nents is given below. The results obtained are given in Tables III and IV.

Spot 2, marein, was isolated from a methanolic extract of fresh blossoms by banding with 30% HOAc and was purified by rechromatography in BAW and 40% HOAc.

Spot 7, maritimein, was isolated from solution B by banding in 40% HOAc and was purified by rechromatography twice in BAW.

Spot 8, maritimetin, was isolated from the hydrolyzed solution C by banding with 40% HOAc and was purified by rechromatography three times in BAW. Difficulty was experienced in separating it from spot 8a because of dark and similar colors, so in the final bandings only the central portion of the band was cut out.

Spot 8a, okanin, was isolated exactly as spot 8, from which it was separated with great difficulty.

Spot 9, sulfurein, was isolated by solution B by banding in 40% HOAc and was purified by rechromatography four times in BAW.

Spot 10, coreopsin, was isolated from the methanolic extract of fresh blossoms by banding in 30% HOAc and was purified by rechromatography in BAW, 40% HOAc and BAW.

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LOS ANGELES 24, CALIFORNIA

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

Anthochlor Pigments. XII. Maritimein and Marein

BY J. B. HARBORNE AND T. A. GEISSMAN

RECEIVED JULY 25, 1955

The structures of maritimein and marein, two new anthochlor pigments of *Coreopsis maritima*, have been shown to be 6-glucosidoxy-7,3′,4′-trihydroxyaurone (III, R = Gl) and 4′-glucosidoxy-2′,3′,3,4-tetrahydroxychalcone (II, R = Gl), respectively.

An investigation of the major constituents of *Coreopsis maritima*, using paper chromatography and ultraviolet spectroscopy, is described in the previous communication.[1] This work revealed the presence of two new anthochlor pigments, which were called maritimein and marein. These two glycosides and their respective aglycones were also found at the same time to occur in *Coreopsis gigantea*. Neither pigment was present in sufficient quantity to permit its isolation by classical methods, so that structure determinations have had to be carried out on the minute quantities available from the use of paper chromatographic isolations. In this communication is described the elucidation of structure of maritimein and marein.

The two pigments were present as a single band in butanol:acetic acid:water (band 5, R_f = 0.47) in the preliminary separation on thick paper of the *C. maritima* extract. Chromatography of the eluate of this band in the same solvents and then in 30% aqueous acetic acid eventually led to its separation into two components. The faster moving component, maritimein, (R_f = 0.28, in 30% acetic acid) was readily identified as an aurone from its color reactions in alkali (yellow to purple red), its ultraviolet spectra (visible max. at 419 mμ, alkaline max. at 500 mμ) and the spectra of its acetate.[2] Its spectrum did not shift in the presence of aluminum ions, indicating the absence of a 4-hydroxy group.[3] Hydrolysis of maritimein gave glucose and a rather unstable aglucone, maritimein. The properties of maritimein did not correspond to those of any of the five known naturally occurring aurone glucosides (leptosin,[4] sulfurein,[5] aureusin[6] and cernuoside[6a] and palastrin[6b]).

(1) T. A. Geissman, J. B. Harborne and M. K. Seikel, THIS JOURNAL, **78**, 825 (1956).

(2) M. K. Seikel and T. A. Geissman, *ibid.*, **72**, 5725 (1950).

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

(4) T. A. Geissman and C. D. Heaton, THIS JOURNAL, **65**, 677 (1943); **66**, 486 (1944).

(5) M. Shimokoriyama and S. Hattori, *ibid.*, **75**, 1900 (1953).

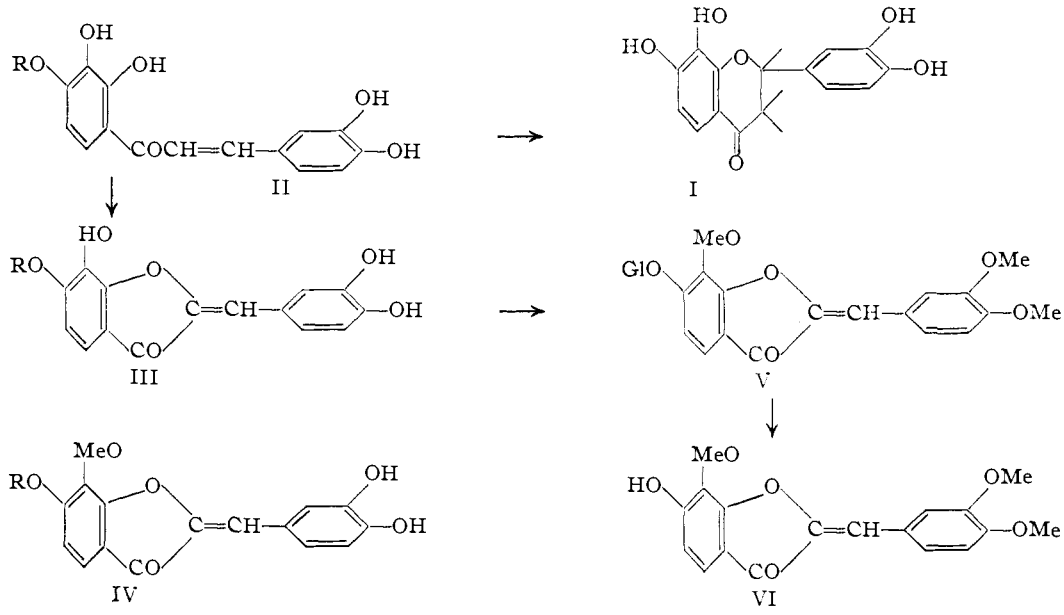
(6) (a) T. A. Geissman and J. B. Harborne, *ibid.*, **78**, 832 (1956); (b) B. Puri and T. Seshadri, *J. Chem. Soc.*, 1589 (1955).

The slower moving component, marein (R_f = 0.22, in 30% acetic acid), was also a glucoside and its ultraviolet spectrum was that of a chalcone, with a visible max. at 382 mμ. Its spectral shifts in the presence of alkali and aluminum ion were also indicative of a chalcone structure. During the course of the investigation, it was found that marein was closely related to maritimein. Paper strips of the marein band, after keeping for several weeks, were eluted with alcohol and the eluate was found to contain only maritimein. It was then clear that marein and maritimein were a chalcone–aurone pair, interrelated in the same way as lanceolin and leptosin,[5] and coreopsin and sulfurein.[1]

Marein, on heating in dilute acid, underwent isomerization to give a flavanone, which was different from any of the commonly occurring 7-hydroxylated or 5,7-dihydroxylated flavanones, since it gave a characteristic blue fluorescence in ultraviolet light in the presence of ammonia vapor. It was eventually identified as 7,8,3′,4′-tetrahydroxyflavanone (I), by comparison of its spectra in ethanolic and alkaline solutions (see Fig. 1) and from the comparison of R_f values in three solvent systems (Table I) with authentic material. The spectra and R_f values of a number of other flavanones are given in Table I. As will be seen, the majority of flavanones do not undergo ring opening in the presence of traces of alkali, but give quite stable peaks of increased intensity and the whole spectra are moved over toward the visible region by 20–60 mμ. This behavior is useful for distinguishing flavanones with different hydroxyl groups in the A ring, and has not previously been described. From the above evidence, it follows that marein is a glucoside of 2′,3′,4′,3,4-pentahydroxychalcone (II, R = H). This chalcone has earlier been isolated[7] from the tropical hardwood, *Cyclicodiscus gabunensis* Harms. in the free state as the *cis* and *trans*-isomers, named isookanin and okanin.

Since marein, a glucoside of (II, R = H), is con-

(7) F. E. King and T. J. King, *ibid.*, 569 (1951).



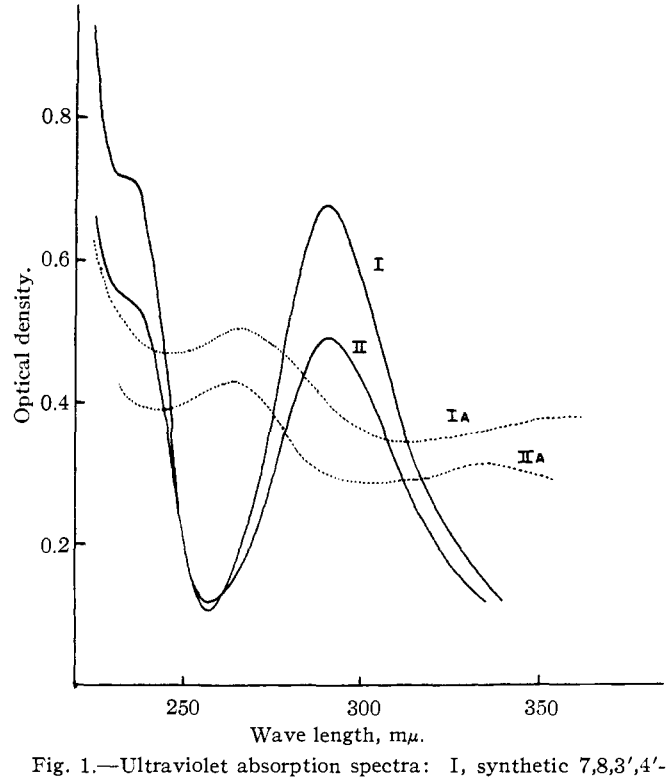


Fig. 1.—Ultraviolet absorption spectra: I, synthetic 7,8,3′,4′-tetrahydroxyflavanone in 90% ethanol; II, "marein flavanone" in 90% ethanol; IA and IIA, corresponding spectra in ethanolic sodium ethoxide.

verted by aerial oxidation to maritimein, then the latter must have the structure of the corresponding aurone and be a glucoside of 6,7,3′,4′-tetrahydroxyaurone (III, R = H). This fact was confirmed in a number of different ways. Careful acid hydrolysis of maritimein gave the aglucone, maritimetin, which was identical in all respects with synthetic 6,7,3′,4′-tetrahydroxyaurone (III, R = H), prepared from 6,7-dihydroxycoumaranone and protocatechualdehyde. The methylated derivative of maritimetin was similarly shown to be the same as 6,7,3′,4′-tetramethoxyaurone. Maritimetin (III, R = H) is very unstable in alkali, giving a violet color which rapidly disappears, and is also decomposed slowly by hot dilute acid. This instability is presumably due to the presence of two *o*-dihydroxyl groupings in the same molecule, since the glucoside, maritimein, is more stable. Comparison of the spectra of leptosin (IV, R = Gl) and leptosidin (IV, R = H) with the spectra of maritimein and maritimetin (Fig. 2) shows the great similarity in structure of the two aurone glycosides. In particular, the effect of introducing a sugar molecule into the aglucones causes spectral shifts not often encountered in phenolic plant pigments.[8] This effect, which results in a small (*ca.* 5 mμ) bathochromic shift of the color band and a hypsochromic shift, with increase of intensity, of the 320–360 mμ band, is exactly the same in both pairs of compounds.

The position of the sugar molecule in maritimein, and thus in marein, was determined as follows. Complete methylation of maritimein gave a compound, presumably V, which on acid hydrolysis gave a product, identical spectrally and chromatographically with 6-hydroxy-7,3′,4′-trimethoxyaurone (VI). The spectrum of the isomeric 7-hydroxy-6,3′,4′-trimethoxyaurone was quite different (see Table II). That the sugar in maritimein is not in the 3′- or 4′-position follows from a consideration of its properties (*e.g.*, stability to alkali) and its relationship to leptosin. Furthermore, experience in the 4,6,3′,4′-tetrahydroxyaurone (aureusidin) series[6a] shows that the spectral identity of the hydrolysis product of methylated maritimein with VI is quite conclusive, and hence glucosidation is in the 6-position. The complete structures of maritimein and marein are thus (III, R = Gl) and (II, R = Gl), respectively.

(8) *Cf.* ref. 2; also in unpublished work on the ultraviolet spectra of aurones.

TABLE I

ULTRAVIOLET SPECTRA AND R_f VALUES OF "MAREIN FLAVANONE" AND OTHER FLAVANONES

Flavanone	λ_{max} 95% EtOH			λ_{max} EtOH–NaOEt[d]	R_f BAW	R_f 5% HAc	R_f 30% HAc	Color[e] UV	Color[e] UV + NH_3
From marein	234[a]	291		264[c]	0.72	0.19	0.55	C	1B
7,8,3′,4′-Tetrahydroxy	234[a]	291		265[c]	.72	.19	.55	C	1B
7-Methoxy-8,3′,4′-trihydroxy	237	285		259, 360	.76	.23	.63	C	C
8-Methoxy-7,3′,4′-trihydroxy	234	281		261, 340	.82	.29	.65	C	C
7,8,4′-Trihydroxy	238[a]	294		[b]	.81	.24	.62	C	1B
6,7,3′,4′-Tetrahydroxy	239	282	348	260, 362	.73	.12	.46	C	C
7,3′,4′-Trihydroxy (butin)	233	278	312	254, 337	.84	.26	.64	C	Y
5,7,3′,4′-Tetrahydroxy (eriodictyol)	226	288	325[a]	245, 328	.85	.14	.56	C	Y
5,7,4′-Trihydroxy (naringenin)	224	290	325	247, 327	.69	..	.81	C	Y

[a] Inflection. [b] Feeble max. in 330–360 mμ range, which disappears with time. [c] Immediately ring opening occurs with chalcone formation. [d] Approximately 0.01 *N* NaOEt in EtOH. [e] All these flavanones gave a blue spot after spraying with a ferric chloride–potassium ferricyanide reagent [Barton, Evans and Gardner, *Nature*, 170, 249 (1952)].

Experimental

Abbreviations.—The following abbreviations are used throughout: BAW for *n*-butanol–27% acetic acid (1:1); %HOAc for water containing the given % of glacial acetic acid. Colors are denoted thus: B, brown; Bl, blue; Bk, black; C, colorless; G, green; l, light; M, mauve; O, orange; P, purple; Pk, pink; R, red; Y, yellow.

The precautions described under the heading, general remarks, in the Experimental section of the previous paper[1] apply here also.

Separation of Maritimein and Marein.—The initial separation of *C. maritima* extract is described in the previous paper in this series.[1] Band 5 (R_f 0.47 in BAW) of this separation was cut off, eluted with 70% ethanol and concentrated. On chromatography in BAW on thick paper, it was partially separated into two bands (5.1, R_f 0.52, colors in ultraviolet and ultraviolet/NH_3, Y → R; 5.2, R_f 0.45, Bk → NB). These two bands were eluted separately and rechromatographed in 30% HOAc on Whatman No. 3 paper. Complete separation was not achieved. Band 5.1 gave a major aurone component (R_f 0.28, Y → R) and a minor chalcone component (R_f 0.20, Bk → Bk). Band 5.2 was mostly chalcone (R_f 0.22, Bk → Bk) with a minor aurone component (R_f 0.28, Y → R). Elution of these bands with 70% ethanol and appropriate combination gave pure solutions of the chalcone, marein and the aurone, maritimein. The purity of these solutions was checked spectroscopically.

Structure of Marein.—The pure eluate of marein was diluted with 95% ethanol to an appropriate concentration and its spectrum was determined against a paper blank. It had λ_{max} at 268, 382 mμ, alkaline λ_{max} at 448 mμ, aluminum chloride λ_{max} at 422 mμ. Okanin, 2′,3′,4′,3,4-pentahydroxychalcone, had λ_{max} 259, 382 mμ,[9] alkaline λ_{max} decomposes, aluminum chloride λ_{max} at 420 mμ.

A fresh aqueous ethanolic eluate of marein (*ca.* 50 cc.) and concentrated hydrochloric acid (5 cc.) were heated on a steam-bath for three hours and the ethanol was allowed to evaporate. The cooled solution was extracted four times with ether. The aqueous residue was tested chromatographically[10] for sugars and showed the presence of glucose, by cochromatography in three solvent mixtures. The dried ethereal extract was evaporated to a residue, which was chromatographed in 30% HOAc. Some unchanged chalcone remained near the origin; the flavanone appeared as a light blue band at R_f 0.57 on fuming the paper with ammonia. It was cut off, eluted, and its spectrum was measured. This "marein flavanone" was identified as 7,8,3′,4′-tetrahydroxyflavanone from R_f values and the spectral results shown in Table I and Fig. 1.

The ultraviolet spectra and R_f values of a number of other hydroxylated flavanones are included in Table I. The majority of compounds were available in these laboratories. The spectra of some of these flavanones have been recorded previously, and our values are in agreement with these figures. The spectra in ethanolic sodium ethoxide are similar in simple cases to the alkaline spectra of the acetophenone from which the flavanone is derived. The spectra of 7-hydroxy and 5,7-dihydroxyflavanones in alkali have two main bands of increased intensity.

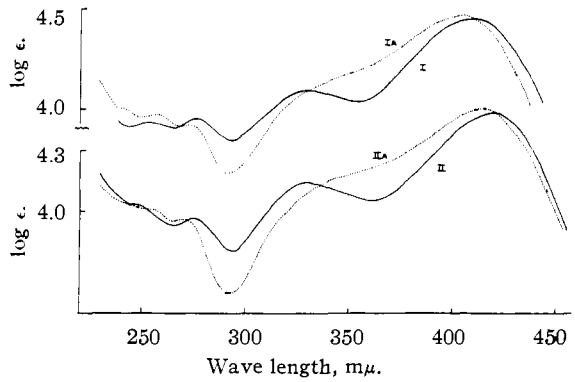


Fig. 2.—Ultraviolet absorption spectra: I, leptosin; IA, leptosidin; II, maritimein; IIA, maritimetin (in 95% ethanol).

Conversion of Marein to Maritimein.—Paper strips of band 5.2, after keeping for one to two months, were eluted with 70% ethanol for 36 hours. The eluate was banded in 30% HOAc and showed only one band (R_f 0.32, Y → R) and a complete absence of the dark chalcone band. An eluate of this band gave clean spectral maxima in 95% ethanol at 327 and 418 mμ. (See Table II for the spectrum of maritimein.) This conversion was observed a number of different times.

Structure of Maritimein (III, R = Gl).—An eluate containing maritimein was evaporated to dryness and the residue was heated with acetic anhydride (2 cc.) and sodium acetate (trace) for one hour at 100°. It was poured into water, and the resulting solution was extracted into ether. The ethereal extract was washed with water, dried and evaporated. The residue was made up to a suitable volume with 95% ethanol, and its spectrum was measured: λ_{max} 251, λ_{min} 282, λ_{max} 328, inflection 365 mμ. Spectra of other aurone acetates are given in ref. 2.

An ethanolic solution of pure maritimein was heated for 1.5 hours[11] with an equal volume of 2 *N* aqueous hydrochloric acid. After evaporation of the ethanol, the cooled solution was exhaustively extracted with ethyl acetate. The aqueous residue was chromatographed in three solvent systems and showed the presence of glucose.[10] The dried ethyl acetate extract was evaporated and the residue chromatographed in 30% HOAc. The aglucone, maritimetin, readily separated from traces of unchanged glucoside. The eluate from the maritimetin band was identical with 6,7,3′,4′-tetrahydroxyaurone (see Table II). Maritimetin was methylated with methyl sulfate, potassium carbonate and

(9) A. Russell, J. Todd and C. L. Wilson, *J. Chem. Soc.*, 1940 (1934), report λ_{max} 270, 289, 386 mμ.

(10) *Cf.* E. Lederer and M. Lederer, "Chromatography," Elsevier Publishing Co., Houston, Texas, 1953, p. 158, and many other manuals.

(11) Longer heating appreciably reduced the amount of aglucone formed.

TABLE II

ULTRAVIOLET SPECTRA AND R_f VALUES OF MARITIMEIN AND RELATED AURONES

Aurone	λ_{max}[d] 95% EtOH				EtOH–NaOEt	R_f BAW	R_f 30% HOAc	R_f 50% HOAc	Colors UV	Colors UV/NH_3	Colors 1% aq. Na_2CO_3 spray
Maritimein	242	274	330	419	505	0.42	0.21	0.37	Y	R	P
Maritimetin	..	270	355[a]	413	Dec.	.53	.10	.24	Y	R	Pk
6,7,3′,4′-Tetrahydroxy	252	268	355[a]	415[c]	Dec.	.53	.09	.24	Y	R	Pk
Methylated maritimein			[b]	408	No shift	.88	.53	.75	YG	YG	..
Methylated maritimetin			[b]	403	No shift	.82	.17	.54	YG	YG	..
6,7,3′,4′-Tetramethoxy	256	268[a]	340[a]	404	No shift	.82	.18	.54	YG	YG	..
Leptosin	257	276.5	328.5	411	522	.51	.33	.52	Y	R	P
Leptosidin	257	272	340	406	396, 459	.76	.19	.41	Y	OR	Pk
6-Methoxy-7,3′,4′-trihydroxy	245	272	337	413	355, 477	.69	.18	.40	Y	R	P
6,7-Dimethoxy-3′,4′-dihydroxy	257	272	332.5	406	312, 487	.78	.19	.47	Y	R	P
Hydrolyzate of methylated maritimein	[b]		...	401	425	.83	.17	.50	YG	O	..
6-Hydroxy-7,3′,4′-trimethoxy	255	268	...	401	424	.83	.18	.50	YG	O	..
7-Hydroxy-6,3′,4′-trimethoxy	243	266	358	411	362, 447	.83	.20	.52	G	B	..

[a] Inflection. [b] Not measured in this region. [c] Max. at 413 in presence of trace of acid. [d] The spectra of these compounds do not shift on adding EtOH–$AlCl_3$.

acetone and the product was identical with authentic 6,7,-3′,4′-tetramethoxyaurone.

Maritimein was methylated with methyl sulfate, potassium carbonate and acetone. The spectrum of the methylated product did not shift in the presence of alkali, indicating that complete methylation had taken place. This product was hydrolyzed for three hours with dilute 2 *N* acid at 100°. The cooled solution was extracted with ethyl acetate and the organic extract was dried and evaporated. The residue was identical with authentic 6-hydroxy-7,3′,4′-trimethoxyaurone (VI) (see Table II).

Synthesis of Aurones.—The method used was exactly that described earlier.[6] The preparation of 6,7,3′,4′-tetrahydroxyaurone will be described elsewhere.[3] The other new aurones are described here.

6-Hydroxy-7-methoxycoumaranone and veratraldehyde gave **6-hydroxy-7,3′,4′-trimethoxyaurone**, yellow needles, m.p. 204–205°, from aqueous acetic acid.

Anal. Calcd. for $C_{18}H_{16}O_6$: C, 65.85; H, 4.92. Found: C, 65.82; H, 5.19.

7-Hydroxy-6-methoxycoumaranone and veratraldehyde gave **7-hydroxy-6,3′,4′-trimethoxyaurone**, yellow needles, m.p. 210–211°, from aqueous acetic acid.

Anal. Calcd. for $C_{18}H_{16}O_6$: C, 65.85; H, 4.92. Found: C, 65.79; H, 5.30.

Acknowledgments.—The authors thank the U. S. Public Health Service for a research grant (G-3667), and Eli Lilly and Company for a Fellowship to J. B. H. A gift of 6,7,3′,4′-tetrahydroxyflavanone from Dr. M. K. Seikel is gratefully acknowledged.

LOS ANGELES 24, CALIFORNIA

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

Anthochlor Pigments. XIII. The Ultraviolet Absorption Spectra of Phenolic Plant Pigments. Polyhydroxyaurones

BY T. A. GEISSMAN AND J. B. HARBORNE

RECEIVED JULY 25, 1955

As an aid in the identification of aurone plant pigments, the ultraviolet spectra of twenty-seven hydroxylated or methoxylated aurones have been measured in both neutral and in alkaline solution. While the presence of a 4- or 3′-hydroxyl-, or a 5-hydroxyl- in a 6-hydroxyaurone, does not change the spectra appreciably, a 6-hydroxyl group has a pronounced hypsochromic effect on aurone spectra. The following hydroxyl groups are bathochromic: 2′-, 4′-, 7- in the presence of 6-, and 3′ in the presence of 4′-. These results are interpreted as being partly due to cross conjugation. Comments are included on the spectra of aurone glycosides and the spectra in alkaline solution.

Although aurones (benzalcoumaran-3-ones) (*e.g.*, I) have been known for a long time, it is only recently that they have been found to occur in nature. Three such compounds have been isolated as glycosides from the flower petals of certain *Compositae*, especially the genus *Coreopsis*, and other plants in these laboratories[1] and elsewhere.[2-4] They are leptosidin (I, R = H, R′ = OMe), aureusidin (I, R = OH, R′ = H) and sulfuretin (I, R = R′ = H). Because of their bright golden yellow colors, these substances are important contributors to the pigmentation of the flowers in which they occur.

Recent chromatographic and spectral studies of the petal extracts of *Coreopsis maritima* Hook. have revealed the presence of a new aurone glycoside, maritimein, in this plant. As only micro quantities of this new compound were available, the absorption spectra of a number of polyhydroxyaurones were measured in order to provide a means of identifying its structure. No comprehensive study has previously been made of the spectra of these

(1) (a) T. A. Geissman and C. D. Heaton, THIS JOURNAL, **65**, 677 (1943); **66**, 486 (1944); (b) T. A. Geissman and M. K. Seikel, *ibid.*, **72**, 5725 (1950); (c) T. A. Geissman and W. Mojé, *ibid.*, **73**, 5765 (1951).

(2) M. Shimokoriyama and S. Hattori, *ibid.*, **75**, 1900 (1953).

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

(4) C. G. Nordstrom and T. Swain, *Chemistry and Industry*, 823 (1953).