Δ^4-Pregnene-17α,21-diol-3,20-dione (Substance S) 17-Acetate 21-Phenyl Ether (XVI).—Aluminum isopropoxide (1.1 g.) was added to a solution of 2.2 g. of the 17-acetate 21-phenyl ether XVg in 90 cc. of dry toluene and 30 cc. of cyclohexanone and the mixture was boiled under reflux for 1 hour. The volatile components were then removed by steam distillation, the solid was collected and well extracted with hot acetone. Evaporation of the extract, followed by crystallization of the residue from acetone–pentane, led to 1.78 g. (81%) of substance S 17-acetate 21-phenyl ether (XVI) with m.p. 146–150°. A further purified specimen showed m.p. 149–151°, $[\alpha]D$ +49°, λ_{max} 240 and 276 mμ, log ϵ 4.25 and 3.20, respectively.

Anal. Calcd. for $C_{29}H_{36}O_5$: C, 74.97; H, 7.81. Found: C, 74.73; H, 7.62.

Attempts to remove the phenyl ether function under a variety of conditions, both acidic and alkaline, either left this group intact or resulted in decomposition of the side chain.

MEXICO D. F., MEXICO

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

Anthochlor Pigments. XI. The Constituents of *Coreopsis maritima*. Reinvestigation of *Coreopsis gigantea*

BY T. A. GEISSMAN, J. B. HARBORNE AND MARGARET K. SEIKEL

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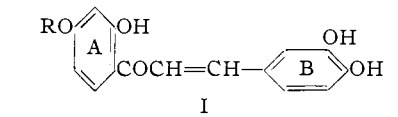
By means of paper chromatography and ultraviolet spectroscopy, the constituents of the flowers of *Coreopsis maritima* have been identified as butein, coreopsin, marein, maritimein, luteolin-7-glucoside and sulfurein. Reinvestigation of *C. gigantea*, a plant morphologically similar to *C. maritima*, has shown the presence in the flowers of all of the same pigments, except that the luteolin glucoside appears to be absent. The biogenetic significance of the pigmentation of these two species of the genus *Coreopsis* is discussed.

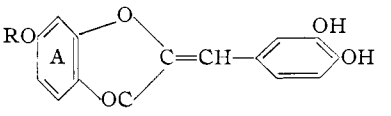
The anthochlor pigments of species of the genus *Coreopsis* have been the subject of investigations in these laboratories[1] and elsewhere.[2] In this earlier work, the pigments, mainly chalcone and aurone glycosides, were isolated by classical methods and no attempt was made to identify all the pigments occurring in a single species. Recently, flowers of *Coreopsis maritima*, a hitherto uninvestigated species, became available, and an examination of all the major constituents was undertaken with the use of a combination of paper chromatography and ultraviolet spectroscopy. As a part of an investigation of flavonoid biogenesis, the main object was to study all of the pigments present in one plant in order to gain more detailed information on their interrelationships.

A fresh, concentrated extract of *C. maritima* flowers was separated by chromatography on thick paper into five main bands. These bands were then eluted separately with aqueous ethanol, and purified by rechromatography. The spectral characteristics and R_f values of the purified eluates were then studied. Comparison of these spectral results, combined with the R_f values in several different solvent systems, with measurements on known compounds served to identify the majority of the constituents. The compounds identified were butein (I, R = H), chlorogenic acid (II), coreopsin [I, R = Gl (glucosyl)], sulfurein (III, R = Gl), luteolin 7-glucoside (IV), marein (V, R = Gl) and maritimein (VI, R = Gl).

Butein (I, R = H) and its 4′-glucoside, coreopsin, have been isolated from several other *Coreopsis* species[1,2] and together constitute perhaps the most commonly occurring of all anthochlor pigments. The corresponding aurone, sulfurein (III, R = Gl) previously has been found in *Cosmos sulfureus*[2,3] and as the aglucone, sulfuretin, in yellow Dahlia.[4] The presence of chlorogenic acid (II) has not previously been detected in the genus *Coreopsis*, but it occurs in all members of this species that have



I



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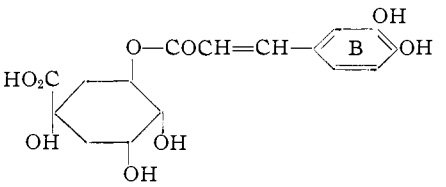
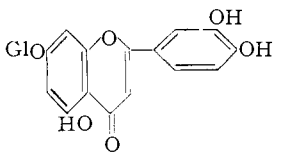
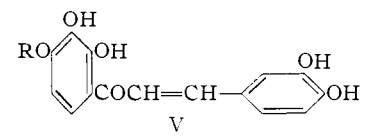


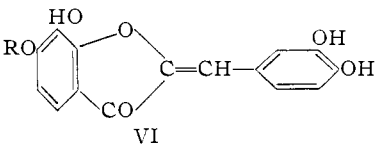
II



IV



V



VI

(1) (a) T. A. Geissman, THIS JOURNAL, **63**, 656 (1941); (b) **63**, 2689 (1941); (c) T. A. Geissman and C. D. Heaton, *ibid.*, **65**, 677 (1943); **66**, 486 (1944); (d) M. K. Seikel and T. A. Geissman, *ibid.*, **72**, 5720 (1950).

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

(3) T. A. Geissman and L. Jurd, *ibid.*, **76**, 4475 (1954).

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

been examined, and its presence has biogenetic significance as will be mentioned later. Luteolin 7-glucoside (IV) occurs in *Sophora angustifolia*,[5a] *Humulus japonicus*[5b] and probably in several other plants. Luteolin itself has been reported in *Cosmos sulfureus*,[6] a related composite, but not previously in the genus *Coreopsis*. The two new anthochlor glucosides, marein and maritimein, were present in minor amounts, and the determination of their structures as (V, R = Gl) and (VI, R = Gl), respectively, will be the subject of the following paper.[7]

Coreopsis gigantea, which has earlier been shown to contain butein[1b] and coreopsin,[6] was reinvestigated by the newer methods. In morphology, taxonomy and culture, it is very closely related to *C. maritima*. The new, more detailed study of its flower pigments showed that it contained the same anthochlors as are present in *C. maritima*. In addition to butein and coreopsin and the corresponding aurone pigments, *C. gigantea* contains the newly discovered marein and maritimein. The corresponding aglucones, okanin (V, R = H) and maritimetin[7] (VI, R = H) were also present. Although no flavone pigments have been studied in detail in this species, two-dimensional chromatography failed to reveal a spot identical with the luteolin 7-glucoside found in *C. maritima*. There was a fairly close correspondence otherwise between the spots obtained on two-dimensional chromatograms of extracts of these two species.

Extracts of freshly picked flowers of both plants show distinctly the presence of both glucosides and aglucones of the chalcones and aurones and it is considered unlikely that any of these pigments are artifacts, formed during the extraction process. On the other hand, the concentration of aurones and the presence of flavanones, barely visible in fresh extracts, increases at the expense of the chalcones present in extracts kept for a period of a few weeks. It is clear that the chalcone anthochlors, on keeping, slowly isomerize to the related flavanone and oxidize to the corresponding aurone. For example, the butein spot of "aged" extracts may be mostly sulfuretin, but no attempt was made to confirm this due to the difficulty of separation (see ref. 4). It has been ascertained, however, that the chalcone marein slowly oxidized on keeping to the related aurone, maritimein.[7]

The presence of these pigments in *C. maritima* and *C. gigantea* suggests several interesting biogenetic conclusions. (a) The presence of coreopsin with sulfurein, and marein with maritimein, confirms the interrelationship of chalcones and aurones, discussed by Hattori and Shimokoriyama.[2] (b) The fact that all of the compounds present in these species, and also in the other species investigated earlier, have the same hydroxylation pattern in the B ring (*cf.* formulas I, II and III) and that the related cinnamic acid is also present,[8] suggests that there is a genetic factor present in the genus *Coreopsis* responsible for 3′,4′-dihydroxylation. (c) Glycosidation is not necessarily a haphazard process in any one particular plant, since all of the compounds isolated here have the sugar attached in the same position, *i.e.*, in the A ring *para* to the carbonyl group. (d) The flavone present in *C. maritima*, namely, luteolin, has a different hydroxylation pattern in the A ring from the chalcones and aurones. This observation suggests that in flavonoid biosynthesis, the pathway to flavones and the pathway to chalcones and aurones diverge at a fairly early stage. If the pathways were identical, one would expect luteolin to be accompanied by the related aurone, aureusidin, and that the flavones related to sulfuretin and maritimetin would also be present in *C. maritima*. The methods used here are reliable enough to detect these compounds, if they occurred in appreciable quantities. Furthermore it is significant that flavones with hydroxyl groups in only the 7- and 8-positions in the A ring are unknown in nature. Similar conclusions about the biogenesis of aurones have been derived from a study of the pigments of *Antirrhinum majus*.[9]

Experimental

General Remarks.—Solutions of chalcones and aurones obtained by elution from paper are particularly unstable (because of oxidation, isomerization and decomposition) so the manipulations necessary in their study were carried out as quickly as possible. During their elution, it was common practice to introduce a trace of hydrochloric acid into the eluting chamber to counteract any basic vapors retained on the paper strips.

The R_f values were determined on non-equilibrated papers and in non-equilibrated tanks, so that they varied slightly with the experiment, the investigator, and the concentration of the spot. Therefore, wherever possible, cochromatography with authentic material was carried out. The solvents used for development are abbreviated as follows: BAW for *n*-butanol (reagent grade) and 27% (v./v.) aqueous acetic acid (1:1); CAW for *m*-cresol, acetic acid, water (50:2:48) (v./v.); BW for *n*-butanol saturated with water; 30% HOAc for 30% (v./v.) aqueous acetic acid (similarly for other concentrations). The colors of the spots and bands are abbreviated as follows: B, brown; Bl, blue; Bk, black; C, colorless; G, green; Gr, gray; M, mauve; P, purple; Pk, pink; R, red; O, orange; W, white; Y, yellow. The tones of the colors are: b, bright; d, dark; l, light; p, pale.

In preparation for spectral measurements, the final purified eluates were half-banded on sheets of Whatman No. 1 or No. 3 paper and after development, the band and a blank cut from the other half of the paper were cut out and eluted separately with 95 or 70% ethanol. In determining the shifts in the absorption spectra, three drops of either a 5% ethanolic solution of aluminum chloride or of 1 *N* ethanolic sodium ethoxide were added to the cell solution (*ca.* 3 cc.). The main maximum of the alkaline shift was read immediately after addition of the base, since many of these compounds decompose under basic conditions.

Coreopsis maritima

Materials.—Flowers of *C. maritima* were collected along the coast road in northern Baja California, Mexico, in the spring of 1954. The fresh flower heads (*ca.* one kilogram) were extracted in a Waring blendor with one liter of ethanol. After centrifuging and filtering this solution, the residue was reëxtracted with a further liter of ethanol. The filtrates were combined and evaporated carefully under reduced pressure to remove the ethanol. The residual aqueous concentrate was filtered through Celite to remove cellular material, washed with benzene and then light petroleum, and finally exhaustively extracted with ethyl acetate (8 times). This dried extract was concentrated to 150 cc. and used as

(5) (a) S. Hattori and M. Matsuda, THIS JOURNAL, **76**, 5792 (1954); (b) *Acta Phytochim.* (*Japan*), **15**, 133 (1949).

(6) T. A. Geissman, THIS JOURNAL, **64**, 1704 (1942).

(7) Paper XII of this series, T. A. Geissman and J. B. Harborne, *ibid.*, **78**, 829 (1956).

(8) T. A. Geissman and J. B. Harborne, *Arch. Biochem. Biophys.*, **55**, 447 (1955).

(9) E. C. Jorgensen and T. A. Geissman, *ibid.*, **55**, 389 (1955).

the stock solution. On chromatography, it showed the same spots as a fresh petal extract, except that in the latter, the butein spot was very faint. An extract of the stem and leaves of this plant showed the presence of chlorogenic acid and the absence of any of the anthochlor pigments.

Chromatographic Separation.—The stock solution was banded on thick (Whatman No. 3) paper and on chromatography in BAW, separated into six distinct bands. The bands were then cut off separately, eluted with 70% ethanol, concentrated and purified by a process of rechromatography in BAW, 30% HOAc and other solvent systems as mentioned below. The main results are tabulated in the Tables I and II. Comments on the individual bands follow. The R_f's refer to the primary separation in BAW.

TABLE I

ULTRAVIOLET SPECTRA OF THE CONSTITUENTS OF *C. maritima*

Compound	95% EtOH	λmax[a] EtOH/NaOH	EtOH/AlCl₃
Band 0	277.5	275, 334	.....
Band 1	265, 382	448	448
Butein	262, 382	447	445
Band 2[b]	232, 300,[d] 323	264, 383	343
Chlorogenic acid[b]	236, 300,[d] 324	263, 384	344
Band 3	270, 384	448	424
Coreopsin[c]	263, 398, 385	447	428
Band 4.1	257, 277, 340,[d] 403	*ca.* 515	No shift
Sulfurein[c]	257, 277, 340,[d] 404	*ca.* 515	No shift
Band 4.2	257, 353	405	360, 390
Luteolin	256, 353	407	360, 388.5
Band 5.1 (maritimein)	242, 274, 330, 419	505	No shift
Band 5.2 (marein)	268, 382	448	422

[a] The position of the minima and the relative intensities of maxima and minima of the spectra of unknown and authentic pigments were also in close agreement with each other. [b] Measured in aqueous solutions. [c] Sample kindly supplied by Professor S. Hattori. [d] Inflection.

TABLE II

R_f VALUES OF THE CONSTITUENTS OF *C. maritima*

Substance	R_f values[a] BAW	CAW	BW	30% HOAc	50% HOAc	UV	Colors UV + NH₃
Band 0	0.95	..	0.92	..	0.87	C	Bl
Band 1	.83	..	..	..	..	Y	O
Butein	.83	..	..	..	..	Y	O
Band 2	.65	0.10	..	0.72	0.73	Bl	G
Clorogenic acid	.66	.11	..	.72	.73	Bl	G
Band 3	.56	.18	.11	..	..	B	OR
Coreopsin	.56	.18	.11	..	..	B	OR
Band 4.1	.49	.41	.10	.28	.49	Y	R
Sulfurein	.49	.41	.10	.27	.49	Y	R
Aglucone of 4.1	.80	..	.65	.14	.35	Y	O
Sulfuretin	.80	..	.65	.14	.35	Y	O
Band 4.2[b]	.49	..	.15	.25	.47	P	dG
"Luteolin 7-glucoside"[c]	.55	..	.21	.32	.56	P	YG
Aglucone of 4.2	.85	.47	.65	..	..	B	YG
Luteolin	.85	.48	.66	..	..	B	YG
Band 5.1 (maritimein)	.52	..	..	.28	.37	Y	R
Band 5.2 (marein)	.45	..	..	.22	.33	Bk	B

[a] Whatman No. 1. [b] S. Hattori and H. Matsuda, ref. 6a, gives R_f 0.47 in BAW (4:1:1) for luteolin 7-glucoside from *Sophora angustifolia*. [c] Impure material, isolated from partially hydrolyzed "crude apiin." R_f values agree with those of Nordström and Swain, ref. 11.

Band O (R_f 0.90) appeared only after the stock solution had been kept for several weeks. From its spectrum, it appeared to be one or more flavanones, formed by isomerization of the chalcones. It was not further studied.

Band 1 (R_f 0.83) was identified as butein.

Band 2 (R_f 0.69) after rechromatography in 30% HOAc and then water was identified as chlorogenic acid.

Band 3 (R_f 0.63) was identified as coreopsin.

Band 4 (R_f 0.56) was found by examining its spectrum to be a mixture of two substances. The two components were eventually separated by rechromatography in 5% HOAc for 24 hours, allowing the solvent to flow off the end of the descending chromatogram. The faster moving component (band 4.1) was identified as sulfurein. On hydrolysis with dilute aqueous alcoholic mineral acid, it gave sulfuretin. The slower moving component (band 4.2) was identified as luteolin 7-glucoside. On acid hydrolysis it gave luteolin and a sugar undistinguishable chromatographically from glucose in two different solvent systems.[10] For comparison, luteolin 7-glucoside was also isolated from partially hydrolyzed "crude apiin," as described by Nordström and Swain.[11] This material differed slightly in R_f values from our material (see Table II). Furthermore, its spectra in neutral, alkaline and ethanolic aluminum chloride solutions indicated the presence of some impurity, which could not be removed. As a final confirmation, band 4.2 was subjected to complete methylation, followed by acid hydrolysis. The product had exactly the spectral characteristics and color properties of 7-hydroxy-5,3',4'-trimethoxyflavone,[11] proving that the sugar was attached in the 7-position.

Band 5 (R_5 0.47) was separated into two components, by rechromatography in BAW and then 30% HOAc. The proof of the structure of band 5.1 (maritimein) and band 5.2 (marein) is given in the following paper.[7]

Coreopsis gigantea

Materials.—*Coreopsis gigantea* was collected on the bluffs of the Pacific Ocean near Malibu, California. For isolation of the chalcones the petals were suspended in methanol and this fresh methanolic extract was used for banding without further treatment. In another collection the whole flower heads were extracted in a Waring Blendor with 95% ethanol and the residue, after filtration, was washed with hot ethanol. These solutions were concentrated under reduced pressure to remove the ethanol. Two-dimensional chromatography showed that this solution contained the glycosides and aglycones, which were separated to a considerable extent by the following extractions. The aqueous residue was saturated with ammonium sulfate and extracted with ether. The ether extract was purified by extraction with many small volumes of water. This dark brown water solution was resaturated with the salt and the extractions repeated. The complete cycle was done four times and then all the yellow ether solutions were combined (solution A). The brown and yellow aqueous solutions were also combined. The glycosides were extracted from the latter with propanol, the propanol evaporated to dryness, the residue taken up in water and purified by extraction with ethyl acetate. This residual aqueous solution is solution B. A solution of the aglycones was prepared by hydrolyzing solution B with a 1:1 mixture of methanol and 2 *N* hydrochloric acid for 11 hours on the steam-bath. The methanol was boiled off, the aqueous solution extracted with ether and the ether washed with saturated ammonium sulfate. The brown oil remaining when the ether was evaporated was dissolved in methanol and traces of ammonium sulfate were removed by filtration. This methanolic solution is solution C.

Two-dimensional Chromatography.—In order to catalog all of the pigments present in *C. gigantea* and to determine how best to isolate them chromatographically, two-dimensional chromatography was applied to all of the solutions described above. BAW was used as the first solvent and 30% HOAc was found to be a suitable second solvent.[12] In the following discussion, figures in parentheses refer to the R_f values of spots in these two solvents, respectively. The papers (Whatman No. 1) were washed for two or more days in the chromatography tank with BAW in order to remove impurities, which prevent good definition of spots with R_f values greater than 0.80/0.50. The solutions were applied in amounts large enough to reveal substances present in traces without causing streaking of the main components. The spots were studied in visible and ultraviolet light both with and without the presence of ammonia vapor and after spraying with 1% sodium carbonate.

A total of forty-five spots were clearly discernible, but even more were present in minute, hardly distinguishable traces. The spots could be classified by their color reac-

(10) *Cf.* E. and M. Lederer, "Chromatography," Elsevier Publishing Co., New York, N. Y., 1953, p. 158; and many other manuals.

(11) C. G. Nordström and T. Swain, *Chemistry and Industry*, 85 (1953); *J. Chem. Soc.*, 2764 (1953).

(12) Phenolic solvents were avoided in all this work since even traces of phenols interfere with spectra.

TABLE III

CHROMATOGRAPHY OF *C. gigantea*

Spot No.	R_f[a] BAW / 30% HOAc	Colors[b]	Na_2CO_3 colors[b]	Rel. amount present in: Fresh	Sol. A (ether)	B (aq.)	C (hyd.)	Type of compd.	Identity	R_f[a] of purified eluate: BAW	30% HOAc
2	0.45/0.20	Y-PBk 10-Bk (PB)	pO-B	++++	−−	++	−−	Chalcone glycoside	Marein[c]	0.38	0.22[d]
5a	.45/ .47	C-pB pY-bOPk	pY-pKkO	+	−−	++	−−	Chalcone glycoside	....		
6	.47/ .53	pY-pdB pO-10R	pOY-1Pk	−−	−−	++	−−	Aurone glycoside	....		
7	.52/ .27	1Y-GY Pk-B	1PPk-P	++?	+	+++	−−	Aurone glycoside	Maritimein[c]	.58	.29
8a	.59/ .05	pY-Bk Y-P	1PB-pBk	+	−−	−−	++	Chalcone aglycone	Okanin	.56	.08
8	.65/ .12	1Y-GY OPk-PkO	10Pk-PR	+	+++	−−	++	Aurone aglycone	Maritimetin	.57	.11
9	.60/ .41	Y-bY pPk-RO	dPPk-bOR	++	+++	++++	−−	Aurone glycoside	Sulfurein[e]	.59	.41
10	.65/ .42	1Y-B PkO-OBR	OPk-RO	++++	++	+	−−	Chalcone glycoside	Coreopsin[e]	.62	.43[f]
17	.76/ .52	C-PB 1Y-1O	1Y-1YO	++	+	−−	−−	Chalcone aglycone	....		
19a	.86/ .22	Y-YB OY-RO	BY-bY	++	?	−−	?	Chalcone aglycone	Butein		
19	.86/ .26	bY-bGY O-bOY	OY-bO	+	++++	−−	+++	Aurone aglycone	Sulfuretin		

[a] The leading edges of the spots were measured. [b] First line, color in visible light, in ultraviolet light; second line, color when fumed with NH_3 in visible light, in ultraviolet light. [c] Cochromatographed with authentic solutions from *C. maritima*. [d] In 50% HOAc, 0.41. [e] Cochromatographed with authentic material. [f] In 50% HOAc, 0.60.

tions. The anthochlor spots, chalcones and aurones, which are listed in Table III were the most highly colored, changing from yellow in visible light to red, brown and orange shades in the ultraviolet or with base. The twelve flavanone spots were not discernible until the sheets were sprayed with sodium carbonate and allowed to stand for hours or days; the vivid basic colors of the corresponding chalcones slowly developed. Six other spots were classed as flavonoid because of their light yellow or orange-yellow colors; some of these may be flavones, others may be anthochlors in low concentrations. Sixteen spots were non-pigment in nature, appearing as various shades of blue or green in the ultraviolet light either with or without ammonia. Three spots at least seemed to be degradation products since they did not appear in the fresh extracts. They looked very similar, were destroyed by carbonate, and were difficult to elute from paper; one showed a strong absorption band (λ_{max} 271 mμ). Glycosides and aglycones could be distinguished by their relative R_f values (the former moving slower in BAW and faster in acetic acid solutions) as well as by their presence before and after hydrolysis.

An extract of fresh flowers, chromatographed at once, showed twenty-one spots, the most prominent by far being those of the two chalcone glucosides, coreopsin and marein. However, small amounts of the corresponding aglucones (butein and okanin) and of the corresponding aurone glucosides and aglucones (sulfurein, maritimein, sulfuretin and maritimetin) were present. Flavanone glycosides but not aglycones also appeared. Aqueous solution B apparently contained only glycosides (and/or esters) since on hydrolysis all the original spots (except some chlorogenic acid and a second blue spot) disappeared and a new set appeared. The ether extract, solution A, contained aglycones in higher concentration than glycosides, although many of the latter were present.

Six of the antichlor pigments were isolated and identified as described below. Spots 19 and 19a, generally appearing as one spot, were discernible as two in the fresh extract, where 19a is present in the higher concentration. They are considered to be sulfuretin and butein, since the R_f values of authentic samples were 0.85 and 0.84, respectively, in BAW and 0.25 and 0.22 in 30% HOAc and since butein has already been shown to be present in this flower.[1b]

Other spots provisionally identified from their R_f values, color reactions and general behavior are as follows: butin (0.89/0.61) (authentic, 0.91, BAW, 0.62, 30% HOAc), a butin glycoside (0.71/0.79), chlorogenic acid (0.78/0.81) (authentic, 0.79/0.77) and caffeic acid (three spots,[7] 0.83/0.56, 0.88/0.67, 0.86/0.71) (authentic, 0.89/0.58, 0.89/0.67, 0.89/0.76). No spot was differentiated which was identical with the luteolin 7-glucoside found in *C. maritima*. It may have been concealed by the large sulfurein spot. However, two-dimensional chromatograms of *C. maritima* showed it clearly (0.63/0.28) while all of the other spots were identical with those obtained from *C. gigantea*.

Chromatographic Separation.—Appropriate solutions, preferably containing large amounts of the spot under investigation, were striped on sheets of Whatman No. 3 paper and developed with solvents chosen with reference to the two-dimensional chromatograms. The crude bands so obtained were cut out, the material eluted and purification effected by rechromatography in suitable solvent systems. Elutions were carried out with methanol, over periods of 12–24 hours. The purification of the individual compo-

TABLE IV

ABSORPTION SPECTRA OF PURIFIED SPOTS FROM *C. gigantea*[a]

Spot no.	Identity	Main band[b] λ_{max}, mμ	Subsidiary bands[b] λ_{max}, mμ
2	Marein	384	267, 330 infl.
	+$AlCl_3$	422	274, 327
	+NaOEt	467	
7	Maritimein	418	276, 325
	+$AlCl_3$	Spectrum unchanged[c]	
	+NaOEt	506	
8	Maritimetin	413	256, (267), 360 infl.
	+$AlCl_3$	Spectrum unchanged[c]	
	+NaOEt	497	
8a	Okanin	381	*ca.* 255, 350 infl.
	+$AlCl_3$	420	249, 271
	+NaOEt	*ca.* 446	
9	Sulfurein	404	276, 340, infl.
	+$AlCl_3$	Spectrum unchanged[c]	
	+NaOEt	510	
10	Coreopsin	385	245, 265, 305 infl.
	+$AlCl_3$	435	243, 274, 318
	+NaOEt	*ca.* 450	

[a] Determined in 95% alcohol with a blank from the paper as reference solution. [b] See Table I, footnote *a*. [c] Because of impurities from the paper or the bands, the curves after addition of aluminum chloride were not always superimposable on the original, but the essentials were unchanged; in fact the maxima were often sharpened.

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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Anthochlor Pigments. XII. Maritimein and Marein

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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).