VII was cyclized in 76% yield with polyphosphoric acid to 4,5-phenanthrylene ketone (X).

Experimental[11]

1,2-Dihydrophenanthrene-4-carboxylic Acid (IV).—Diethyl oxalate (219 g., 1.5 moles) was added to an anhydrous ether (1 liter) slurry of anhydrous powdered potassium ethoxide prepared from 43 g. (1.1 moles) of potassium in absolute ethanol.[12] The solution was cooled to 15° and 242 g. (1 mole) of I dissolved in 300 ml. of anhydrous ether was added under a nitrogen atmosphere. After 24 hours at room temperature the reaction mixture was poured on a mixture of ice and concentrated hydrochloric acid (130 ml.). The ether layer was washed three times with water, dried with sodium sulfate and the ether removed *in vacuo*. The oxalic ester condensation product and excess diethyl oxalate remained as a reddish oil.

After the crude oil was stirred vigorously with 3.5 liters of 20% sulfuric acid at reflux temperature for 96 hours, the aqueous portion was decanted and the remaining semi-crystalline mass dissolved in concentrated potassium carbonate solution. This basic solution was extracted with ether twice and poured slowly with stirring on a mixture of ice and excess hydrochloric acid. The crude acid was filtered and dried. Recrystallization from acetone–Skellysolve F (petroleum ether, b.p. 30–65°) yielded 165 g. (75%) of IV, m.p. 227–233°. A second recrystallization produced colorless needles, m.p. 234.0–234.5°.

Anal. Calcd. for $C_{18}H_{12}O_2$: C, 80.3; H, 5.4; neut. equiv., 224. Found: C, 80.2; H, 5.2; neut. equiv., 227.

Methyl 1,2-Dihydrophenanthrene-4-carboxylate (V).—IV was converted to V in 87% yield by boiling 36 hours with methanolic hydrogen chloride. V crystallized from Skellysolve F in colorless needles, m.p. 70.5–71.5°.

Anal. Calcd. for $C_{16}H_{14}O_2$: C, 80.7; H, 5.9. Found: C, 80.7; H, 5.9.

Methyl 4-Phenanthrenecarboxylate (VI).—V was dehydrogenated by heating with an equivalent of sulfur at 250–260°. The dehydrogenation runs were carried out in 70-g. lots. When hydrogen sulfide evolution slowed measurably (20–30 min.), a small amount of powdered zinc was added and the ester was distilled *in vacuo*. The addition of 50 ml. of cold ethanol to the distillate promoted instantaneous crystallization of VI. The yields ranged from 65–72% of VI, m.p. 78–82°. One recrystallization from methanol yielded colorless plates, m.p. 84.8–85.5°.

Anal. Calcd. for $C_{16}H_{12}O_2$: C, 81.3; H, 5.1. Found: C, 81.3; H, 4.9.

4-Phenanthrenecarboxylic Acid (VII).—Saponification of VI with 10% sodium hydroxide yielded VII quantitatively. Recrystallization of VII from chloroform–Skellysolve F produced colorless plates, m.p. 173.5–174.5°. Melting points of 171.5–173°,[5b] 170–171°[4] and 169–171°[6] have been reported.

4-Phenanthryl Isocyanate (VIII).—VII (5 g., 22.5 mmoles) was dissolved in 100 ml. of a solution which contained equal volumes of trifluoroacetic acid and trifluoroacetic anhydride. The flask was stoppered with a cotton plug, cooled to 0–5° and excess sodium azide was added portionwise with stirring. The reaction proceeded rapidly. Within 5 minutes gas evolution ceased and the isocyanate VIII crystallized. The reaction mixture was allowed to stand at 0–5° with swirling for an additional 5 minutes and then poured on ice. A yield of 4.7 g. (95.5%) of VIII, m.p. 66–68°, was obtained. Recrystallization from Skellysolve F yielded colorless needles, m.p. 68.6–69.0° (strong absorption at 4.55 μ).[9] The reaction did not proceed either in pure trifluoroacetic acid or trifluoroacetic anhydride alone. VIII reacts slowly with moisture and care was needed to obtain the analytical sample.

Anal. Calcd. for $C_{15}H_9ON$: C, 82.1; H, 4.1; N, 6.3. Found: C, 82.0; H, 4.1; N, 6.4.

4-Aminophenanthrene (IX).—A solution of 4.7 g. (21.0 mmoles) of VIII, 3 g. of potassium hydroxide and 130 ml. of 70% ethanol was refluxed for 5 hours. The reaction mixture was poured on ice whereupon IX precipitated. Recrystallization from Skellysolve F afforded 3.5 g. (84%) of IX, m.p. 65–66°. Melting points of 55°[9] and 62.5–63.5°[13] have been reported. IX was prepared independently from the oxime of 4-keto-1,2,3,4-tetrahydrophenanthrene[9] (XI). The melting point coincided with that obtained from the hydrolysis of VIII. The melting point of a mixture was not depressed. The infrared curves were identical.

4,5-Phenanthrylene Ketone (X).—VII (1 g., 4.5 mmoles) was stirred for 60 hours at 100–105° with approximately 30 ml. of polyphosphoric acid.[14] The reaction mixture was then poured on a mixture of ice and potassium carbonate. The precipitate was filtered and dried. Recrystallization from acetone which contained a small amount of chloroform afforded 0.7 g. (76%) of X, m.p. 170.5–171.5° (Kruber[4] reported 170°).

(11) All melting points are corrected unless otherwise noted. Analyses by Galbraith Laboratories, Knoxville, Tenn.

(12) K. G. Rutherford and C. L. Stevens, THIS JOURNAL, **77**, 3279 (1955).

(13) J. W. Krueger and E. Mosettig, *J. Org. Chem.*, **3**, 345 (1938).

(14) We wish to thank the Victor Chemical Works, Chicago, Ill., for a generous sample of polyphosphoric acid.

COLUMBUS 10, OHIO

[CONTRIBUTION FROM THE BOTANICAL INSTITUTE, FACULTY OF SCIENCE, UNIVERSITY OF TOKYO]

Anthochlor Pigments of *Coreopsis tinctoria*

BY MASAMI SHIMOKORIYAMA

RECEIVED JUNE 20, 1956

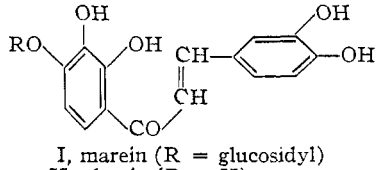
From the ray flowers of *Coreopsis tinctoria*, crystals of two anthochlor pigments have been isolated and identified as 4′-glucosidoxy-2′,3′,3,4-tetrahydroxychalcone (marein) (I) and 6-glucosidoxy-7,3′,4′-trihydroxyaurone (maritimein) (V). Marein tends to isomerize into a flavanone glycoside, flavanomarein (III). The latter has also been obtained in crystalline form. Distribution of these three glucosides and other substances in various parts of flowers was studied.

The anthochlor pigments in the composite plants of the sub-tribe *Coreopsidinae*, to which the genera *Coreopsis*, *Cosmos*, *Dahlia*, *Bidens*, etc., belong, have been the subject of many investigations. *Coreopsis tinctoria*, a very commonly cultivated annual or biennial *Coreopsis* in Japan, usually has yellow rays with a red-brown base. A garden variety is often found, in which the red base is spread all over the right surface of the ray flower. The epidermis of the reverse side as well as the mesophyll which lies just under the red part are generally tinged with pale yellow and clearly give a red anthochlor reaction with alkalies. The red part of the upper epidermis contains an anthocyanin which has been identified by Hayashi and Abe[1] as chrysanthemin (cyanidin-3-glucoside).

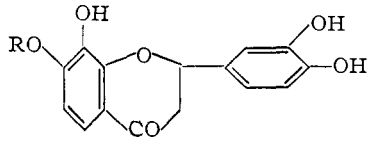
From the ray flowers two anthochlor glycosides have been isolated which are regarded as major

(1) K. Hayashi and Y. Abe, Miscellaneous Reports of the Institute for Natural Resources, No. 29, 1 (1953).

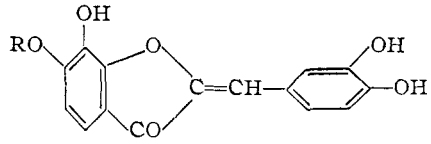
constituents in the yellow colored part. The presence of these two orange-yellow pigments has been demonstrated readily by means of paper chromatography prior to isolation. One of them has the properties of a chalcone and the other has those of a benzalcoumaranone. The former gave a brown and the latter a golden-yellow spot on a paper chromatogram when seen under ultraviolet light. The chalconase-test[2] was also effective in this case, showing that these two glycosides are of corresponding structure. Not only the chalcone but also the aurone glycoside have oxygen-sensitive polyhydroxyl groupings in the molecule which are very liable to oxidation by polyphenoloxidase plus oxygen even in the presence of potassium cyanide in a high concentration (0.05 *M*).



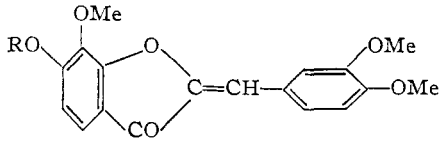
I, marein (R = glucosidyl)
II, okanin (R = H)



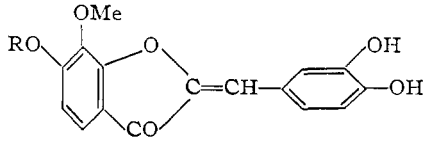
III, flavanomarein (R = glucosidyl)
IV, probably isoökanin (R = H)



V, maritimein (R = glucosidyl)



VI, heptamethylmaritimein (R = tetramethylglucosidyl)



VII, leptosin (R = glucosidyl)
VIII, leptosidin (R = H)

Though it has neither the phloroglucinol nor the 2′,6′-dihydroxyl-type structure, as will be shown below, the chalcone glycoside has a great tendency to isomerize during the course of its isolation, into the colorless flavanone glycoside, for which the author proposes the name flavanomarein (III). The flavanone glycoside (III) showed a bluish-purple coloration with magnesium and concd. hydrochloric acid, a reaction well known for flavanones, and a green color with ferric chloride, suggesting that no hydroxyl group is present in the position *ortho* to the carbonyl. Actually it was impossible to obtain from it any partial acetate. As far as the author is aware, this is the first example of the chalcone–flavanone isomerization in which the equilibrium is shifted quite in favor of the flavanone without any effect of stabilization due to a hydrogen-bonding between the 5-hydroxyl group[3] and the carbonyl.

The chalcone glycoside gave an acetate by the usual method under mild conditions. It gave no coloration with ferric chloride, but there resulted immediately a vivid red color when a few drops of 1 *N* sodium hydroxide were added to an alcoholic solution. These properties suggest that the acetate here obtained may be regarded as the octaacetate of the chalcone itself. Spectrophotometric as well as analytical data proved, however, that it possessed the flavanone structure.

The fact that the chalcone glycoside also showed a great tendency to convert to its flavanone form under the acetylating process is reminiscent of the real structure of the most closely related chalcone glycoside, lanceolin,[4] which was isolated in its acetate form. Spectrophotometric reinvestigation of lanceolin acetate revealed that it has the flavanone form, too. Only when acetylated under drastic conditions is it possible to get an octaacetate of marein (I).[6]

The flavanone glycoside gave on hydrolysis with mineral acids one mole of glucose and a flavanone accompanied by a considerable amount of a chalcone ($^1/_4$), as a result of re-opening of the oxygen ring. On the other hand, hydrolysis of the chalcone glycoside gave in addition to the chalcone the corresponding flavanone to the same or sometimes to a greater extent. The aglycone in the chalcone form is most probably identical with the okanin (2′,3′,4′,3,4-pentahydroxychalcone) (II) isolated by King and King[5] from the tropical hardwood, *Cyclicodiscus gabunensis*. The flavanone is probably identical with 7,8,3′,4′-tetrahydroxyflavanone, which corresponds to it. The flavanone, "marein flavanone" of Harborne and Geissman,[6] gave a characteristic blue fluorescence under ultraviolet light in the presence of ammonia vapor as described by them, and its absorption spectrum is that of a characteristic flavanone. It melted at 130–135°, gave an acetate which melts at 120–122°, much resembling isoökanin (m.p. 140°, its acetate 113°) described as the *cis* form of okanin by King and King.[5] When the flavanone prepared by us was tested with ferric chloride, it gave a pure green coloration without the slightest tint of brown. We feel that isoökanin may not be a *cis* form of the chalcone but an isomeric flavanone. Prof. Hattori kindly wrote a letter to Prof. F. E. King suggesting that isoökanin should be regarded as a flavanone. Prof. King replied that he also had come to the same conclusion and is now preparing an article on this subject.

Geissman and co-workers[6] identified the coloring

(2) M. Shimokoriyama and S. Hattori, THIS JOURNAL, **75**, 2277 (1953).

(3) N. Narasimhachari and T. R. Seshadri, *Proc. Ind. Acad. Sci.*, **27A**, 223 (1948); *C. A.*, **44**, 1493d (1950).

(4) M. Shimokoriyama and S. Hattori, THIS JOURNAL, **5**, 1900 (1953).

(5) F. E. King and T. J. King, *J. Chem. Soc.*, 569 (1951).

(6) T. A. Geissman, J. B. Harborne and M. K. Seikel, THIS JOURNAL, **78**, 825 (1956); J. B. Harborne and T. A. Geissman, *ibid.*, **78**, 829 (1956).

TABLE I

DATA ON ABSORPTION SPECTRA ([a] Indicates Inflection)

		λmax and λmin (log ε) in 98% EtOH			
Marein	Max.	266(3.91)	320[a](4.09)	383(4.47)	
	Min.	283(3.75)			
Marein octaacetate	Max.	226(4.19)	313(4.42)		
	Min.	254(3.68)			
Okanin	Max.	260(3.89)	330[a](4.11)	384(4.49)	
	Min.	284(3.50)			
Okanin tetraacetate	Max.	315[a](4.18)	347(4.40)		
	Min.	260(3.67)			
Okanin pentaacetate	Max.	227(4.07)	310(4.33)		
	Min.	250(3.72)			
Flavanomarein	Max.	283(4.23)	321(3.64)		
	Min.	315(3.63)			
Flavanomarein heptaacetate	Max.	269(4.21)	315(3.82)		
	Min.	292(3.80)			
Flavanoökanin	Max.	235(4.15)	291(4.12)		
	Min.	257(3.30)			
Flavanoökanin tetraacetate	Max.	258(4.03)	315(3.57)		
	Min.	284(3.18)			
Maritimein	Max.	245(4.00)	274(3.92)	329(4.10)	418(4.45)
	Min.	264(3.87)	292(3.76)	355(4.04)	
Maritimein heptaacetate	Max.	247(4.00)	329(4.37)	370(4.36)	
	Min.	277(3.83)	351(4.32)		
Heptamethylmaritimein	Max.	256(3.90)	269(3.92)	327(4.06)	406(4.38)
	Min.	266(3.89)	291(3.70)	346(4.02)	
Maritimetin	Max.	270(3.83)	255[a](4.05)	418(4.37)	
	Min.	294(3.44)			
Maritimetin tetraacetate	Max.	246(4.05)	320(4.28)	374(4.37)	
	Min.	275(3.90)	340(4.12)		
Trimethylmaritimetin	Max.	274(4.03)	314(3.92)	409(4.39)	
	Min.	295(3.86)	340(3.81)		
Tetramethylmaritimetin	Max.	256(4.04)	268[a](4.01)	340[a](4.18)	405(4.48)
	Min.	290(3.69)			

constituents of the flowers of *C. maritima* by means of paper chromatography and ultraviolet spectra as butein, coreopsin, sulfurein, marein (4′-glucosidoxy-2′,3′,3,4- tetrahydroxychalcone) (I), maritimein (6-glucosidoxy-7,3′,4′-trihydroxyaurone) (V) and luteolin-7-glucoside. The author has planted some *Coreopsis maritima* raised from the seeds sent to Prof. Hattori from the Bergianska Botanical Garden and independently studied the anthochlor pigments of the flowers. A small quantity of coreopsin was isolated easily from the ray flowers. During the course of its isolation and of later investigation, we became aware of the identity of the tinctoria anthochlor glycosides with the paper chromatographically slower moving glycosides of *C. maritima* and the presence of two further pairs of anthochlor pigments in the ray flowers of *C. maritima*, as was demonstrated easily by the chalconase test.

Thus, the chalcone glycoside of *C. tinctoria* is now to be regarded as completely identical with marein and the flavanone glycoside as having the same hydroxyl pattern.

The benzalcoumaranone glycoside, which was isolated in a small amount and synthesized oxidatively from the chalcone glycoside, marein, was easily proven to be maritimein. Geissman and co-workers have reinvestigated *C. gigantea*, a plant morphologically similar to *C. maritima*, and shown the presence in its flowers of all of the same pigments, except luteolin glucoside. The two species investigated by them contain, thus, two pairs of anthochlor pigments. In the ray flowers of *C. tinctoria*, however, there was not detected any anthochlor pair of coreopsin and sulfurein.

The position of the sugar molecule in maritimein, and hence in marein, was determined by Geissman[6] to be 6 from the fact that complete methylation of maritimein gave a methyl derivative which, on hydrolysis, gave a product identical spectrally and chromatographically with synthesized 6-hydroxy-7,3′,4′-trimethoxyaurone (VIII). When maritimein was methylated, it gave a heptamethyl ether VI. The compound was completely identical with the hexamethyl ether of leptosin (VII). The methyl derivative gave on hydrolysis with mineral acids an aglycone, which was identical with the 6-hydroxy-7,3′,4′-trimethoxyaurone described by Geissman.

The absorption maxima and minima of the compounds measured in 98% ethanol are presented in Table I. As was shown by Geissman and co-workers,[7] absorption spectra of the acetates, either of the aglycones or the glycosides, of anthochlor pigments have been found readily to differentiate the two types. According to them, Band I, the longer wave length band of the acetyl derivatives of polyhydroxychalcones and of their glycosides, has

(7) M. K. Seikel and T. A. Geissman, THIS JOURNAL, **72**, 5720, 5725 (1950).

TABLE II

++++ means 10–15 mg./g. fresh weight, +++ 5–10 mg./g., ++ 1–5 mg./g., + below 1 mg./g., ± trace.

Rf value	An unknown pigment 0.18	Marein 0.34	Maritimein 0.38	Chrysanthemin 0.40	Flavanomarein 0.48	Okanin 0.56	Maritimetin 0.62	Chlorogenic acid 0.68	Caffeic acid 0.87
Ray flower									
Upper part of corolla (yell.)		++++	+		+	+	±		
Lower part of corolla (red)		++	+	+	+	+	±		
Ovary (colorless)								++	++
Bract (pale brown)	++	+++	+	±	+	+			+
Tube flower									
Stigma (yellow)		+++	+						
Style (pale yell.)		+							
Ovary (colorless)								++	++
Anther (brown)		+							
Pollen (yellow)									
Filament (colorless)									
Upper part of corolla (or.-red)		+		+					
Lower part of corolla (pale yell.)		+	+		+				
Bract (pale red)		+		+					
Involucre (pale green)	+	+						±	±
Disk (pale green)	+	±						±	±
Leaf (green)	+	+						+	+
Stem (green)	+	+						+	+

one maximum and the corresponding compounds of the benzalcoumaranone series, two maxima. This law is directly applicable to the case with the acetates of marein and maritimein and the acetates of okanin and maritimetin.

It is to be noted that for the partially acetylated okanin (tetraacetate) the position of the maximum has been shifted by 37 mμ to longer wave lengths than that of the pentaacetate, which is known to appear at 310 mμ. Therefore it occupies an intermediate position between okanin[3] (384 mμ) and its pentaacetate (310 mμ). As the partial acetate of okanin was more readily recrystallizable than the peracetate, it is the most suitable form for the purification and identification. The chemical and spectral behaviors of the free hydroxyl group in the position 2′ of chalcone and its partial acetate are of much interest.

As part of the investigation, an attempt was made to make clear the localization in the plant of the pigments as well as related compounds in order to obtain some information about flavonoid biogenesis. For this purpose, two types of flowers, ray and tube, were carefully separated and as described in the Experimental part, were carefully dissected into several parts as far as possible and the constituents studied by means of paper chromatography. The results obtained are summarized in the Table I. The presence of marein (I) accompanied by a small quantity of maritimein (V) and flavanomarein (III) is most prominent in the corolla of both ray and tube flowers and in the bract of ray flowers. A small quantity of free okanin was always detected in the corolla and bract of ray flowers. Marein was found in almost every part of the plant. Filament and ovary do not, however, contain even a trace of the pigment. The bract, involucre, disk, stem and foliage leaf contain a small quantity of an unknown anthochlor-like pigment which as yet has not been isolated. There were found caffeic acid in the bract of the ray and both caffeic and chlorogenic acid in considerable amount in the ovary, both fertile and sterile, in a small quantity in the leaf and stem and in a trace amount in the involucre and bare flower disk. Chrysanthemin was found in the lower part of the corolla of the ray, in the upper part of the corolla of the tube and in the upper part of the small bract of the tube flowers.

The results thus obtained as to the presence in *C. tinctoria* of anthochlor pigments and related compounds may suggest several important conclusions. (1) The presence of marein and maritimein in *C. tinctoria* confirms the biogenetical interrelationship of two types of anthochlor pigments, which was at first found by us in *Cosmos sulphureus* (coreopsin and sulfurein), *Coreopsis lanceolata* and *C. saxicola* (lanceolin and leptosin). A more complicated case was found recently by Geissman and coworkers in *C. maritima* and *C. gigantea*, in which two pairs of anthochlor pigments, namely, coreopsin–sulfurein and marein–maritimein, are concerned. Of all these anthochlor pairs, the enzyme "chalconase" may be always active in converting chalcones to aurones. (2) Although the reason why marein shows an unusual tendency to isomerize into the flavanone form is as yet not interpreted, the co-existence of chalcone, flavanone and aurone of corresponding structure in the flowers of *C. tinctoria* suggests a close connection among them. (3) The anthocyanin present in *C. tinctoria* has a different hydroxylation pattern in the A ring (C_6(A)–C_3–C_6(B)) from anthochlors. It suggests that the chemical pathway leading to anthocyanin and the way to anthochlors diverge from each other at an early stage of the formation of these substances, and it reminds us of a similar relationship between flavones and anthochlors suggested by Geissman. Otherwise it may be due to the production in quite different tissues or cells of anthocyanin and anthochlors, since the red epidermis contained almost no trace of anthochlor.

Experimental[8]

Isolation of Marein, Flavanomarein and Maritimein.—Fresh ray flowers (850 g.) of *Coreopsis tinctoria* were boiled with 4, 3, 2 and 2 l. of 95% ethanol, respectively, each time for 10 min., and the ethanolic extract was concentrated under reduced pressure to about 250 ml. After extracting 4–5 times with ether to remove ether-soluble carotenoids as well as a small quantity of free okanin, the concentrate was extracted exhaustively with ethyl acetate about 10 times. The acetate solution was evaporated and the residue dissolved in 200 ml. of hot water, filtered and allowed to stand for a few days. From the solution, marein gradually precipitated in an amorphous agar-like state, yield about 5.9 g. The mother liquor was evaporated *in vacuo* to about 100 ml.; marein accompanied by a considerable amount of flavanomarein (2.9 g.) was further obtained in a semi-crystalline state. When the mother liquor was further evaporated and allowed to stand, maritimein separated in a brownish-yellow powder (0.4 g.). The solution, from which the glycosides had separated, was evaporated to dryness and acetylated with acetic anhydride and pyridine. Acetates of both flavanomarein (0.9 g.) and maritimein (0.4 g.) were fractionated by repeated recrystallizations from ethanol.

Marein.—When reprecipitated several times from 50% ethanol, orange needles were obtained which melted at 130–132° (effervescence). The glycoside is difficultly crystallizable and has a great tendency to isomerize into its flavanone form. When 1 drop of the yellow ethanolic solution was added to 1 *N* sodium hydroxide solution, a red color appeared. With ferric chloride, marein gave a brown coloration.

Anal. Calcd. for $C_{21}H_{22}O_{11}\cdot 3H_2O$: C, 50.00; H, 5.60; H_2O, 10.7. Found: C, 50.18; H, 5.53; H_2O (dried under reduced pressure over P_2O_5 at 110°), 10.7, 10.8.

Marein Octaacetate.—One-tenth gram of marein was acetylated by boiling 3 min. with 1 ml. of acetic anhydride and 0.1 g. of sodium acetate. The product was recrystallized from 10 ml. of 50% ethanol as colorless needles, m.p. 162–165°.

Anal. Calcd. for $C_{37}H_{38}O_{20}$: C, 55.36; H, 4.77. Found: C, 55.11; H, 5.08.

Flavanomarein.—As noted above, the chalcone glycoside marein has an unusual tendency to take its flavanone form. An effort to crystallize marein as such from dilute ethanol resulted in almost colorless flavanomarein. It is noteworthy that such a chalcone which is not hydroxylated both in the positions 2′ and 6′ tends to ring closure. Flavanomarein is readily recrystallized from 30 times its weight of 50% ethanol in colorless crystals of m.p. 237–243°, which showed a bluish-green coloration with ferric chloride in an alcoholic solution, a bluish-purple one with magnesium and concd. hydrochloric acid and an orange-yellow halochromy with concd. sulfuric acid. When 1 *N* NaOH solution was added to the alcoholic solution, it changed almost instantly into vivid red. Similarly the flavanone eriodictyol, which is hydroxylated in the positions 3′ and 4′, shows a yellow color when treated likewise, gradually turning orange-red; the coloration in the case of flavanomarein suggests its rapid conversion to its chalcone form.

Anal. Calcd. for $C_{21}H_{22}O_{11}\cdot{}^1/_2H_2O$: C, 54.90; H, 5.05; H_2O, 1.96. Found: C, 54.97; H, 5.29; H_2O, 2.00.

Flavanomarein Heptaacetate.—Fifty mg. of flavanomarein was acetylated with 1 ml. of acetic anhydride and 2 drops of pyridine. As the reaction did not take place at room temperature, the mixture was heated on a boiling water-bath. The acetate was recrystallized from 15 ml. of ethanol. After 3 recrystallizations, it was obtained as colorless needles which melted at 194–198°.

Anal. Calcd. for $C_{35}H_{36}O_{18}$: C, 56.45; H, 4.87. Found: C, 56.21; H, 5.02.

Fifty mg. of marein was acetylated as above. When the product was recrystallized, the same acetate was obtained which melted at 194–198° both alone and on admixture with the acetate from flavanomarein.

Hydrolysis of Flavanomarein.—Flavanomarein (0.530 g.) was suspended in 30 ml. of water, 1.3 g. (4%) of concentrated sulfuric acid added and the mixture was boiled for 1 hr., giving a clear yellow solution after 10 min. heating. On cooling, the aglycone gradually separated, at first in a resinous and afterwards in an orange-yellow crystalline form. After standing overnight, the product was filtered and weighed (0.329 g.) (found 62.2%, theor., 64.0%). The filtrate separated from the aglycone, was neutralized with barium hydroxide (4 g.), filtered, transferred into a volumetric flask and the flask filled up with water to 200 ml. With 1 ml. of this solution the sugar was quantitatively determined according to the method of Sumner, using 3,5-dinitrosalicylic acid as oxidizing agent.[9] It was found that 1.15 mg. of sugar as glucose was contained per 1 ml. of the solution (found 43.4%, theor. 40.0% glucose). The solution was then evaporated under reduced pressure on a boiling water-bath to about 10 ml. Chromatograms were prepared using 1-butanol–acetic acid–water (4:1:1) as solvent and the benzidine solution as a developing agent. The filtrate gave only a single spot, which corresponded to glucose (R_f 0.17). When the filtrate was again concentrated to 5 ml. and heated on a boiling water-bath with phenylhydrazine hydrochloride (0.4 g.) and sodium acetate (0.6 g.) an osazone was formed after 8 min. After 30 min. heating, the osazone was filtered (50 mg.) and recrystallized from 90% ethanol. The osazone decomposed at 204–208°, both alone and on admixture with an authentic specimen of glucosazone.

When the aglycone was recrystallized from 30% ethanol (10 ml.), orange crystals (okanin, 0.10 g.) were obtained, which after 3 recrystallizations from dilute ethanol melted at 235–242° and exhibited the properties of okanin.

Anal. Calcd. for $C_{15}H_{12}O_6\cdot 2H_2O$: C, 55.55; H, 4.97; H_2O, 11.1. Found: C, 55.34; H, 5.34; H_2O, 11.8.

When the mother liquor separated from okanin was mixed with water, pale yellow needles gradually separated (0.15 g.). This substance was recrystallized from 5 ml. of 10% ethanol and obtained in pale yellow needles, which melted at 129–132° (effervescence). It showed a green coloration with ferric chloride and a bluish-purple one with magnesium and concd. hydrochloric acid, showing thus the properties of the flavanone corresponding to the chalcone, okanin. It exhibited a yellow coloration with 1 *N* NaOH solution, which turned immediately through orange to reddish-brown.

Anal. Calcd. for $C_{15}H_{12}O_6\cdot 1{}^1/_2H_2O$: C, 57.14; H, 4.80; H_2O, 9.57. Found: C, 57.76; H, 4.84; H_2O, 9.43.

Hydrolysis of Marein.—Marein (1.012 g.) was hydrolyzed by boiling with 30 ml. of 4% sulfuric acid for 1 hr. The aglycone was filtered and weighed (0.566 g.) (found 55.9%, $C_{15}H_{12}O_6/C_{21}H_{22}O_{11}\cdot 3H_2O$ requires 57.1%). When the aglycone was recrystallized from 20 ml. of 30% ethanol, 0.10 g. of okanin was obtained. From the mother liquor 0.32 g. of flavanoökanin separated, after addition of 20 ml. of water, almost in the same way as above.

Okanin Tetraacetate.—Fifty mg. of okanin was acetylated with 0.5 ml. of acetic anhydride and 2 drops of pyridine at room temperature. The product was recrystallized from 5 ml. of ethanol and obtained in yellow prisms of m.p. 168–173°. It gave a brown color reaction with ferric chloride.

Anal. Calcd. for $C_{23}H_{20}O_{10}$: C, 60.52; H, 4.42. Found: C, 60.12; H, 4.64.

Okanin Pentaacetate.—Fifty mg. of okanin was acetylated with acetic anhydride and a droplet of sulfuric acid as usual. The pentaacetate crystallized from ethanol in colorless needles, which melted at 138–141° (141° with 1.5 moles of H_2O).[5]

Anal. Calcd. for $C_{25}H_{22}O_{11}$: C, 60.24; H, 4.45. Found: C, 59.88; H, 4.42.

Flavanoökanin Tetraacetate.—Seventy mg. of the flavanone was put in a small test-tube and acetylated with acetic anhydride (0.7 ml.) and a droplet of pyridine at room temperature. The reaction mixture was mixed with water and the product was filtered, followed by recrystallization from 3 ml. of 50% ethanol. The tetraacetate was obtained in an aggregate composed of colorless minute needles of m.p. 120–122° (113°).[5]

Anal. Calcd. for $C_{23}H_{20}O_{10}$: C, 60.52; H, 4.42. Found: C, 60.96; H, 4.53.

(8) All melting points are uncorrected.

(9) J. B. Sumner, *J. Biol. Chem.*, **62**, 287 (1924); **65**, 393 (1925).

Maritimein.—Maritimein melted at 208–214°. It showed a purplish coloration with aqueous alkali and a brown one with ferric chloride.

Anal. Calcd. for $C_{21}H_{20}O_{11}\cdot 2H_2O$: C, 52.07; H, 4.99; H_2O, 7.44. Found: C, 52.34; H, 5.16; H_2O, 7.34.

Maritimein from Marein.—One gram of marein in 25 ml. of 50% ethanol was mixed with sodium bicarbonate (0.16 g., 1 mole) and refluxed on a boiling water-bath for 10 min. After standing for a week, the conversion of marein to maritimein was complete, and when 1 drop of the solution was added to 1 *N* NaOH solution, it gave a purplish color. The above reaction mixture which had become red was slightly acidified with acetic acid and extracted with ethyl acetate. The acetate solution was evaporated and the residue dissolved in 100 ml. of 50% ethanol. Yellow crystals of maritimein gradually precipitated, yield about 0.25 g., m.p. 208–214°.

Maritimein Heptaacetate.—Maritimein (0.1 g.) was acetylated with 1 ml. of acetic anhydride and 2–3 drops of pyridine by heating 5 min. on a boiling water-bath. The acetate, recrystallized from 15 ml. of ethanol, crystallized in minute needles which melted at 207–210°.

Anal. Calcd. for $C_{35}H_{34}O_{18}$: C, 56.60; H, 4.62. Found: C, 56.77; H, 4.83.

Hydrolysis of Maritimein.—Maritimein (0.452 g.) was suspended in 20 ml. of 50% ethanol, 0.8 g. of sulfuric acid added and the glycoside was hydrolyzed by boiling under reflux. Sparingly soluble maritimein dissolved and was decomposed gradually, resulting in a red clear solution after 6 hr. boiling. The solution was neutralized with sodium hydroxide and extracted 5 times with ethyl acetate. The acetate solution was evaporated under reduced pressure to dryness (0.280 g., 62%; $C_{15}H_{10}O_6/C_{21}H_{20}O_{11}\cdot 2H_2O$ requires 59%), and the aglycone was recrystallized from 10% ethanol (10 ml.) and then from water (6 ml.). Orange needles blackened and decomposed at 280–292° (292°).[6]

Anal. Calcd. for $C_{15}H_{10}O_6\cdot 2H_2O$: C, 55.90; H, 4.38; H_2O, 11.2. Found: C, 56.19; H, 4.63; H_2O, 11.4.

Enzymatic Hydrolysis of Maritimein with Emulsin.—Fifty mg. of maritimein suspended in 3 ml. of water was added to 3 ml. of a solution of 30 mg. emulsin, prepared from Japanese apricot. The mixture was covered with toluene and incubated at 30°. The hydrolysis was almost completed in three days. The mixture was heated on a boiling water-bath in order to inactivate emulsin and mixed with 2 ml. of ethanol followed by filtration. The aglycone was filtered and recrystallized from 10% ethanol as above; m.p. 280–290°.

Maritimetin Tetraacetate.—Fifty mg. of maritimetin was acetylated with acetic anhydride and 2 drops of pyridine at room temperature. The acetate, recrystallized from ethanol, melted at 190–192°.

Anal. Calcd. for $C_{23}H_{18}O_{10}$: C, 60.79; H, 3.99. Found: C, 60.63; H, 3.93.

Tetramethylmaritimetin.—To a solution of 0.2 g. of maritimetin tetraacetate in 20 ml. of warm methanol, 1 ml. of dimethyl sulfate and then 3 g. of 50% sodium hydroxide were added. When the reaction came to an end, the solution was diluted with water and extracted with ether. The ethereal solution was evaporated, and the crystalline residue was recrystallized from aqueous ethanol. The yellow needles melted at 156–157°, alone and on admixture with leptosidin trimethyl ether.[10]

Anal. Calcd. for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30. Found: C, 66.94; H, 5.14.

Heptamethylmaritimein.—Maritimein (0.3 g.) was taken in a glass mortar and mixed thoroughly with 3 ml. of dimethyl sulfate. Six grams of hot 50% sodium hydroxide solution was added in 5 portions to the mixture and brayed with a pestle incessantly. When the reaction ended, a yellow crystalline solid separated, which was filtered, washed with water and recrystallized from 80% ethanol (6 ml.). Yellow needles of heptamethylmaritimein melted at 118–120°, both alone and on admixture with leptosin hexamethyl ether, which was prepared by the same method as above.

Anal. Calcd. for $C_{28}H_{34}O_{11}$: C, 61.53; H, 6.27. Found: C, 60.99; H, 6.47.

(10) T. A. Geissman and C. D. Heaton, THIS JOURNAL, **65**, 677 (1943).

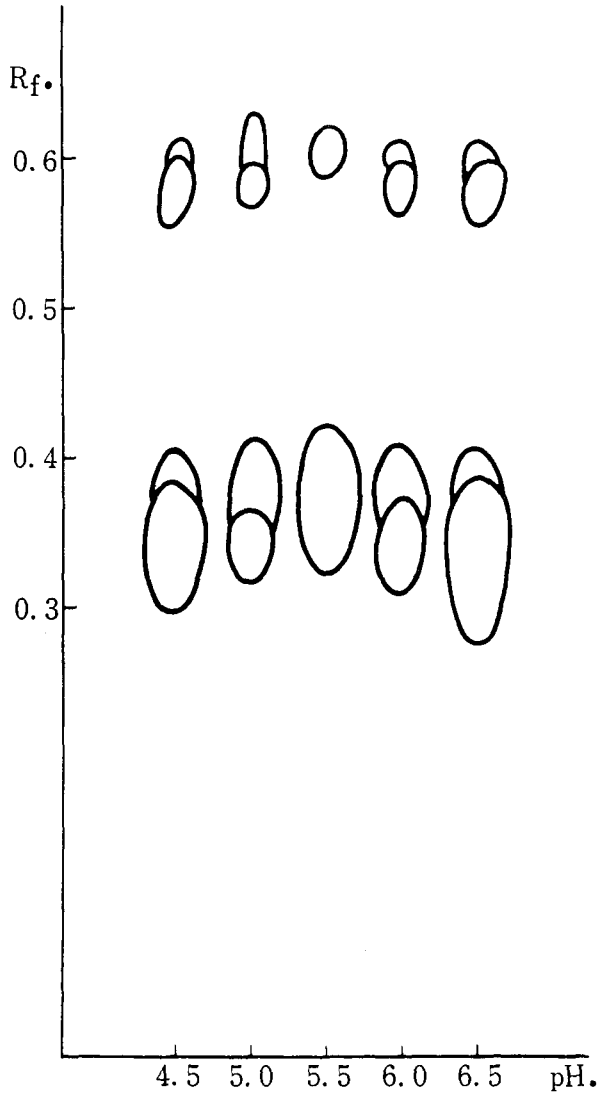


Fig. 1.—Paper chromatograms of the ray flower of *C. tinctoria*, macerated and subjected to the action of chalconase at *p*H 4.5–6.5. The spots represent maritimetin, okanin, maritimein and marein, respectively, from top to bottom.

Maritimetin 7,3′,4′-Trimethyl Ether (Leptosidin 3′,4′-Dimethyl Ether).—Maritimein heptamethyl ether (100 mg.) was hydrolyzed with 50% ethanol which contained 4% sulfuric acid by heating on a boiling water-bath for 2 hr. The hydrolysate was neutralized with sodium bicarbonate and extracted with ethyl acetate. The acetate solution was evaporated *in vacuo* to dryness, and the residue was recrystallized from 50% ethanol as yellow needles of m.p. 198–204° (204–205°).[6]

Anal. Calcd. for $C_{18}H_{16}O_6\cdot H_2O$: C, 62.42; H, 5.24. Found: C, 62.02; H, 5.46.

Maritimetin 7,3′,4′-Trimethyl Ether 6-Acetate.—Trimethyl ether (50 mg.) was acetylated with acetic anhydride and pyridine by the usual procedure. The acetate was obtained from ethanol in yellow needles of m.p. 165–168°.

Anal. Calcd. for $C_{20}H_{18}O_7$: C, 64.86; H, 4.90. Found: C, 64.90; H, 4.76.

Chromatograms of Extract of *Coreopsis tinctoria*.—Chromatograms were prepared, using 1-butanol–acetic acid–water (4:1:2) as solvent and Whatman No. 1 filter paper at 20°. The ethanol extract of the ray flowers of *C. tinctoria*, used for the isolation of the above substances, gave three spots of anthochlor pigments, corresponding to marein (R_f 0.34), maritimein (R_f 0.38) and okanin (R_f

0.56), respectively. The last mentioned free chalcone appeared only in a small quantity and gave a faint spot, while maritimetin was hardly detectable in a fresh extract. A spot (R_f 0.48), which became yellow with ammonia vapor and green with ferric chloride, corresponded to flavanomarein.

So as to make clear the distribution in the plant of these compounds, tube and ray flowers were carefully separated from the flower disks and, as described in the case with *Cosmos sulphureus*,[11] the former were dissected into stigma, style, ovary, anther, pollen, filament, upper and lower part of corolla and bract and the latter into corolla, bract and sterile ovary. One-hundredth to 1.0 g. each of these parts was extracted with 1–10 ml. of boiling ethanol for about 3 min., and the extraction was repeated two or three times. The combined ethanolic extracts were evaporated and the residue was dissolved in 0.01–1.0 ml. of 50% ethanol to give a solution of original volume, which was most adequate for the paper chromatographic detection. Spotting an equal volume of the plant extract and standard solution of control substance side by side, chromatograms were then run. A semi-quantitative determination was made by comparison of the size and depth of color of the spots, in visible or ultraviolet light, with those of known substances in known concentrations on filter paper. For the detection of anthocyanin, each part dissected was extracted with 5 times its weight of 1% methanolic hydrochloric acid in the cold. Naked flower disk was also tested. The results are summarized in Table II. The concentration of chrysanthemin and of an unknown pigment in the table means merely a probable relative amount.

Chalconase Test.—Fresh rays were taken in a glass mortar with an equal volume of water, two-fifths of McIlvaine buffer solutions of various *p*H and 0.1 to 0.04 of 0.05 *M* potassium cyanide and macerated for 10 min. The homogenate was then mixed with an equal quantity of ethanol and heated on a boiling water-bath for a while followed by filtration. Five to ten drops of the filtrate was spotted on a filter paper. The chromatograms were run as above. The optimum *p*H for the conversion of chalcone to aurone was found to be 5.5 in the case of *C. tinctoria* and *C. maritima* or *Cosmos sulphureus* as well. An example for the chalconase-catalyzed reaction is shown in Fig. 1.

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TOKYO, JAPAN

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE RICE INSTITUTE]

A Synthetic Approach to Polycyclic Hydroaromatic Systems Related to the 19-Norsteroids

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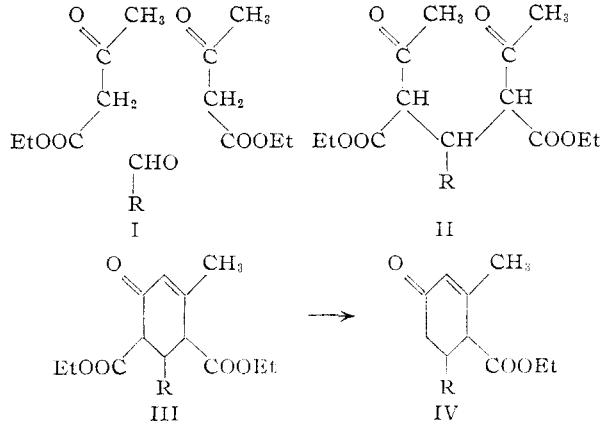
Exploratory work directed toward the total synthesis of physiologically active 19-norsteroids possessing a carbonyl group at C.11 has led to the synthesis of a tricyclic diketone containing the elements of the A, B and C rings with carbonyl groups and a double bond in appropriate positions. Other experiments carried out in this connection suggest that modification of the present synthetic scheme to include incorporation of the D-ring should involve no insurmountable difficulties.

The formation of condensation products of type II in the reaction of aldehydes with two molecular equivalents of ethyl acetoacetate has been studied extensively by Knoevenagel,[2] Rabe,[3] Horning,[4] and others. The results of these investigations have established that the primary condensation products II can be readily cyclized to 5-alkyl-4,6-dicarbethoxy-3-methylcyclohexenones III, which are in turn convertible into monocarbethoxy derivatives IV by partial hydrolysis and decarboxylation under a variety of conditions. When formaldehyde is employed as the aldehyde component,[5] the ultimate product is the well known Hagemann ester (IV, R = H),[6] which has found wide application as an intermediate in various synthetic procedures. In particular, two projected steroid syntheses involving the use of Hagemann's ester, as well as bicyclic and tricyclic analogs of this substance, have been briefly explored by Hogg[7]



and by Mukharji.[8] In both approaches the ring possessing the vinylogous β-ketoester function provides the elements of ring C, although different applications of the functional groups were suggested by the two investigators.

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