Spinochrome A Monoacetate[27] **(10).**—Spinochrome A was treated with ketene and the acetylation products were separated on a column of acid-treated, deactivated silica gel. The principal product crystallized from isooctane to give 2-hydroxy-3-acetyl-7-acetoxynaphthazarin as red needles, mp 185–189° dec. Nmr spectrum in $CDCl_3$ showed C-3 acetyl, δ 2.88; C-7 acetoxyl, 2.39. The ultraviolet spectrum gave λ_{max} 570 mμ (ϵ 1410), sh 525 (3150), 493 (3700), 300 (9100), sh 252 (8620); λ_{min} 387 mμ (ϵ 1150), 276 mμ (ϵ 7160).

(27) Synthesized by I. Singh in this laboratory.

Isolation of Eleven New Spinochromes from Echinoids of the Genus *Echinothrix*

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The Hawaiian echinoids *Echinothrix diadema* Linn. and *E. calamaris* Pallis elaborate some 30 pigments in their spines, many of them in trace amounts. Sixteen of these pigments have now been identified (the previously described echinochrome A, spinochromes A, B, C, and D, six new naphthazarin, four new juglone derivatives, and an unprecedented benzoquinone).

The literature of the pigments derived from sea urchins had long presented a bewildering array of structural proposals based on shaky evidence. Recent work[1] has established the existence of but six authentic compounds (one juglone and five naphthazarin derivatives). The structures of all six have now been proven by synthesis. These six compounds were isolated from a great variety of animals which had been collected in the Atlantic and Pacific Oceans as well as in the Mediterranean Sea, thereby suggesting a concise and simple solution of a complex situation. Our equanimity was jolted when we examined the calcareous portions of two *Echinothrix* species, *viz. E. diadema* Linn. and *E. calamaris* Pallis, from Kaneohe Bay, Oahu, and found that these echinoderms elaborate no fewer than some 30 pigments. We have identified the five known compounds (echinochrome A and spinochromes A, B, C, and D) and now wish to report the characterization of 11 new spinochromes (six naphthazarins, four juglones, and an unprecedented benzoquinone).

The complex mixture of pigments was separated readily on a column of acid-treated, deactivated silica gel into eight bands which could be eluted with benzene, one with chloroform, and one with 5% methanol–chloroform. The first four fractions could be further fractionated into their components by preparative thin layer chromatography; however, for the remaining, slower moving bands it was found necessary to fractionate the mixture after methylation with diazomethane and regenerate the free pigments by acid hydrolysis.[2]

The structures of the 16 identified spinochromes from *Echinothrix*, their R_f values, melting points, and yields are presented in Table I.

Structure Determinations.—In addition to echinochrome A (**12**) and spinochromes A (**9**), B (**16**), C (**13**), and D (**15**), we have isolated 2-hydroxy-3-acetylnaphthazarin (**1**),[3] 2-hydroxy-6-ethyljuglone (**2**),[3,4] 2-hydroxy-6-ethylnaphthazarin (**3**),[3] naphthopurpurin (**5**), 2,7-dihydroxy-6-acetyljuglone (**6**),[3] 2,7-dihydroxy-3-ethylnaphthazarin (**8**),[3] and 2,7-dihydroxynaphthazarin (**11**)[5–8] and have shown their identities by comparison with authentic samples.

The most interesting pigment in this group is 2,5-dihydroxy-3-ethylbenzoquinone (**4**), the first representative benzoquinone to have been discovered in marine invertebrates. Examination of its ultraviolet spectrum (Figure 1) showed that the pigment was not a typical naphthazarin or juglone. The structure of **4** is readily elucidated from its infrared, nmr, and mass spectra. The infrared spectrum showed a sharp band at 1613 cm^{-1} attributed to a quinone carbonyl having an adjacent hydroxyl.[9] Since this was the only carbonyl absorption, both quinone carbonyls have to be flanked by hydroxyl substituents. The molecular weight of 168 determined by mass spectrometry suggested a 2,3- or 2,5-dihydroxybenzoquinone substituted with an ethyl group. The nmr spectrum immediately revealed the presence of the ethyl group and this was substantiated by the small, but nevertheless characteristic doublet at m/e 139 ($M - C_2H_5$) and 140 ($M - CH_2CH_2$) in its mass spectrum.[10] The position of the C-6 proton signal (δ 5.82) is only compatible with the placement of a hydroxyl at C-5. Therefore the pigment must have structure **4**. Compound **4**, a previously unknown substance, was easily synthesized in 27% yield by oxidation of ethylhydroquinone with basic hydrogen peroxide.

The principal pathway of disintegration in the mass spectrum of **4** begins with the loss of a methyl radical from the parent ion, the base peak, to form the second

(1) I. Singh, R. E. Moore, C. W. J. Chang, and P. J. Scheuer, *J. Am. Chem. Soc.*, **87**, 4023 (1965).

(2) Methylation patterns of polyhydroxynaphthoquinones will be the subject of a forthcoming publication by C. W. J. Chang, R. E. Moore, H. Singh, and P. J. Scheuer.

(3) R. E. Moore, H. Singh, C. W. J. Chang, and P. J. Scheuer, *J. Org. Chem.*, **31**, 3638 (1966).

(4) Not isolated in sufficient amount for nmr and mixture melting point with an authentic sample. This point will be clarified in a subsequent paper dealing with the structures of the remaining pigments of *Echinothrix* echinoids.

(5) 2,7-Dihydroxynaphthazarin has also been identified as a minor pigment in the spines of the Hawaiian echinoids *Echinometra oblonga* Blainville and *Tripneustes gratilla* Linn.

(6) Under the conditions of the isolation 2,7-dihydroxynaphthazarin is not formed as an artefact from loss of the acetyl group of spinochrome A owing to acid hydrolysis.

(7) This pigment, called mompain, has recently been isolated from the microorganism *Helicobasidium mompa* Tanaka [S. Natori, Y. Kumada, and H. Nishikawa, *Chem. Pharm. Bull.* (Tokyo), **13**, 633 (1965)]. After completion of our work we became aware of its synthesis by Professor R. H. Thomson (private communication) *via* a tetralone intermediate [*cf.* H. A. Anderson, J. Smith, and R. H. Thomson, *J. Chem. Soc.*, 2141 (1965)].

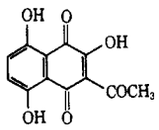
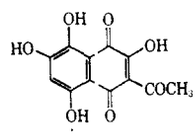
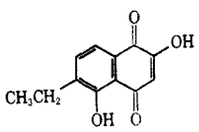
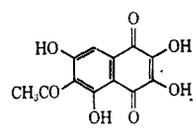
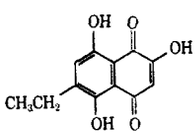
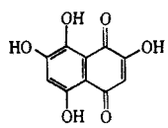
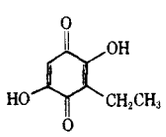
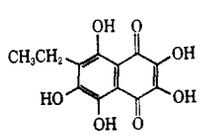
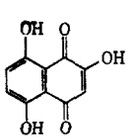
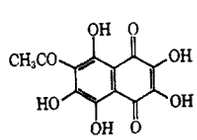
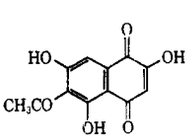
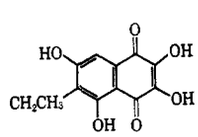
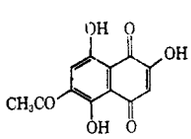
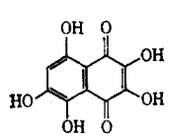
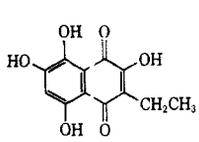
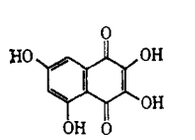
(8) The electronic absorption spectra and synthesis of substituted 1,4-naphthoquinones will be presented shortly in a full paper by C. W. J. Chang, R. E. Moore, R. Ogata, I. Singh, and P. J. Scheuer.

(9) S. Natori, *Chem. Pharm. Bull.* (Tokyo), **13**, 511 (1965).

(10) The m/e 139 and m/e peaks could be due to loss of CHO radical and CO, respectively, from the molecular ion.

TABLE I

PIGMENTS FROM THE SPINES OF *Echinothrix diadema* AND *E. calamaris* IN ORDER OF ELUTION

Pigment	Relative R_f[a]	Mp, °C	Yield, %	Pigment	Relative R_f[a]	Mp, °C	Yield, %
 1	0.692	163–164 dec	0.0001	 9	0.105	192–193	0.0004[e]
 2	0.618	219–220[b]	<0.00001[c]	 10	0.050	245–255 subl	0.00005[e]
 3	0.488	204–204.5	0.00003[c]	 11	0.040	265–275 subl	0.0008[e]
 4	0.458	130–145 subl	0.000025[d]	 12	0.039	222–223	0.002[e]
 5	0.412	200–210 subl	0.000075	 13	0.038	246–248	0.0004[e]
 6	0.366	215 dec	0.00004	 14	0.008	265–269 dec and subl	0.002
 7	0.169	179–180 dec	0.000075	 15	0.005	280–290 subl	0.00001[e]
 8	0.146	190–192	0.00035[e]	 16	0.001	>300	0.00015[e]

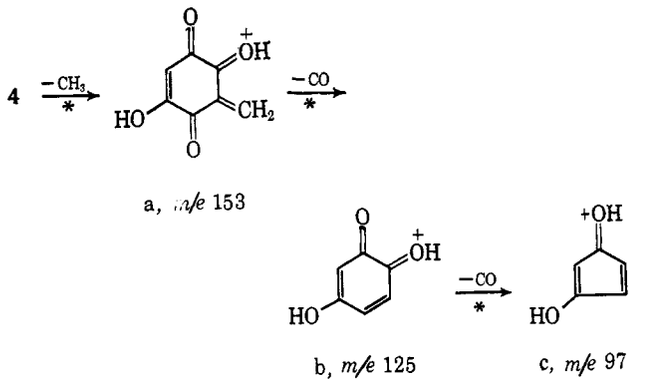
[a] On a thin layer plate of acid-washed, deactivated silica gel with benzene; referred to naphthazarin (R_f 1.000). The R_f values are not reproducible and will vary appreciably depending on the characteristics of the plate. These R_f values are the result of a single accurate experiment and can be compared with those reported in other tables in this manuscript and all other related papers. [b] Insufficient amount of pigment prevented determination of melting point; reported value is that of reduction product of spinochrome A (see ref 3). [c] From spines of *E. calamaris;* pigment not observed in *E. diadema.* [d] From spines of *E. diadema;* pigment not observed in *E. calamaris.* [e] Yield based on methylated derivative.

most abundant ion a (*m/e* 153, 44% relative intensity) followed by two consecutive expulsions of carbon monoxide to give peaks at *m/e* 125 (b, 18% relative intensity) and *m/e* 97 (c, 8% relative intensity). Metastable ions[11] outline this course of fragmentation.

The structure of 2-hydroxy-6-acetylnaphthazarin (**7**) was deduced from spectral and chemical evidence. The mass spectrum[12] confirmed the molecular weight of 248 and the ultraviolet spectrum (Figure 1) revealed

(11) Transitions supported by a metastable ion are marked by asterisks in the proposed fragmentation schemes.

(12) For a discussion of the mass spectrum of **7**, see the following paper: D. Becher, C. Djerassi, R. E. Moore, H. Singh, and P. J. Scheuer, *J. Org. Chem.*, **31**, 3650 (1966).



a naphthazarin with one β-hydroxyl.[8] The nmr spectrum showed the presence of an acetyl group (δ 2.73) and furthermore that both the β-hydroxyl and acetyl substituents have adjacent ring protons. The chemical shift of δ 6.43 for the C-3 proton and 7.63 for the C-7 proton indicated the location of the quinoid and benzenoid properties.[13] The 7-acetyl isomer was discarded as a possibility when **7** was converted to the known **3** by sodium borohydride reduction. Compound **7** was synthesized in 15% yield by the action of boron trifluoride on an acetic anhydride solution of 1,2,4,5,8-pentaacetoxynaphthalene (leucoacetate of naphthopurpurin) followed by mild basic hydrolysis in the presence of air.[14] A small amount of **1** (2% yield) was also formed in the synthesis.

Pigment **10**[15] was rather difficult to handle[16] but the 2,3,7-trihydroxy-6-acetyljuglone structure was suggested as the most probable from the mass spectrum (*m/e* 264) and qualitative electronic spectrum (λ_{max} 305, 388, 490 mμ). The nmr spectrum of the stable dimethyl ether, 2,3-dimethoxy-6-acetyl-7-hydroxyjuglone (**17**) immediately confirms the proposed structure. The signals at δ 4.10 and 4.15 indicate that *both* methoxyls are on the quinoid ring.[13] The C-8 proton appears at δ 7.16 and thus establishes a hydroxyl at C-7.[13] Two hydroxyl signals can be seen at δ 13.95 and 14.20 showing intramolecular hydrogen bonding to proximate carbonyl functions, the C-7 hydroxyl being bonded to the acetyl group at C-6 (δ 2.83).[13] The ultraviolet spectrum of **17** (Figure 2) is similar to that of **6**.[3] Compound **17** could be hydrolyzed with concentrated hydrobromic acid to regenerate **10**, but in poor yield.

The structural elucidation of 2,3,7-trihydroxy-6-ethyljuglone (**14**), the major pigment of *E. diadema*, offered no difficulty. The ultraviolet spectrum (Figure 2) demonstrated the juglone chromogen with three β hydroxyls.[8] The presence of the ethyl group at C-6 was revealed by the nmr spectrum and the signal at δ 7.13 for the C-8 proton could be reconciled only by the attachment of a hydroxyl at C-7.[13] The pigment formed a trimethyl ether and the chemical shifts for the three methoxyls (see the Experimental Section) were compatible only with the assigned structure.[13]

(13) R. E. Moore and P. J. Scheuer, *J. Org. Chem.*, **31**, 3272 (1966).

(14) Synthesized by I. Singh in this laboratory. For similar experimental details see ref 8.

(15) 2,3,7-Trihydroxy-6-acetyljuglone is also present as a minor pigment in the spines of *Echinometra oblonga* Blainville.

(16) Instability of **10** may be due to air oxidation or light-catalyzed reactions in solution as evidenced by thin-layer chromatography and ultraviolet absorption spectrum.

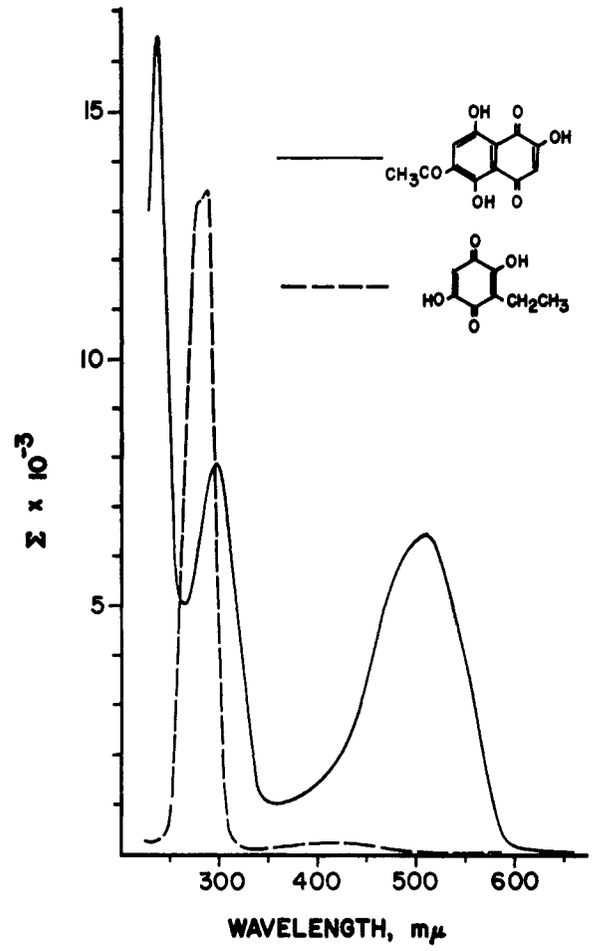


Figure 1.—Electronic absorption spectra of 2,5-dihydroxy-3-ethylbenzoquinone and 2-hydroxy-6-acetylnaphthazarin.

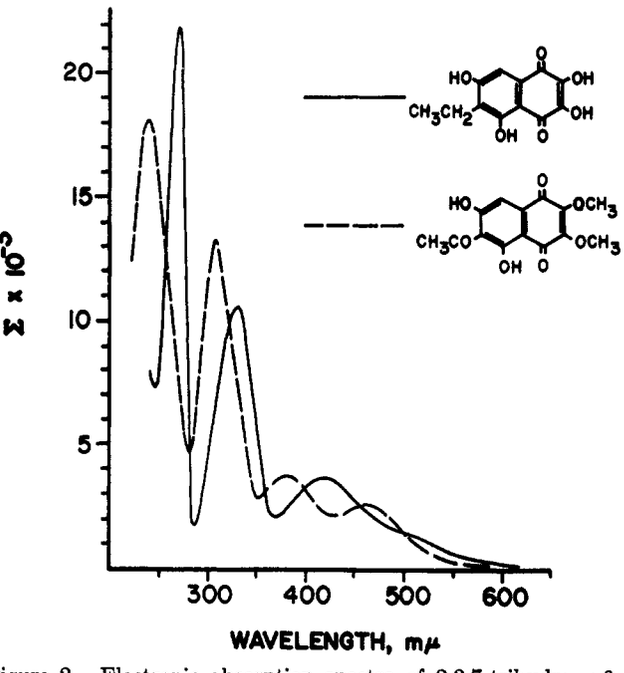
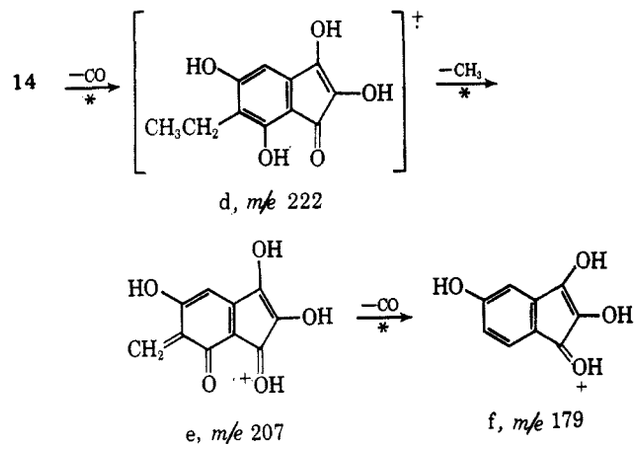


Figure 2.—Electronic absorption spectra of 2,3,7-trihydroxy-6-ethyljuglone and 2,3-dimethoxy-7-hydroxy-6-acetyljuglone.

The mass spectrum of **14** shows the molecular ion (*m/e* 250) as the base peak and its principal disintegration is initiated by expulsion of carbon monoxide to give an *m/e* 222 ion d (18% relative intensity) which subsequently eliminates a methyl radical to form the

m/e 207 ion e (52% relative intensity). Appropriate metastable peaks at m/e 197.1 ($222^2/250 = 197.1$) and 193.0 ($207^2/222 = 193.0$) accompany these fragmentations. Loss of carbon monoxide from ion e followed by rearrangement results in the m/e 179 ion f (5% relative intensity) and a metastable ion at m/e 154.8 ($179^2/207 = 154.8$) supports this transition.



Experimental Section[17]

Isolation of Spinochromes from *Echinothrix diadema*. A. Isolation and Partial Separation of Pigment Mixture by Column Chromatography on Silica Gel.—The animals were collected periodically during June 1963–July 1965 in Kaneohe Bay, Oahu, at a depth of 4–12 ft and the spines were removed by rubbing each of the animals on a wide mesh wire screen. Fleshy parts were separated from the calcareous portions by abrasive agitation in water and decantation of the suspended flocculent matter. After the aqueous decant had become relatively clear, the spines were washed thoroughly with acetone and dried.

A 1-kg batch of spines was distributed among ten 4-l. beakers or flasks and each *ca.* 100-g portion was digested in 250 ml of concentrated hydrochloric acid. Considerable foaming occurred and the addition of acid usually required 1 hr. The mixture was filtered (filtration is slow and usually takes 3 hr) and the filtrate was extracted exhaustively with peroxide-free ether to remove the pigments. The spinochromes were washed into aqueous sodium bicarbonate solution under nitrogen and the ethereal layer was discarded. The aqueous phase which contained a considerable amount of precipitated sodium salts of the pigments was acidified with hydrochloric acid and extracted thoroughly with benzene and finally with ether. The isolation of the crude pigment required about 6–8 hr and the best yields were usually obtained if the isolation to this point was not interrupted and if the crude pigment was introduced onto the silica gel for chromatography (see below) on the same day.

The benzene extract was evaporated and the residue was chromatographed on a 40 × 1.8 cm column of acid-treated, deactivated silica gel.[3] The chromatogram was developed in a continuous fashion[18] with benzene, chloroform, and 5% methanol–chloroform. Eight distinct bands separated with benzene elution and each band plus the intermediate zone preceding the next band constituted a fraction. Two more fractions were collected by continuous elution with chloroform and 5% methanol–chloroform. The results of the chromatography are summarized in Table II. A total of 20 kg of *E. diadema* spines was processed.

TABLE II

Fraction	Eluent	Band color	Elution time
1	Benzene	Pink	0.5 hr
2	Benzene	Orange-yellow	1 hr
3	Benzene	Pink	2–3 hr
4	Benzene	Red	4–6 hr
5	Benzene	Purple	8–12 hr
6	Benzene	Orange	1 day
7	Benzene	Orange-red to brown[a]	1 day
8	Benzene	Orange-yellow	2–3 days
9	Chloroform	Orange to red-orange	2–3 days
10	Methanol–chloroform 5:95	Brown	2–3 hr

[a] Fraction 7 does not separate entirely from 6.

B. Separation of Spinochromes in Fraction 1.—The gummy red solid (fraction 1) was purified by preparative thin layer chromatography (tlc) on acid-treated, deactivated silica gel.[3] The plate (0.5 mm × 20 cm × 20 cm) was developed with benzene to give a rapidly moving pale yellow band followed by a red band. The pigment was recovered from the red band and crystallized from petroleum ether (bp 30–60°) to give impure 2-hydroxy-3-acetylnaphthazarin. The mass spectrum indicated the presence of a substantial amount of impurity.

The pigment was methylated carefully with diazomethane and the progress of the methylation was determined on a tlc plate, on which the methylated pigment travelled slower than the free pigment. The product was purified by preparative tlc as described above. The methylated pigment was somewhat labile and hydrolyzed merely on standing for a few days in chloroform solution to give 2 mg of the natural pigment, **2-hydroxy-3-acetylnaphthazarin** (1), as dark red needles from isooctane, mp 163–164° dec.

C. Separation of Spinochromes in Fraction 2.—The crude pigment in fraction 2 was rechromatographed on a column of acid-treated, deactivated silica gel and elution with benzene removed a pale yellow-brown band (fraction 2A) followed closely by an orange-yellow one (fraction 2B).

Fraction 2A was homogeneous by tlc and vacuum sublimation followed by recrystallization from benzene yielded 5 mg of **2,5-dihydroxy-3-ethylbenzoquinone** (4) as orange prisms, subliming at 130–145° without melting. The ultraviolet spectrum showed λ_{max} sh 282 mμ (ε 13,200), 288 (13,400), 422 (183); λ_{min} 325 mμ (ε 118). Nmr spectrum in acetone-d_6 showed C-3 methylene, δ 2.41 (quartet, J = 7.5 cps); C-3 CH_2CH_3, 1.02 (triplet, J = 7.5 cps); C-6 hydrogen, 5.82. Mass spectrum showed m/e 168 (relative intensity 100), 153 (44), 140 (3), 139 (2), 125 (18), 97 (8), 94 (6), 69 (19).

Fraction 2B was fractionated further by preparative tlc with benzene to give an orange band (fraction 2B1), a yellow band (fraction 2B2), and a yellow band (fraction 2B3). Fraction 2B1 was homogeneous by tlc and recrystallization from chloroform and vacuum sublimation gave 15 mg of **napthopurpurin** (5) as orange-brown crystals, subliming at 200–210° without melting. Fraction 2B2 was also pure and crystallization from chloroform afforded 8 mg of **2,7-dihydroxy-6-acetyljuglone** (6) as small, orange needles, mp 215° dec.

D. Separation of Spinochromes in Fraction 4.—The crude pigment in fraction 4 was separated on preparative tlc plates with benzene into a red band (fraction 4A), an orange band (fraction 4B) which did not separate entirely from fraction 4A, and a red band (fraction 4C).

Fraction 4A was homogeneous by tlc and crystallized readily from chloroform–isooctane to give 15 mg of **2-hydroxy-6-acetylnaphthazarin** (7) as black needles, mp 179–180° dec. The ultraviolet spectrum showed λ_{max} 236 mμ (ε 16,500), 297 (7930), 507 (6450); λ_{min} 266 mμ (ε 4980), 352 mμ (ε 950). Nmr spectrum in $CDCl_3$ gave C-3 hydrogen, δ 6.43; C-6 acetyl, 2.73; C-7 hydrogen, 7.63.

Fraction 4C was slightly impure, but further preparative tlc did not result in satisfactory separation of the pigments. Methylation with diazomethane and preparative tlc of the methylated 4C (4CM) with benzene resulted in ready separation into a red band (fraction 4CM1), a red band (fraction 4CM2), an orange band (fraction 4CM3), and an orange-yellow band (fraction

(17) Analyses were performed by Berkeley Analytical Laboratory, Berkeley, Calif. In view of the notoriously poor behavior of these compounds during combustion, few samples were submitted for elemental analysis. Ultraviolet–visible spectra were determined in chloroform solution on a Cary 14 spectrophotometer, nmr spectra on a Varian A-60 instrument. Mass spectra were determined at Stanford University on an A.E.I. MS-9 instrument operating with an ionization energy of 70 ev. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Previously known compounds and their methyl ethers were compared by ultraviolet and nmr spectra, mixture melting points, and R_f values in two systems. Diazomethane methylations were conducted in ether or methanol–ether solution and the progress of the reaction was followed by tlc. In many instances care had to be exercised to avoid the undesired methylation of *peri* hydroxyls, especially in the methylation of pigments possessing acetyl groups.

(18) H. Rapoport and J. Bordner, *J. Org. Chem.*, **29**, 2727 (1964).

4CM4). Fraction 4CM1 crystallized from isooctane as long, red needles of 2,7-dimethoxy-6-ethylnaphthazarin, mp 145–147°, and fraction 4CM2 crystallized identically to give 2-hydroxy-7-methoxy-3-ethylnaphthazarin,[19] mp 230–232°. Fractions 4CM1 and 4CM2 were combined and hydrolyzed in 1:1 ethanol–12 *N* hydrochloric acid (48 hr of reflux under nitrogen). Recrystallization from absolute methanol afforded 70 mg of the natural pigment, **2,7-dihydroxy-3-ethylnaphthazarin** (**8**) as dark red needles, mp 190–192°.

E. Separation of Spinochromes in Fraction 5.—The crude pigment was rechromatographed on silica gel and partially separated into a purple band (fraction 5A) and a reddish brown band (fraction 5B).

Fraction 5A was mainly spinochrome A but was somewhat impure. The pigment was carefully methylated with diazomethane (5AM) and chromatographed on tlc plates with benzene. The results are summarized in Table III.

TABLE III

Fraction	Band color	Identity with other fractions
5AM1	Orange	Contains some 4CM1
5AM2	Yellow	
5AM3	Red	4CM2
5AM4	Purple	
5AM5	Red	
5AM6	Yellow	
5AM7	Red	
5AM8	Purple	
5AM9	Orange-yellow	

Fraction 5AM2 crystallized from isooctane to give 2,3-dimethoxy-7-hydroxy-6-acetyljuglone as small, orange-yellow needles, mp 134–135°. The ultraviolet spectrum showed λ_{max} 238 mμ (ε 18,000), 307 (13,300), 378 (3740), 462 (2530); λ_{min} 278 mμ (ε 4750), 350 (2840), 423 (2030). Nmr spectrum in $CDCl_3$ showed C-2 methoxyl, δ 4.10; C-3 methoxyl, 4.15; C-5 hydroxyl, 13.95 or 14.20; C-6 acetyl, 2.83; C-7 hydroxyl, 13.95 or 14.20; C-8 hydrogen, 7.16.

Fraction 5AM4 crystallized from chloroform–isooctane as black needles of 2-hydroxy-7-methoxy-3-acetylnaphthazarin (monomethylspinochrome A), mp 246–248°. Fraction 5AM5 crystallized from isooctane to give 2,7-dimethoxy-6-acetylnaphthazarin (dimethylspinochrome A) as long, red needles, mp 181–182°. Fraction 5AM4 and 5AM5 were combined and hydrolyzed in 1:1 ethanol–12 *N* hydrochloric acid at reflux (nitrogen atmosphere). The progress of the hydrolysis was determined by tlc and 40 mg of **spinochrome A** (**9**) was isolated after chromatography.[20]

Fraction 5B was subjected to preparative tlc and separated into a purple band (5A), a light brick-red band (5B1), a yellow band (5B2), and an orange-brown band (5B3). Fraction 5B3 crystallized from chloroform to give 3 mg of **2,3,7-trihydroxy-6-acetyljuglone** (**10**) as brick-red crystals, subliming at 245–255° without melting. An additional amount of the pigment could be obtained by acid hydrolysis of fraction 5AM2 (2 hr of reflux in concentrated hydrobromic acid under nitrogen), but in poor yield.[16]

F. Separation of Spinochromes in Fractions 6 and 7.—Fraction 6 was carefully methylated and subjected to preparative tlc with benzene. The results of the chromatography are summarized as shown in Table IV.

TABLE IV

Fraction	Band color
6M1	Red-orange
6M2	Red-orange
6M3	Red
6M4	Purple
6M5	Red
6M6	Yellow
6M7	Red
6M8	Yellow
6M9	(Remaining bands)

(19) The structural determinations of these partially methylated spinochromes will be discussed in a forthcoming publication (see ref 2).

Fraction 6M1 crystallized readily from isooctane as long, red needles of 2,3,6-trimethoxy-7-ethylnaphthazarin (trimethylechinochrome A), mp 131–132° (lit.[21] mp 130°). Fraction 6M2 crystallized from isooctane to give 2-hydroxy-3-ethyl-6,7-dimethoxynaphthazarin as small, red needles, mp 153–154°.[19] Fraction 6M5 crystallized from isooctane to give 2-hydroxy-3,7-dimethoxy-6-ethylnaphthazarin, mp 152–154°.[19] Fractions 6M1, 6M2, and 6M5 were combined and demethylated in concentrated hydrobromic acid (2 hr of reflux under nitrogen) to give *ca.* 350 mg of **echinochrome A** (**12**), as red needles from chloroform, mp 222–223°.

Crystallization of fraction 6M3 from chloroform afforded 2,3,6-trimethoxy-7-acetylnaphthazarin (trimethylspinochrome C) as brown needles from isooctane–chloroform, mp 126–127° (lit.[22] mp 116–117°), and fraction 6M4 gave 6,7-dimethoxy-2-hydroxy-3-acetylnaphthazarin,[19] as reddish brown needles from isooctane–chloroform, mp 224–227° dec. Fractions 6M3 and 6M4 were combined and hydrolyzed in 1:1 ethanol–12 *N* hydrochloric acid (22 hr of reflux under nitrogen) to yield after tlc **spinochrome C** (**13**)[23] as red-orange needles from absolute methanol, mp 246–248°.

Crystallization of fraction 6M7 gave 2,7-dimethoxynaphthazarin as dark red needles from chloroform–isooctane, mp 273–275° with sublimation, which after hydrolysis in concentrated hydrobromic acid (2 hr reflux under nitrogen) resulted in 150 mg of **2,7-dihydroxynaphthazarin** (**11**) as small, red needles after vacuum sublimation and crystallization from chloroform, subliming at 265–275° without melting (lit.[7] mp >300° dec).

Fraction 7 was treated in exactly the same manner as fraction 6 since they consisted of the same pigments, but in different amounts. Fraction 6 contained most of the echinochrome A whereas fraction 7 contained most of the spinochrome C and 2,7-dihydroxynaphthazarin.

G. Separation of Spinochromes in Fraction 8.—The pigment in fraction 8 was essentially pure and vacuum sublimation followed by two recrystallizations from chloroform gave *ca.* 400 mg of **2,3,7-trihydroxy-6-ethyljuglone** (**14**) as brick-red needles, mp 265–269° dec with partial sublimation. The ultraviolet spectrum gave λ_{max} 270 mμ (ε 21,900), 330 (10,600), 417 (3740), 485 sh (1510); λ_{min} 246 mμ (ε 7180), 286 (1440), 367 (1970). Nmr spectrum in acetone-d_6 showed C-5 hydroxyl, δ 12.08; C-6 methylene, 2.71; C-6 CH_2CH_3, 1.14; C-8 hydrogen, 7.13. Mass spectrum showed *m/e* 250 (relative intensity 100), 235 (7), 222 (18), 207 (52), 179 (5).

Anal. Calcd for $C_{12}H_{10}O_6$: C, 57.6; H, 4.0. Found: C, 57.1; H, 3.6.

Methylation with diazomethane formed a trimethyl ether, mp 113–114°, as orange needles from isooctane. Nmr spectrum in $CDCl_3$ showed C-2 methoxyl, δ 4.07; C-3 methoxyl, 4.09; C-5 hydroxyl, 12.23; C-6 methylene, 2.72; C-6 CH_2CH_3, 1.10; C-7 methoxyl, 3.96; C-8 hydrogen, 7.20.

H. Separation of Spinochromes in Fraction 9.—The crude pigment was methylated with diazomethane (9M) and chromatographed on preparative thin layer plates with benzene. The results of the chromatography are outlined as shown in Table V.

Fraction 9M1 was essentially pure and crystallization from isooctane and from absolute methanol yielded 2,3,6-trimethoxynaphthazarin (trimethylspinochrome D) as red needles, mp 161–162°. Hydrolysis in 1 ml of 1:1 ethanol–12 *N* hydrochloric acid (125° for 24 hr in sealed tube) afforded 1 mg of **spinochrome D** (**15**) as brick-red crystals after vacuum sublimation, subliming at 280–290° without melting.

Fraction 9M12 was a mixture and separation was better achieved by rechromatography on plates using chloroform (see Table VI).

Fraction 9M12G crystallized from benzene as orange crystals of 2,3-dimethoxy-7-hydroxyjuglone (dimethylspinochrome B), mp 204–205°.[19] Hydrolysis in concentrated hydrobromic acid (2 hr of reflux under nitrogen) gave 15 mg of **spinochrome B** (**16**) as brick-red crystals after vacuum sublimation, mp >300°.

(20) The yield of spinochrome A is no greater than 60% (based on methylated derivative) due to hydrolytic loss of the acetyl group. Appreciable amounts of 2-hydroxy-7-methoxynaphthazarin and 2,7-dihydroxynaphthazarin are formed during the reaction.

(21) K. Nishibori, *Nature*, **184**, 1234 (1959).

(22) C. W. J. Chang, R. E. Moore, and P. J. Scheuer, *Tetrahedron Letters*, 3557 (1964).

(23) The yield of spinochrome C based on the trimethyl derivative is *ca.* 50%. Prolonged hydrolysis (45 hr) leads to spinochrome D in 95% yield.

TABLE V

Fraction	Band color
9M1	Red-orange
9M2	Yellow
9M3	Yellow
9M4	Yellow
9M5	Yellow
9M6	Yellow
9M7	Purple
9M8	Red
9M9	Pink
9M10	Orange
9M11	Purple
9M12	Orange-brown (remaining bands)

TABLE VI

Fraction	Band color
9M12A	Orange
9M12B	Orange
9M12C	Purple
9M12D	Yellow
9M12E	Brown
9M12F	Yellow
9M12G	Yellow
9M12H	Brown
9M12I	Brown

Isolation of Spinochrome from *E. calamaris*.—A 700-g batch of spines was processed for crude pigments as described above. Essentially the same array of pigments that was found in *E. diadema* was displayed. The only significant differences that were noted are (1) 2,5-dihydroxy-3-ethylbenzoquinone could not be detected and (2) fraction 1 exhibited seven pigments instead of one for *E. diadema*.

Fraction 1 was chromatographed on a thin-layer plate with benzene and separated into a red band (1A), three yellow bands (1B, 1C, and 1D), a red band (1E), a yellow band (1F), and a red-orange band (1G). Fraction 1E proved to be identical with **2-hydroxy-3-acetylnaphthazarin** (1). Fraction 1F was too small to crystallize, but its ultraviolet spectrum and R_f value were identical with those of **2-hydroxy-6-ethyljuglone** (2). Fraction 1G crystallized from isooctane to give 0.25 mg of **2-hydroxy-6-ethylnaphthazarin** (3), mp 204–204.5°.

Conversion of 2-Hydroxy-6-acetylnaphthazarin to 2-Hydroxy-6-ethylnaphthazarin.—Two milligrams of the natural pigment, 2-hydroxy-6-acetylnaphthazarin, was carefully methylated with diazomethane and after purification by tlc the resulting 2-methoxy-6-acetylnaphthazarin, red crystals from isooctane, mp 185–186° dec, was dissolved in 2 ml of methanol and *ca.* 1 mg of sodium borohydride was added (red color immediately discharged). The yellow solution was acidified with hydrochloric acid (red color slowly returns) and the reduction product was extracted with benzene and separated from unreduced material by thin layer chromatography. The 2-methoxy-6-ethylnaphthazarin, which was not isolated, was hydrolyzed to 2-hydroxy-6-ethylnaphthazarin by a 2-min boiling in 1 ml of ethanol–12 *N* hydrochloric acid (1:1). The product was identified as 2-hydroxy-6-ethylnaphthazarin by comparison of its ultraviolet spectrum and R_f value (tlc) with those of an authentic sample. On a thin layer plate of acid-treated, deactivated silica gel, 2-hydroxy-6-ethylnaphthazarin moves faster than the 7-ethyl isomer in carbon tetrachloride.

Synthesis of 2,5-Dihydroxy-3-ethylbenzoquinone.—The quinone was prepared using a modified procedure of Jones and Shonle[24] for 2,5-dihydroxybenzoquinone. A stirred mixture of 40 mg of ethylhydroquinone in 1 ml of 60% aqueous sodium hydroxide was treated with 0.5 ml of 30% hydrogen peroxide. The temperature rose to 45° and was maintained at 45–50° for 2 hr during which time the mixture became a thick paste. It was poured onto ice and acidified with hydrochloric acid. The product was extracted from the resulting yellow solution with ether and purified by vacuum sublimation. After crystallization from benzene 13 mg of 2,5-dihydroxy-3-ethylbenzoquinone (27%) was obtained as orange prisms, subliming at 130–145° without melting. The synthetic quinone was identical in every respect (nmr, infrared and ultraviolet spectra, and R_f values is two systems) with the natural pigment.

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(24) R. G. Jones and H. A. Shonle, *J. Am. Chem. Soc.*, **67**, 1034 (1945).

Mass Spectrometry in Structural and Stereochemical Problems.[1] CXI. The Mass Spectrometric Fragmentation of Substituted Naphthoquinones and Its Application to Structural Elucidation of Echinoderm Pigments[2]

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The mass spectra of a series of acetyl, and higher substituted naphthoquinones are presented. The generalizations set out by Beynon and Williams and by Williams and co-workers have been expanded by considering the fragmentation patterns characteristic for acetylnaphthoquinones. The acetyl function surpasses the hydroxy and methoxy function in its ability to direct fragmentation upon electron impact. Its location in the quinoid or benzenoid moiety of the molecule and in the vicinity of various other substituents yields different spectra, which can be used successfully for structural elucidation of unknown pigments of this group.

Naphthoquinone pigments occur widely in nature among higher plants and microorganisms, yet in the animal kingdom this class of compounds has been encountered only in echinoderms. Even within this phylum only the echinoids (sea urchins) have been the prime producer of these substances. There they occur as structural pigments (spinochromes) and in the eggs, ovaries, and perivisceral fluid of the animals (echinochromes). The first research in this field dates back to 1885,[4] but it was more than 50 years later when a pure,

(1) Paper CX: A. M. Duffield, S. D. Sample, and C. Djerassi, *Chem. Commun.*, 193 (1966).

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