aurones⁶

bearing a 4-hydroxyl group are shifted by the addition of aluminum chloride.⁷ The spectral shifts brought about by the addition of alkali gave further information, and are discussed below.

The spectra of aureusin, which was prepared for the first time by the mild alkaline hydrolysis of its heptaacetate,⁸ showed that the sugar was not in either position 4 (since the long wave length maximum was shifted bathochromically 60 mμ by aluminum chloride) or 4′ (since there was an 85 mμ shift in alkali). Methylation of aureusin, followed by removal of the sugar residue, gave a product which was spectrally and chromatographically identical with an authentic sample of 6-hydroxy-4,3′,4′-trimethoxyaurone (I, R = H, R′ = Me) and different from the isomeric compounds, 4′-hydroxy-4,6,3′-trimethoxyaurone (III, R = OMe) and 3′-hydroxy-4,6,4′-trimethoxyaurone (IV, R = OMe). The three synthetic aurones were prepared by the condensation of the appropriate coumaranone⁹ with veratraldehyde, vanillin and isovanillin, respectively.

The spectrum of cernuoside was not altered by the addition of aluminum chloride solution, an indication that the compound is the 4-glucoside of aureusidin (II, R = glucosyl, R′ = H). This was confirmed by the methylation of cernuoside,¹⁰ followed by acid hydrolysis. The ultraviolet spec-

(1) M. K. Seikel and T. A. Geissman, This Journal, 72, 5725 (1950).

(2) T. A. Geissman, E. C. Jorgensen and B. L. Johnson, *Arch. Biochem. Biophys.*, 49, 368 (1954).

(3) E. C. Jorgensen, Ph.D. Thesis, University of California, Los Angeles, 1953.

(4) A. Ballio, S. Dittrich and G. B. Marini-Bettolo, *Gazz. chim. ital.*, 83, 224 (1953).

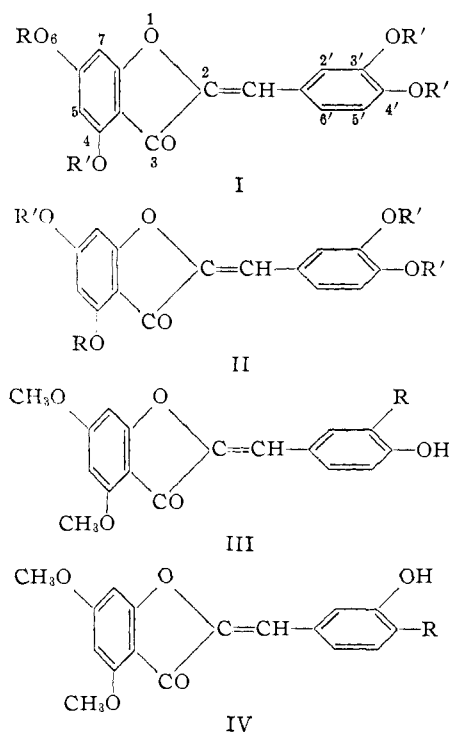(5) R. Lamonica and G. B. Marini-Bettolo, *Ann. Chim.*, 42, 496 (1952).

(6) E. C. Bate-Smith and T. A. Geissman, *Nature*, 167, 688 (1951).

(7) J. B. Harborne, *Chemistry and Industry*, 1142 (1954).

(8) A sample was kindly furnished by Dr. M. K. Seikel.

(9) T. A. Geissman and E. H. Hinreiner, This Journal, 73, 785 (1951); E. H. Hinreiner, Ph.D. Thesis, University of California, Los Angeles, 1951.

(10) A sample of cernuoside from *Oxalis cernua* was kindly furnished by Dr. Marini-Bettolo.
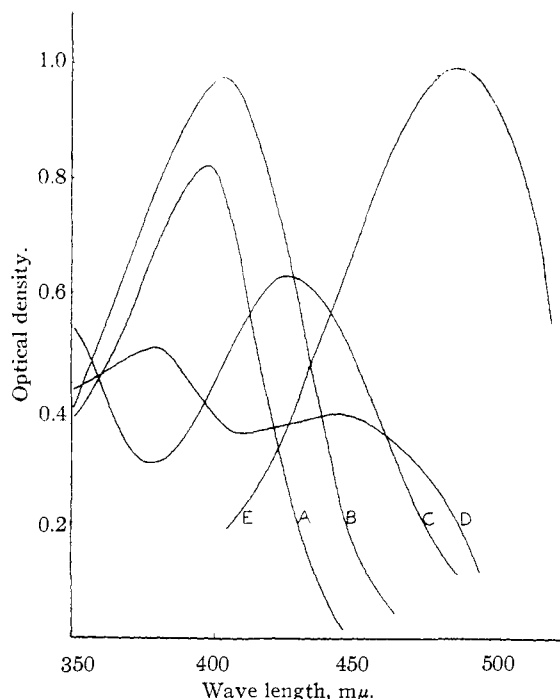
Fig. 1.—Long wave length ultraviolet absorption spectra of: A, 3',4',4,6-(monohydroxytrimethoxy)-aurone in ethanol (see text); B, 3',4',4-trimethoxy-6-hydroxyaurone, alkaline; C, 3',4',6-trimethoxy-4-hydroxyaurone, alkaline; D, 4,6,4'-trimethoxy-3'-hydroxyaurone; E, 3',4,6-trimethoxy-4'-hydroxyaurone, alkaline.

trum of the product was shifted (60 mμ) by aluminum chloride and (28 mμ) by sodium ethoxide; the compound is thus shown to be 4-hydroxy-6,3',-4'-trimethoxyaurone (II, R = H, R' = Me).

The relevant spectral and chromatographic data are collected in Table I. In Fig. 1 are shown the effects of the addition of sodium ethoxide upon the long wave length maximum of the four monohydroxy-trimethoxyaurones: I, R = H, R' = Me; II, R = H, R' = Me; III, R = OMe; and IV, R = OMe. Curve A in the figure can be regarded as representing the absorption near 400 mμ of all of the aurones in neutral (ethanol) solution, since all four have their maximum at 395–399 mμ. Curves B-E represent the spectra of 6-OH-4,3',4'-triOMe, 4-OH-6,3',4'-triOMe, 3'-OH-4,6,4'-triOMe and 4'-OH-4,6,3'-triOMe, respectively. The large (85 mμ) alkaline shift in the case of III (R = OMe) (curve E) is clearly the result of the conjugation of the

anionic 4'-oxygen atom with the C=C—C=O system. A part of this shift is caused by the influence of the 3'-methoxyl group, since in 4,6-dimethoxy-4'-hydroxyaurone (III, R = H) the alkaline shift is only 69 mμ. This marked influence of a substituent in the non-conjugated meta (3') position is observed in other cases. For example, 3',4'-dihydroxyaurone forms a purple salt in alkali, while that of 4'-hydroxyaurone is red. In the case of 3'-hydroxy-4,6,4'-trimethoxyaurone (IV, R = OMe) (curve D), the presence of the (low intensity) maximum at 443 mμ is in part due to the presence of the 4'-methoxy group (or it may be said that ionization of 3'-hydroxy enhances the contribution of the 4'-

TABLE I

ULTRAVIOLET SPECTRA AND R_f VALUES OF AUREUSIDIN AND ITS DERIVATIVES

					λ_{max}				Rf^c		Color^d	
	Aurone		95% EtOH		EtOH–NaOEt^a	EtOH–AlCl$_3$^{b}		BAW	30% HAc	BN	UV	UV + NH$_3$
1	Aureusidin	254	269	335^e	398.5	^{f}	405^e　458		0.57	0.10	0.00	Y	O
2	4,6,3',4'-Tetramethoxy	254	267^e	338^e	397	No shift	No shift		.80	.17	..	G	G
3	6-Hydroxy, 4,3',4'-trimethoxy	..	268	..	395	403	No shift		.77	.11	.11	BG	BG
4	4'-Hydroxy, 4,6,3'-trimethoxy	253	268	329	399	484	No shift		.77	.11	.33	Y	O
5	3'-Hydroxy, 4,6,4'-trimethoxy	..	272	328	397	380　443	No shift		.81	.22	.59	BG	BG
6	Aureusin	..	272	322	405	490	404　466		.28	.16	.00	Y	O
7	Methylated aureusin	254	267	322	401	No shift	No shift		.91	.40	..	G	G
8	Hydrolysis product of 7	..	267^e	342	396	404	No shift		.77	.12	.11	BG	BG
9	Cernuoside	255	267^e	..	405	ca. 450^g	No shift		.49	.25	.00	Y	O
10	Methylated cernuoside	254	..	335	398	No shift	No shift		.88	.60	..	G	G
11	Hydrolysis product of 10	..	268	333	397	425	396　454		.79	.14	.13	BG	BG

^{a} Approximately 0.01 N NaOEt in EtOH.　^{b} Three drops of 5% ethanolic AlCl$_3$ was added to each 3-ml. cell.　^{c} BAW = n-butanol–27% aqueous acetic acid, 1:1; 30% HOAc = 30% aqueous acetic acid; BN = n-butanol-2 N ammonia, 1:1. (Whatman No. 1 paper was used in descending chromatograms.)　^{d} Y = yellow, O = orange, G = green, B = blue.　^{e} Shoulder.　^{f} Rapidly decomposes.　^{g} Slowly decomposes.

methoxy group) as shown by the observation that the aurone IV (R = H) shows no alkaline shift (although the addition of alkali causes some general increase in the level of absorption above 376 mμ).

The small alkaline shift (8 mμ) in the maximum in the case of 6-hydroxy-4,3',4'-trimethoxyaurone (I, R = H, R' = Me) is unexpected in view of the considerable shift (58 mμ) in the case of 6-hydroxy-aurone itself, and must be an effect of the presence of the 3',4'-methoxyl groups in I (R = H, R' = Me). These substituents, in particular the 4'-methoxy group, contribute an opposing, or crossed, conjugation to the system that includes the 6-hydroxyl group, as in V, and thus diminish the importance of structures such as in VI:

V

VI
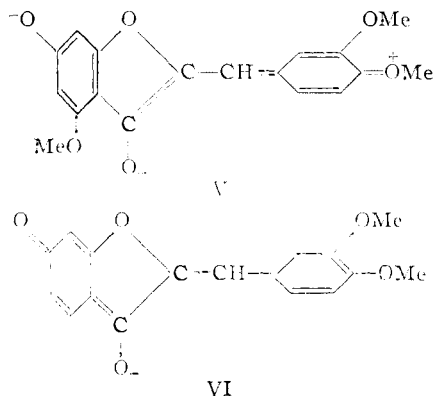
Experimental

Aureusin: Preparation and Methylation.—Aureusin heptaacetate (141 mg.) in acetone (5 ml.) and ethanol (1 ml.) was shaken for one-half hour with 30 ml. of 0.5 N aqueous sodium hydroxide. The deep red solution was acidified and unchanged acetate was removed by filtration. The filtrate was evaporated under reduced pressure to remove the volatile solvents and the aqueous residue was saturated with ammonium sulfate and extracted ten times with 20-ml. portions of ethyl acetate. The extract was dried and evaporated to dryness. A portion of the residue was dissolved in ethanol and chromatographed with butanol–acetic acid–water (BAW) on Whatman No. 3 paper. The band at R_f 0.31 was cut out, eluted with 70% ethanol, and the eluate made up to a suitable volume for spectrophotometric measurement. A similar eluate of an area of corresponding R_f from a blank chromatogram was used as the solvent blank in the spectrophotometer. Rechromatography of the R_f 0.31 component in two different solvents showed that it contained a trace of an impurity that was not aureusin, nor was it anthochlor-like in nature.

The major portion of the ethyl acetate-extracted material was dissolved in a mixture of 5 ml. of acetone and 5 ml. of benzene and refluxed for four hours with 2 g. of potassium carbonate and 1 ml. of dimethyl sulfate. After the addition of a further 2 g. of potassium carbonate and 1 ml. of di-

methyl sulfate, refluxing was continued for another 20 hours. The potassium carbonate was removed by filtration and washed with acetone, and the filtrate evaporated to dryness. An ether solution of the residue was washed with 0.5 N aqueous alkali and with water, dried and evaporated. A portion of the residue was removed for the determination of its absorption spectrum. The spectrum was unaffected by the addition of ethanolic sodium ethoxide and alcoholic aluminum chloride, showing that the methylation of the phenolic hydroxyl groups was complete.

The major portion of the methylated glucoside was taken up in 5 ml. of ethanol and the solution, after the addition of 5 ml. of 1 N hydrochloric acid, heated for five hours. The ethanol was then allowed to escape and the aqueous residue cooled and extracted with ethyl acetate. The extract was dried and evaporated and the residue chromatographed on Whatman No. 3 paper (BAW). Two bands appeared: a major one at R_f 0.83 and a minor one (the unhydrolyzed methylated glucoside) at R_f 0.94. The 0.83 band was removed, eluted, and its absorption spectrum measured with an eluted blank in the reference cell.

Methylation of Cernuoside and Hydrolysis of the Product.—Cernuoside (31 mg.) was methylated and the product hydrolyzed in exactly the manner described for aureusin. The products were purified by chromatography and the spectra measured on the eluates from the chromatograms.

Preparation of the Aurones.—Equimolecular amounts (ca. 100 mg.) of the required coumaranone and the appropriate aldehyde were dissolved in 5 ml. of glacial acetic acid and 0.2 ml. of concentrated hydrochloric acid added. After three–five hours at room temperature, the solution was poured into water and the product collected and recrystallized from aqueous ethanol.

6-Hydroxy-4-methoxycoumaranone and veratraldehyde gave **6-hydroxy-4,3',4'-trimethoxyaurone**, yellow needles, m.p. 268–70° dec.

Anal. Calcd. for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91. Found: C, 65.89; H, 5.18.

4,6-Dimethoxycoumaranone and vanillin gave **4'-hydroxy-4,6,3'-trimethoxyaurone**, yellow needles, m.p. 232–233°.

Anal. Calcd. for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91. Found: C, 66.11; H, 5.05.

4,6-Dimethoxycoumaranone and isovanillin gave **3'-hydroxy-4,6,4'-trimethoxyaurone**, yellow needles, m.p. 190°.

Anal. Calcd. for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91. Found: C, 66.02; H, 4.96.

4,6-Dimethoxycoumaranone and p-hydroxybenzaldehyde gave **4,6-dimethoxy-4'-hydroxyaurone**, bright yellow needles, m.p. 274°.

Anal. Calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.71; H, 5.00.

4,6-Dimethoxycoumaranone and m-hydroxybenzaldehyde gave **4,6-dimethoxy-3'-hydroxyaurone**, yellow needles, m.p. 214–215°.

Anal. Calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.22; H, 4.75.

LOS ANGELES 24, CALIFORNIA