Sodium Borohydride Reduction of Spinochrome A. Removal of Phenolic Hydroxyls in the Naphthazarin System[1a]

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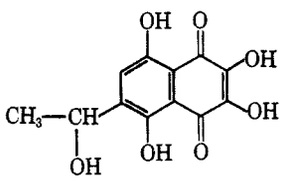
In the attempted sodium borohydride reduction of 2,7-dihydroxy-3-acetylnaphthazarin to 2,7-dihydroxy-3-(1′-hydroxyethyl)naphthazarin the anticipated product was not isolated. Instead, a mixture of 11 products was obtained (six juglone and five naphthazarin derivatives). Four of the naphthazarins had the acetyl group reduced to ethyl and had lost none, one, or two phenolic hydroxyls. One naphthazarin had an intact acetyl, but lost one hydroxyl. Four of the juglones had the acetyl group reduced to ethyl and had lost one or two hydroxyls, while two juglones had acetyl groups intact with loss of one or two hydroxyls. The structures of all 11 products were determined largely by spectroscopic methods. Possible mechanisms for this reaction are considered.

Spinochrome P is reported as one of the pigments in the spines of the Mediterranean echinoid *Paracentrotus lividus* Lam.[2] and based on insufficient evidence Musaio and Minchilli[3] proposed structure **1**. The presence of a hydroxyethyl side chain in their structure prompted us to study the reduction of spinochrome A (**2**), a pigment which was readily accessible to us from the Hawaiian echinoids *Echinometra oblonga* Blainville and *Colobocentrotus atratus* Linn.,[4,5] in the hope that the properties of the reduction product **3** would coincide with those described for spinochrome P.



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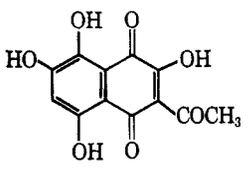

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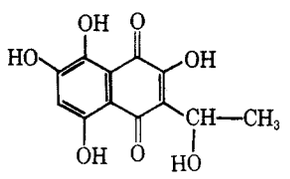

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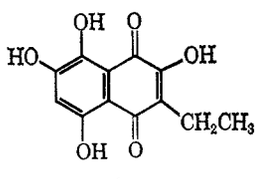
We chose sodium borohydride as the reducing agent. Following the reduction, the quinone system could be regenerated by air oxidation. For example, the intense purple-red color of spinochrome A in methanol was immediately discharged by sodium borohydride and a yellow solution of presumably 1,2,4,5,7,8-hexahydroxy-3-acetylnaphthalene ensued. Upon acidification, the naphthohydroquinone was reconverted by air oxidation to spinochrome A in quantitative yield.

The presence of a slight excess of sodium borohydride did not appear to affect the acetyl carbonyl. To our surprise, however, in the presence of a large excess of sodium borohydride the reduction of spinochrome A did not only involve the reduction of the acetyl carbonyl and proceed cleanly to a single product, but rather produced a mixture of at least 11 components. A preliminary examination of the thin layer chromatogram of the mixture indicated the presence of juglones (yellow spots) as well as naphthazarins (orange, red, and purple spots). Even though the reduction engendered a complex situation, further investigation seemed worthwhile for two reasons. The previously reported[6] sodium borohydride reduction of phenolic hydroxyls had never been fully studied. Furthermore, we strongly suspected that some of the reduction products would prove identical with trace pigments which we were encountering in echinoids (sea urchins). This proved to be the case.[7]

To avoid rapid hydrolysis of the borohydride, the reduction was conducted in basic, aqueous methanol. In this medium an anionic species of 1,2,4,5,7,8-hexahydroxy-3-acetylnaphthalene was present after initial reduction and possessed a red color. When essentially all of the spinochrome A had disappeared, the reduction was terminated and the mixture of products was separated by column and preparative thin layer chromatography on acid-treated deactivated silica gel. Only the colored products were investigated and six juglones and five naphthazarins were isolated and characterized (Table I).

Structure Determination.—The naphthazarins will be discussed first. The principal reduction product is 2,7-dihydroxy-3-ethylnaphthazarin (**4**). Its electronic absorption spectrum (Figure 1) clearly depicts the 2,7-dihydroxynaphthazarin chromophore[8] and the presence of the ethyl group is demonstrated by its nmr spectrum. The position of the methylene signal (δ 2.66), as well as the C-6 proton (δ 6.67), shows that **4** is the predominant tautomer.[9] The triplet signal at δ 1.16 for the methyl protons indicates the askew nature of the ethyl substituent.[9]



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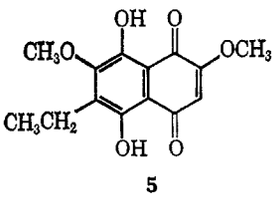

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(1) (a) Supported by a Public Health Service Grant GM-10413 from the Institute of General Medical Sciences, Public Health Service; (b) National Defense, Education Act Fellow, 1960–1963; National Institutes of Health Predoctoral Fellow, 1963–1964.

(2) L. Musaio and M. DiFonzo, *Boll. Sci. Fac. Chim. Ind. Bologna*, 231 (1940); *Chem. Abstr.*, **37**, 124 (1943).

(3) L. Musaio and M. Minchilli, *Boll. Sci. Fac. Chim. Ind. Bologna*, **31**, 113 (1942); *Chem. Abstr.*, **38**, 3277 (1944).

(4) C. W. J. Chang, R. E. Moore, and P. J. Scheuer, *J. Am. Chem. Soc.*, **86**, 2959 (1964).

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

(6) G. I. Fray, *Tetrahedron*, **3**, 316 (1958).

(7) Accompanying manuscript: R. E. Moore, H. Singh, and P. J. Scheuer, *J. Org. Chem.*, **31**, 3645 (1966).

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

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

TABLE I

SODIUM BOROHYDRIDE REDUCTION PRODUCTS OF SPINOCHROME A, IN ORDER OF SEPARATION

Product	No.	Relative R_f[a]	Mp, °C	Yield, %
Ethylnaphthazarin	6	1.120	123–124	0.06
2-Hydroxy-3-ethyljuglone	14	1.020	185–186	0.1
2-Hydroxy-3-ethylnaphthazarin	7	1.000	190.5–191.5	0.2
2-Hydroxy-3-acetylnaphthazarin	9	0.692	163–164 dec	0.05
2-Hydroxy-6-ethyljuglone	15	0.618	219–220	0.9
2-Hydroxy-6-ethylnaphthazarin	8	0.488	204–204.5	2
2,7-Dihydroxy-6-acetyljuglone	16	0.366	215 dec	1.5
2-Hydroxy-6-acetyljuglone	17	0.177	193–196 dec	0.7
2,7-Dihydroxy-3-ethylnaphthazarin	4	0.146	190–192	11
2,7-Dihydroxy-3-acetylnaphthazarin (starting material)	2	0.105	192–193	
2,7-Dihydroxy-3-ethyljuglone	13	0.082	219–220	1
2,7-Dihydroxy-6-ethyljuglone	12	0.048	237 dec	4

[a] On thin layer plate of acid-treated 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.

Compound **4** readily forms the dimethyl ether (**5**) on treatment with diazomethane. As expected[9] the nmr spectrum of **5** displays the C-2 methoxy at δ 3.94 and the C-7 methoxyl at δ 4.07, but the position of the C-6 methylene (δ 2.73) suggests attachment of the ethyl substituent to a rather aromatic ring and the C-3 proton signal (δ 6.26), in the region for a quinoidal-type hydrogen, lends support to this conjecture. Thus **5** is apparently the major representative of the dimethyl ether in chloroform solution.

To synthesize **4**, ethylnaphthazarin was converted to a mixture of ethylnaphthopurpurins by a method introduced by Zahn and Ochwat[10] and modified by Wallenfels.[11] Subjection of the 6-ethyl-2-hydroxynaphthazarin to another Zahn–Ochwat reaction afforded **4**, identical in every respect with the borohydride reduction product.

The structural elucidation of the reduction products, ethylnaphthazarin (**6**), 2-hydroxy-3-ethylnaphthazarin (**7**), and 2-hydroxy-6-ethylnaphthazarin (**8**), was straightforward from examination of the respective ultraviolet and nmr spectra.[8,9] Furthermore, comparison with the synthetic compounds left no doubt as to their respective structures.

The structure of the last naphthazarin compound, 2-hydroxy-3-acetylnaphthazarin (**9**), also resulted readily from spectral examination. The electronic absorption spectrum not only showed the presence of the naphthazarin chromophore, but the spectrum was essentially identical with that of spinochrome A monoacetate [2-hydroxy-3-acetyl-7-acetoxynaphthazarin (**10**)], see Figure 2. As the acetoxyl group has been shown to have essentially no effect on the chromophore of many naphthazarins,[8] one must conclude that the most probable structure for this borohydride product is **6**. Comparison of the R_f values of **9** and its methyl

(10) K. Zahn and P. Ochwat, *Ann.*, **462**, 72 (1928).
(11) K. Wallenfels, *Chem. Ber.*, **75**, 785 (1942).

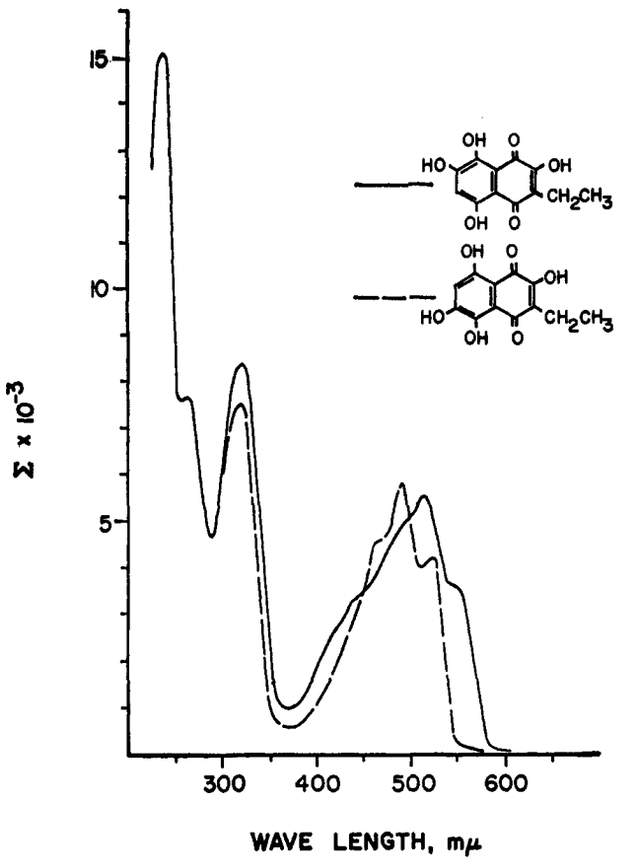


Figure 1.—Electronic absorption spectra of 2,6-dihydroxy-3-ethylnaphthazarin and 2,7-dihydroxy-3-ethylnaphthazarin.

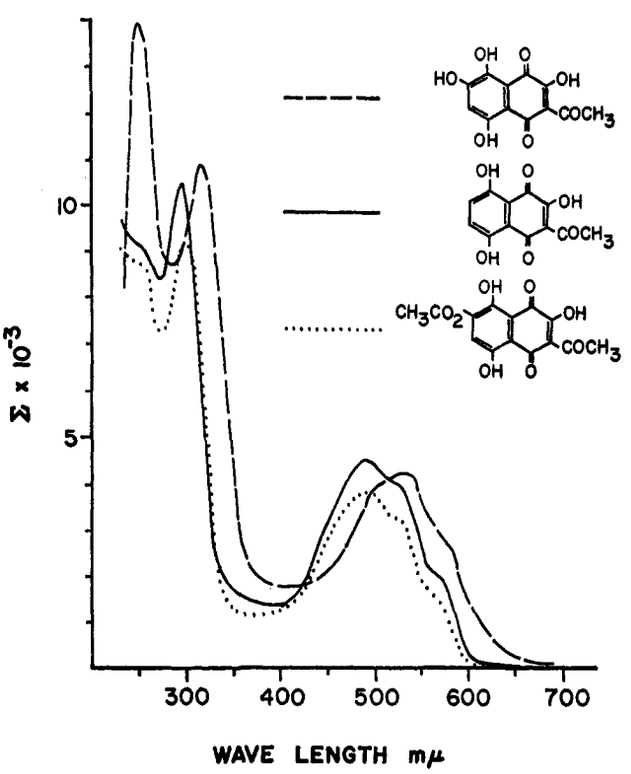


Figure 2.—Electronic absorption spectra of spinochrome A, 2-hydroxy-3-acetylnaphthazarin, and spinochrome A monoacetate.

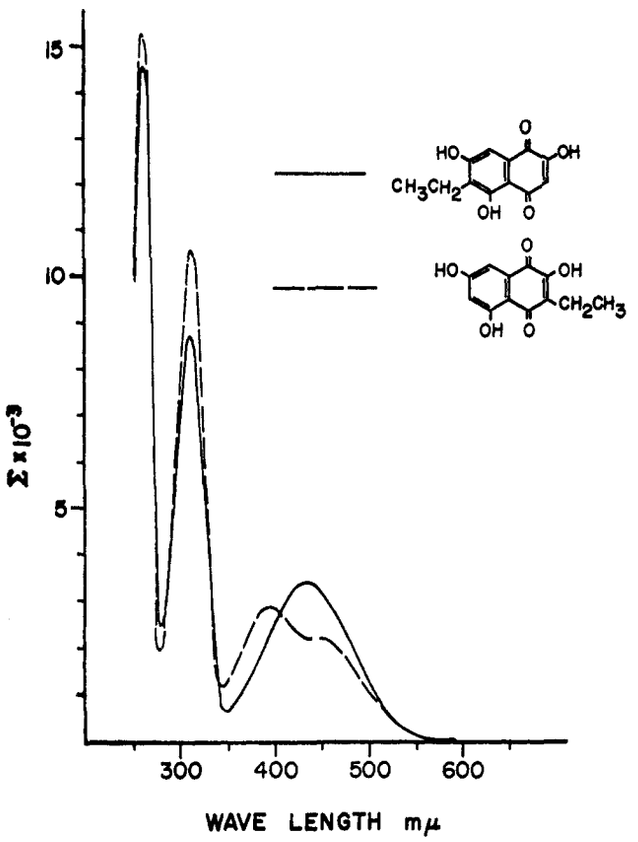


Figure 3.—Electronic absorption spectra of 3-ethyl-2,7-dihydroxyjuglone and 6-ethyl-2,7-dihydroxyjuglone.

ether **11** (Table II) showed that the latter moves more slowly, a behavior noted already for mono- and dimethylspinochrome A. Apparently after methylation the combined polarities of the acetyl and methoxyl groups result in increased adsorption on the column, whereas prior to methylation the polarities of the hydroxyl and acetyl functions are somewhat reduced by the intramolecular hydrogen bonding of the two groups. Even the behavior of the compound and its methyl ether on a thin layer plate suggested structure **9** as the most probable structure.

TABLE II

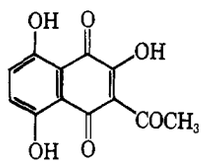
COMPARATIVE R_f VALUES OF SOME ACETYLNAPHTHAZARINS

Compound	Relative R_f[a]	Mp, °C
2-Hydroxy-3-acetylnaphthazarin (9)	0.692	163–164 dec
2-Methoxy-3-acetylnaphthazarin (11)	0.563	*b*
2-Hydroxy-3-acetyl-7-methoxynaphthazarin (10) (monomethylspinochrome A)	0.390	246–248
2,7-Dimethoxy-6-acetylnaphthazarin (dimethylspinochrome A)	0.361	181–182
2,7-Dihydroxy-3-acetylnaphthazarin (2) (spinochrome A)	0.105	192–193

[a] See footnote *a* in Table I. [b] Not determined.

Examination of the nmr spectrum confirmed the structural conclusions. The signal at δ 2.86 affirms the presence of an acetyl with a β hydroxyl adjacent to it and the two doublets (AB quartet) at δ 7.25 and 7.42 (J = 10.5 cps) show the unsubstituted ring of a naphthazarin. Furthermore, the chemical shifts of the two ring protons reflect their aromatic environment and hence the ring bearing the two substituents is predominately quinoidal. The methyl ether (**11**) exhibits the methoxyl signal at δ 4.13 and the acetyl signal at 2.52 as expected.[9,12]

Finally, the mass spectra of **9** and **11** are in perfect agreement with the assigned structures.[13]



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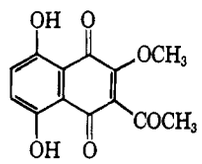

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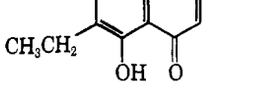
Of the juglone derivatives 2,7-dihydroxy-6-ethyljuglone (**12**) is the major reduction product. The electronic absorption spectrum (Figure 3) is typical for a juglone but it does not correspond to that of 2,7-dihydroxyjuglone.[14] One would suspect at first that **12** does not possess the 2,7-dihydroxyjuglone chromogen.

The nmr spectrum of **12** (in acetone-d_6), however, can only be rationalized with the assigned structure. The signal at δ 7.16 is typical for the *peri*-C-8 proton in the environment of the C-7 hydroxyl.[9] A quinoidal proton with an adjacent hydroxyl is shown by the singlet at δ 6.08 (C-3 proton) and an aromatic ethyl substituent flanked by a β-hydroxyl (at C-7) is evident from the respective quartet and triplet signals at δ 2.72 and 1.14. The C-5 hydroxyl appears at δ 12.93. Compound **12** forms a dimethyl ether with diazomethane and the two methoxyls give nmr signals in the expected region (δ 3.93 and 3.86 for the C-7 and C-2 methoxyls, respectively).

The electronic absorption spectrum of the borohydride product, 2,7-dihydroxy-3-ethyljuglone (**13**), is similar to that of 2,7-dihydroxyjuglone (Figure 3). The reason for the doublet appearance of the visible band of **13** and its collapse to a single peak on the alkylation at C-6 as in **12** is unknown at this time. The structure of **13** is immediately apparent from its nmr spectrum in acetone-d_6 by the doublets at δ 7.08 and 6.58 for the C-8 and C-6 hydrogens, respectively, the C-5 *peri*-hydroxyl signal at 12.62, and the quinoidal ethyl signals at 2.55 and 1.12. Methylation of **13** with diazomethane gives a dimethyl derivative and the nmr spectrum in acetone-d_6 exhibits the C-2 and C-7 methoxyls at δ 4.11 and 3.95, respectively, as expected.[9]



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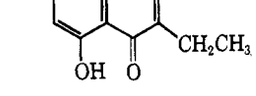

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The structures of 2-hydroxy-3-ethyljuglone (**14**) and 2-hydroxy-6-ethyljuglone (**15**) are also readily deduced from spectral examination. The electronic absorption spectra are typical of juglones and the positions of the ultraviolet bands suggest monohydroxy substitution[8]

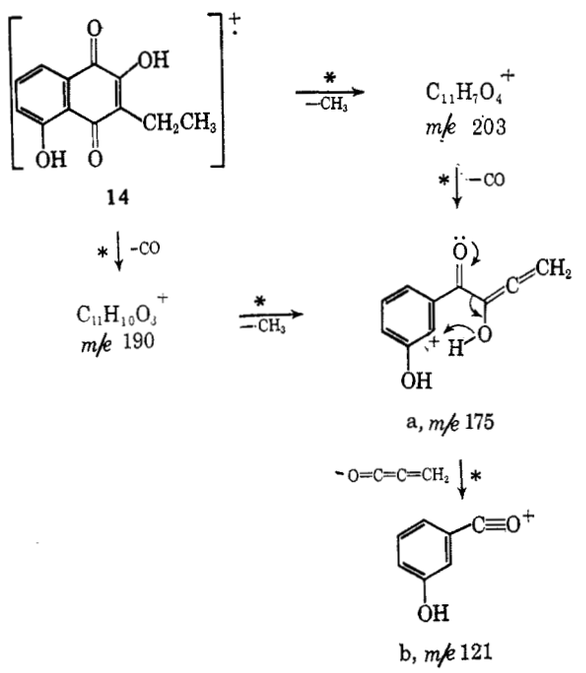
(12) Insufficient material prevented observation of the C-6 and C-7 proton signals, hence the structure of the predominant tautomer is unknown at this time.

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

(14) B. W. Bycroft and J. C. Roberts, *J. Chem. Soc.*, 2063 (1962).

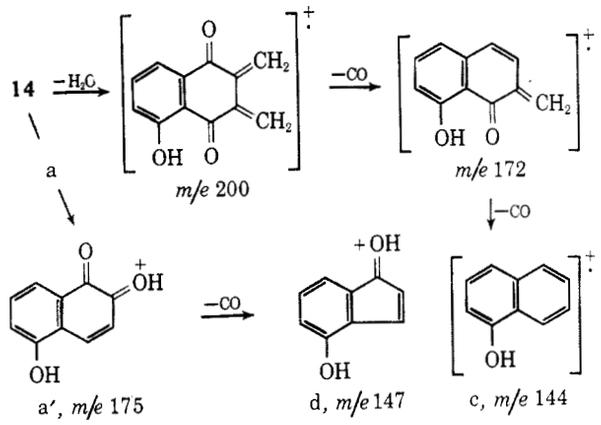
which must be on the quinoidal ring because of the solubility of the compounds in aqueous sodium bicarbonate. The nmr spectra (see the Experimental Section) of **14** and the methyl ether of **15** (2-methoxy-6-ethyljuglone) are consistent[9] with the assigned structures. The structural determination of **14** was based on the position of the *peri*-hydroxyl nmr signal (δ 12.50) showing the attachment of the quinoidal hydroxyl at C-2 rather than at C-3.[9] Absolute proof of structure was obtained upon comparison with a synthetic sample of **14**, produced in 55% yield from the reaction of 2-hydroxyjuglone and propionyl peroxide.

The mass spectra of **14** and **15** confirm the molecular weights with the parent ions forming the base peaks at *m/e* 218. The fragmentation of **14** proceeds essentially as expected[13,15] with the expulsion of methyl radical and carbon monoxide to give the second most abundant ion a at *m/e* 175 (20% relative intensity) which subsequently loses a fragment of 54 mass units *via* a hydrogen-rearrangement mechanism to give the *m/e* 121 ion b (15% relative intensity). An appropriate metastable *ion*[16] accompanies the disintegration of a to



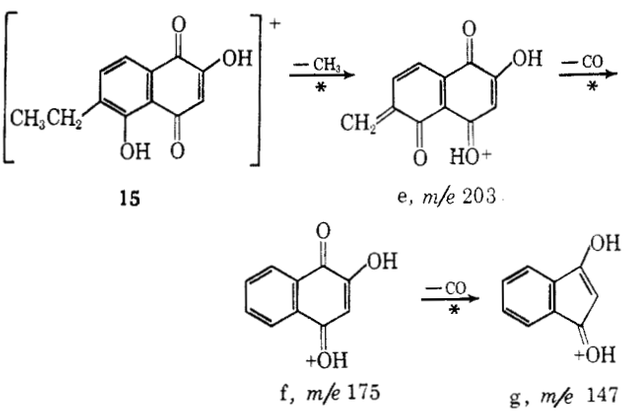
b, but metastable peaks at *m/e*, 140.5 ($175^2/218 = 140.5$), *m/e* 151.0 ($175^2/203 = 151.0$) and *m/e* 161.3 ($175^2/190 = 161.3$) show that ion a can arise by the simultaneous loss of carbon monoxide and methyl radical from the parent ion as well as by loss of carbon monoxide from the M − 15 species and by loss of methyl radical from the M − CO ion. The loss of methyl radical and carbon monoxide from the molecular ion are supported by metastable peaks at *m/e* 189.0 ($203^2/218 = 189.0$) and *m/e* 165.6 ($190^2/218 = 165.6$), respectively. Ethyl radical (possibly CHO) can also be expelled from the parent ion as shown by the *m/e* 189 ion.

Finally, a *m/e* 144 ion (possibly c, 4% relative intensity) and a *m/e* 147 species (possibly d, 7% relative intensity) are present in the mass spectrum of **14**. Although no metastable ions were found to indicate



their formation, c and d could presumably arise in the following manner.

The fragmentation of **15** upon electron impact proceeds in a completely different manner. Elimination of a methyl radical from the molecular ion to form the *m/e* 203 ion e (29% relative intensity) followed by successive losses of carbon monoxide to form ions f (*m/e* 175, 16% relative intensity) and g (*m/e* 147, 4% relative intensity) outlines the principal course of disintegration, and metastable ions at *m/e* 189.0 ($203^2/218 = 189.0$), *m/e* 150.9 ($175^2/203 = 151.0$), and *m/e* 123.5 ($147^2/175 = 123.4$), respectively, accompany these transitions.

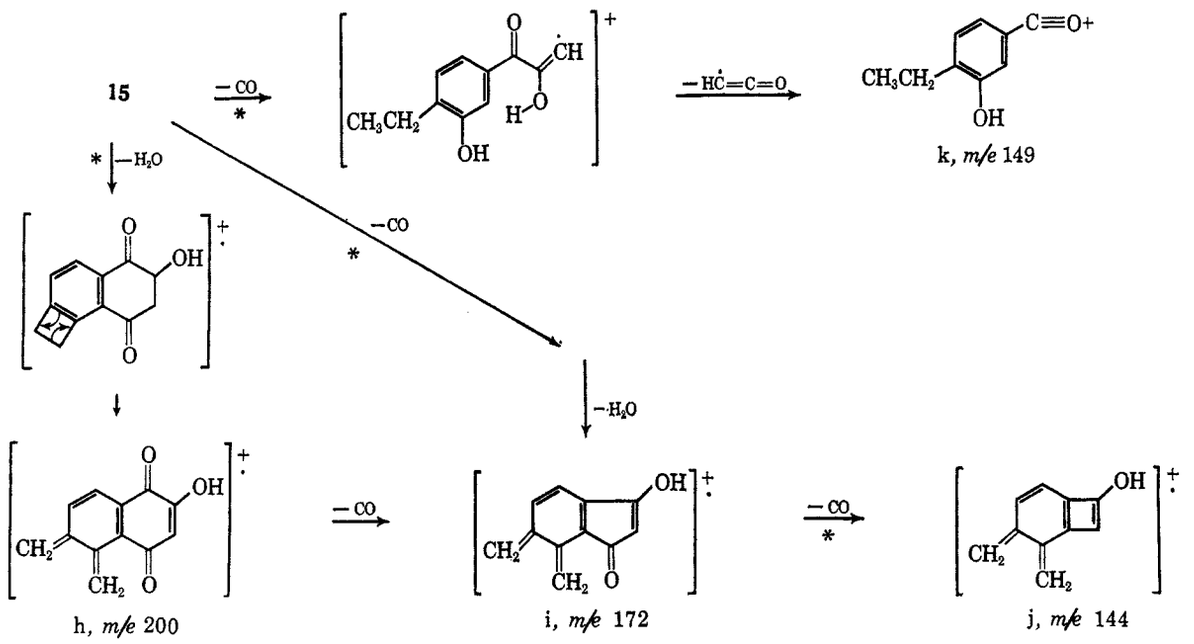


A second pathway of degradation is also discernible in the mass spectrum of **15**. After the initial expulsion of water from the parent ion to form ion h (*m/e* 200, 3.5% relative intensity), carbon monoxide is lost to form the *m/e* 172 ion i (6% relative intensity) which in turn expels carbon monoxide to form the ion j (*m/e* 144, 8% relative intensity). A metastable ion at *m/e* 120.6 ($144^2/172 = 120.6$) accompanies the i → j transition. Ion i could also arise by successive losses of carbon monoxide and water from the molecular ion. Metastable ions at *m/e* 165.6 ($190^2/218 = 165.6$) and *m/e* 183.5 ($200^2/218 = 183.5$) accompany the elimination of carbon monoxide and water, respectively, from the molecular ion. Noteworthy is the suppression of the hydrogen rearrangement typical of 2-hydroxyjuglone[13,15] to form the *m/e* 149 ion k (3% relative intensity).

Finally, two juglones possessing acetyl functional groups in their structural makeup are obtained from the reduction mixture. The major one is 2,7-dihydroxy-6-acetyljuglone (**16**) and its assigned structure is based on its spectral features.[9] The position of the C-8 proton (δ 7.08) in the nmr spectrum of **16** (in acetone-d_6)

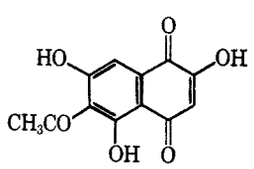
(15) J. H. Bowie, D. H. Cameron, and D. H. Williams, *J. Am. Chem. Soc.*, **87**, 5094 (1965).

(16) Transitions supported by a metastable ions are marked by asterisks in the proposed schemes.



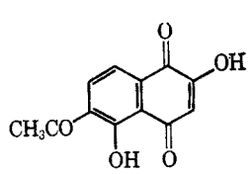
is compatible only with the attachment of a hydroxyl at C-7 and its singlet nature shows substitution at C-6. The signal at δ 2.82 identifies the C-6 substituent as an acetyl group which is strongly hydrogen bonded to the C-7 hydroxyl. The presence of a quinoidal hydroxyl (must be at C-2) is supported by the chemical shift of the C-3 proton signal (δ 6.25).

The electronic absorption spectrum of **16** (Figure 4) shows a twinned visible peak (374 and 469 mμ) and a pronounced rise in the molar extinction coefficients of the ultraviolet absorption bands (ϵ 28,100 for the peak at 241 mμ and ϵ 14,600 for the one at 307 mμ).



16

Examination of the nmr spectrum of 2-hydroxy-6-acetyljuglone (**17**) in acetone-d_6 revealed the structure of this product. The C-8 proton is observed at δ 7.65 as a doublet (J = 8 cps) which demands a proton at C-7. The C-7 proton signal occurs as a doublet at δ 8.02 and the low-field position reflects the C-6 acetyl group, the latter producing a signal at δ 268. The C-3 proton is found at δ 6.30. The mass spectrum of **17** displays the fragmentation pattern described for **16**,[13] but is shifted 16 mass units lower.



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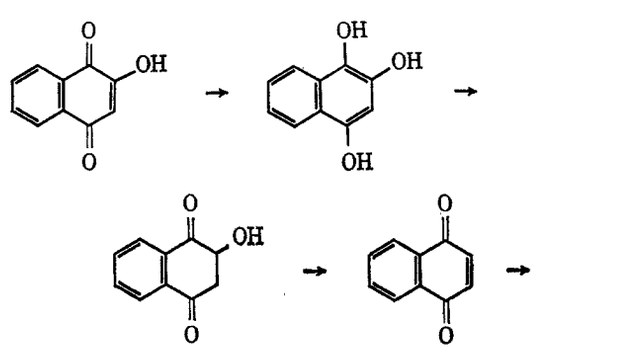
Results and Conclusions.—No compounds possessing the hydroxylethyl side chain were found. Undoubtedly this structural unit can be formed as an intermediate but is probably rapidly reduced to the ethyl group.[17]

Reduction of the acetyl group does not proceed readily as the adjacent β hydroxyl changes the nature of the carbonyl. Tautomerization to the enol form occurs, which is supported by nmr evidence.[9,18] As a result, hydrogenolysis of the ring hydroxyls is a competing reaction and a mixture of products is obtained. The β hydroxyls and the *peri* hydroxyls which have adjacent β hydroxyls can be removed, but the *peri* hydroxyls without adjacent β hydroxyls appear unaffected. Finally, a β hydroxyl and the adjacent *peri* hydroxyl can be removed simultaneously.

The reductive removal of hydroxyl groups from aromatic systems has been observed with stannous chloride[19] and with sodium stannite.[20] Thomson postulates



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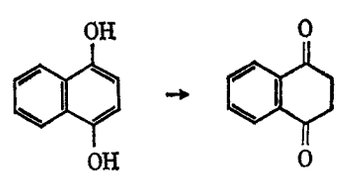


19

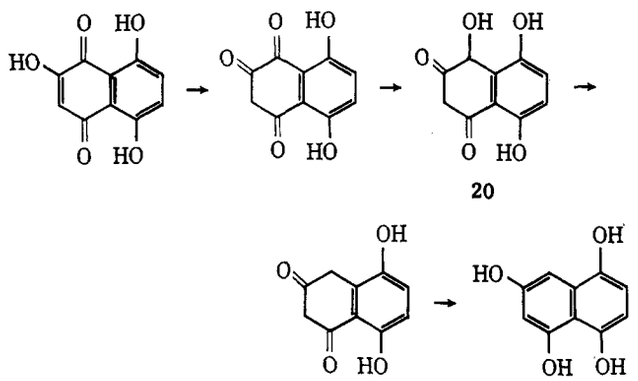
(17) A referee has suggested the following explanation. "In general, borohydride does not reduce benzyl alcohols. Presumably in this case hydrogen bridging of the phenol to the hydroxyl in the hydroxyethyl facilitates dehydration followed by reduction of the benzyl cation with hydride."

(18) This tautomerism is not possible for 2-methoxy-6-acetylnaphthazarin [accompanying manuscript, R. E. Moore, H. Singh, and P. J. Scheuer, ref 7] and this compound is instantly and almost quantitatively reduced to 2-methoxy-6-ethylnaphthazarin with sodium borohydride.

(19) D. B. Bruce and R. H. Thomson, *J. Chem. Soc.*, 1428 (1954).

(20) J. F. Garden and R. H. Thomson, *ibid.*, 2483 (1957).

tautomerization of the intermediate 1,2,4-trihydroxynaphthalene to the diketone **18** followed by dehydration to explain the loss of the β hydroxyl of 2-hydroxy 1,4-naphthoquinone with stannous chloride. In such a scheme the diketone **19** is the end product and can be isolated in good yield.[21] To rationalize the removal of the C-8 *peri* hydroxyl of naphthopurpurin, Thomson suggested the formation of a species **20** which could readily lose its "benzylic" hydroxyl in the reductive medium. Tautomers such as **20** could be responsible for the hydrogenolysis with sodium borohydride and we



20

are at present studying the reduction of simpler compounds to gain some insight into the mechanism of this interesting reaction. Some examples of its use in degradative studies of polyhydroxylated compounds will be presented in forthcoming publications.

Experimental Section[22]

Preparation of the Adsorbent for Chromatography.—The silica gel was wetted with 0.5 hydrochloric acid and allowed to dry at room temperature. All parts of the column were washed with dilute acid prior to use, since glass wool and sintered glass are basic enough to adsorb appreciable amounts of these hydroxynaphthoquinones.

Thin-layer plates of silica gel were prepared using 0.5 *N* hydrochloric acid and were allowed to dry at room temperature.

Sodium Borohydride Reduction of Spinochrome A.—A solution of 1 g of spinochrome A in 150 ml of 70% methanol–water and 25 ml of 10% sodium hydroxide solution was treated with 5 g of sodium borohydride over 1 hr and the reaction mixture was allowed to stand at room temperature. Aliquots were withdrawn periodically and acidified with hydrochloric acid, and the benzene extract was examined on a thin-layer plate of acid-treated, deactivated silica gel to follow the progress of the reduction. When essentially all of the spinochrome A had been reduced, the mixture was acidified with hydrochloric acid and extracted thoroughly with ether. The ether extract and precipitate were shaken with 2 *N* sodium hydroxide solution for a few seconds, the ether layer was discarded, and the basic phase was acidified with hydrochloric acid and extracted with benzene–ether. The benzene–ether extract was concentrated and introduced onto a 80 × 5 cm column of acid-treated, deactivated silica gel. Development of the chromatogram was continued with benzene and eight bands were eluted. The eight fractions had the characteristic colors shown in Table III.

Fraction 6 was unreduced spinochrome A (*ca.* 100–200 mg) which was treated again with sodium borohydride.

(21) See ref 20 for the isolation of such a diketone from the reduction of 3,5,6-trihydroxyjuglone with stannous chloride.

(22) Ultraviolet–visible spectra were determined in chloroform on a Cary 14 spectrophotometer; nmr spectra were obtained on a Varian A-60 instrument. Combustion analyses were generally not determined in order to conserve material and since these highly oxygenated compounds are known to combust poorly. Mass spectrometric analyses (courtesy of Dr. D. Becher and Professor C. Djerassi) were performed on an A.E.I. MS-9 instrument and confirmed the molecular weights of all new compounds. Melting points were determined on a Fisher-Johns apparatus and are uncorrected.

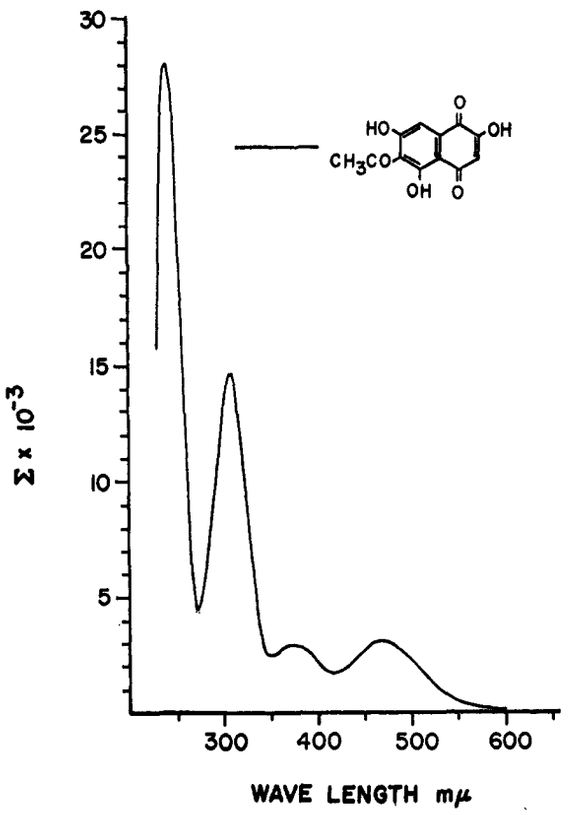


Figure 4.—Electronic absorption spectrum of 2,7-dihydroxy-6-acetyljuglone.

TABLE III

Fraction	Band color
1	Brown
2	Orange
3	Yellow
4	Yellow-brown
5	Red
6	Purple
7	Yellow
8	Yellow

Fraction 1 was distributed between benzene and dilute sodium bicarbonate solution under nitrogen. The product in the benzene phase was chromatographed on a thin layer plate of acid-treated, deactivated silica gel to give a red band from which 0.5 mg of **ethylnaphthazarin** (**6**, 0.06% yield) was obtained, mp and mmp 123–124° (lit.[23] mp 127°). After acidification of the aqueous phase with hydrochloric acid, the reduction products were removed with benzene and separated on a thin-layer plate with benzene into an orange band and a yellow band. The orange band was rechromatographed on a 10 × 1 cm column of water-deactivated silica gel (*not* pretreated with acid). Elution with benzene removed a yellow band and the more strongly adsorbed orange-red band was readily eluted with 5% methanol–chloroform containing a trace of hydrochloric acid. The reduction product in the yellow band crystallized slowly from cold isooctane to give 1 mg of **2-hydroxy-3-ethyljuglone** (**14**, 0.1%) as orange plates, mp 185–186°. Nmr spectrum in $CDCl_3$ showed C-2 hydroxyl, δ 7.42; C-3 methylene, 2.59 (quartet, J = 7.5 cps); C-3 CH_2CH_3, 1.13 (triplet, J = 7.5 cps); C-5 hydroxyl, 12.50; C-6 hydrogen, 7.27 (multiplet, J = *ca.* 8 and 3 cps); C-7 hydrogen, hydrogen, *ca.* 7.57 (multiplet, J = *ca.* 7 and 8 cps); C-8 hydrogen, 7.65 (multiplet, J = *ca.* 7 and 3 cps). The ultraviolet spectrum showed λ_{max} 245 mμ (ϵ 14,800), 288 (12,800), 408 (4200), sh 426 (3990), sh 450 (2940); λ_{min} 263 mμ (ϵ 4070), 263 mμ (ϵ *ca.* 300).

The material in the orange-red band was sublimed at 110° (0.01 mm) and recrystallized from benzene to give 2 mg of

(23) T. Kuroda and M. Wada, *Sci. Papers Inst. Phys. Chem. Res.* (Tokyo), **34**, 1740 (1938); *Chem. Abstr.*, **33**, 2511 (1939).

2-hydroxy-3-ethylnaphthazarin (**7**, 0.2%) as dark red plates, mp 190.5–191.5° (lit.[24] mp 185°). The ultraviolet spectrum gave λ_{max} 296 mμ (ε 7600), sh 385 (1290), sh 476 (5530), sh 490 (6050), 500 (6350), sh 524 (4370), sh 538 (3760); λ_{min} 341 mμ (ε 330).

Fraction 2 was chromatographed on preparative thin-layer plates with benzene and separated into a red band, a yellow band, and an orange band. From the red band 0.5 mg of **2-hydroxy-3-acetylnaphthazarin** (**9**, 0.05%) was obtained as red needles from isooctane, mp 163–164° dec. Nmr spectrum in $CDCl_3$ showed C-3 acetyl, δ 2.86; C-6 hydrogen, 7.25 or 7.42 (doublet, J = 10.5 cps); C-7 hydrogen, 7.25 or 7.42 (doublet, J = 10.5 cps). The ultraviolet spectrum gave λ_{max} sh 250 mμ (ε 9210), 296 (10,500), 490 (4490), sh 525 (3980), sh 568 (1950); λ_{min} 272 mμ (ε 8350), 375–400 mμ (*ca.* ε 1300).

The yellow band crystallized from benzene to give 8 mg of **2-hydroxy-6-ethyljuglone** (**15**, 0.9%) as orange prisms, mp 219–220°. The ultraviolet spectrum showed λ_{max} 237 mμ (ε 8190), 291 (10,300), sh 418 (3460), 432 (3870), sh 455 (3120); λ_{min} 258 mμ (ε 3930), 320 mμ (ε *ca.* 140). Nmr spectrum of 2-methoxy-6-ethyljuglone (mp 137–138°) in $CDCl_3$ gave C-2 methoxyl, δ 3.90; C-3 hydrogen, 6.08; C-7 hydrogen, 7.28 (doublet J = 8 cps); C-8 hydrogen, 7.64 (doublet, J = 8 cps); C-6 methylene, 2.75 (quartet, J = 7.5 cps); C-6 CH_2CH_3, 1.22 (triplet, J = 7.5 cps).

The orange band yielded 20 mg of **2-hydroxy-6-ethylnaphthazarin** (**8**, 2%), brown, fernlike crystals from benzene, mp 204–204.5°. The ultraviolet spectrum showed λ_{max} 302 mμ (ε 8560), sh 479 (5160), 505 (6300), 530 (4150), 544 (4230); λ_{min} 390 mμ (ε 590), 525 (4130), 537 (4020).

Fraction 3 crystallized from chloroform to give 15 mg of **2,7-dihydroxy-6-acetyljuglone** (**16**, 1.5%) as small, orange needles, mp 215° dec. The ultraviolet spectrum gave λ_{max} 241 mμ (ε 28,100), 307 (14,600), 374 (2850), 469 (2960); λ_{min} 273 mμ (ε 4350), 349 (2370), 416 (1600). Nmr spectrum in acetone-d_6 showed C-3 hydrogen, δ 6.25; C-5 or C-7 hydroxyl, 14.75; C-6 acetyl 2.82; C-8 hydrogen, 7.08.

Fraction 4 crystallized from benzene to give 6 mg of **2-hydroxy-6-acetyljuglone** (**17**, 0.7%) as orange-brown leaflets, mp 193–196° dec. The ultraviolet spectrum gave λ_{max} 237 mμ (ε 16,100), 290 (10,700), 425 (4010), 438 (4010); λ_{min} 264 mμ (ε 4570), 323 (470), 432 (3980). Nmr spectrum in acetone-d_6 showed C-3 hydrogen, δ 6.30; C-5 hydroxyl, 13.52; C-6 acetyl, 2.68; C-7 hydrogen, 8.02 (doublet, J = 8 cps); C-8 hydrogen, 7.65 (doublet, J = 8 cps).

The major reduction product was obtained from crystallization of fraction 5 to afford 105 mg of **2,7-dihydroxy-3-ethylnaphthazarin** (**4**, 11%), dark red-brown needles from methanol, mp 190–192°. The ultraviolet spectrum showed λ_{max} 237 mμ (ε 15,100), 265 (7590), 321 (8400), sh 420 (2680), sh 450 (3470), sh 489 (4850), 513 (5510), sh 549 (3540); λ_{min} 256 mμ (ε 7510), 288 (4620), 366 (920). Nmr spectrum in $CDCl_3$ gave C-6 hydrogen, δ 6.67; C-3 methylene, 2.66 (quartet, J = 7.5 cps); C-3 CH_2CH_3, 1.16 (triplet, J = 7.5 cps). Nmr spectrum of 2,7-dimethoxy-6-ethylnaphthazarin (mp 145–147°, long, red needles from isooctane) in $CDCl_3$ showed C-7 methoxyl, δ 4.07; C-6 methylene, 2.73 (quartet, J = 7.5 cps); C-6 CH_2-CH_3, 1.16 (triplet, J = 7.5 cps); C-3 hydrogen, 6.26; C-2 methoxyl, 3.94; C-5 hydroxyl, 13.32; C-8 hydroxyl, 12.80.

Fraction 7 crystallized from benzene to give 10 mg of **2,7-dihydroxy-3-ethyljuglone** (**13**, 1%) as small brick-red crystals, mp 219–220°. The ultraviolet spectrum gave λ_{max} 262 mμ (ε 15,200), 314 (10,500), 391 (2790), 450 (2100); λ_{min} 279 mμ (ε 1850), 346 (1100), 442 (2080). Nmr spectrum in acetone-d_6 showed C-3 methylene, δ 2.55 (quartet, J = 7.5 cps); C-3 CH_2CH_3, 1.12 (triplet, J = 7.5 cps); C-5 hydroxyl, 12.62; C-6 hydrogen, 6.58 (doublet, J = 2.5 cps); C-8 hydrogen, 7.08 (doublet, J = 2.5 cps). Nmr spectrum of 2,7-dimethoxy-3-ethyljuglone (mp 69–70°, red-orange needles from *n*-hexane) in acetone-d_6 showed C-2 methoxyl, δ 4.11; C-3 methylene, 2.58 (quartet, J = 7.5 cps); C-3 CH_2CH_3, 1.10 (triplet, J = 7.5 cps) C-7 methoxyl, 3.95; C-6 hydrogen, 6.71 (doublet, J = 2.5 cps); C-8 hydrogen, 7.08 (doublet, J = 2.5 cps); C-5 hydroxyl, 12.37.

Fraction 8 crystallized from chloroform to give 35 mg of **2,7-dihydroxy-6-ethyljuglone** (**12**, 4%) as red-orange needles, mp 237° dec. The ultraviolet spectrum gave λ_{max} 264 mμ (ε 14,400), 311 (8650), 432 (3410); λ_{min} 280 mμ (ε 2370), 352 mμ (ε 580). Nmr spectrum in acetone-d_6 showed C-3 hydrogen, δ 6.08; C-5 hydroxyl, 12.93; C-6 methylene, 2.72 (quartet, J = 7.5 cps); C-6 CH_2CH_3, 1.14 (triplet, J = 7.5 cps); C-8 hydrogen, 7.16. Nmr spectrum of 2,7-dimethoxy-6-ethyljuglone (mp 189–189.5°, yellow-orange, fernlike crystals from isooctane) in acetone-d_6 gave C-2 methoxyl, δ 4.02; C-3 hydrogen, 6.15; C-5 hydroxyl, 12.68; C-6 methylene, 2.72 (quartet, J = 7.5 cps); C-6 CH_2CH_3, 1.11 (triplet, J = 7.5 cps); C-7 methoxyl, 3.95; C-8 hydrogen, 7.23; nmr in $CDCl_3$ showed C-2 methoxyl, δ 3.95; C-3 hydrogen, 6.08; C-5 hydroxyl, 12.48; C-6 methylene, 2.74 (quartet, J = 7.5 cps); C-6 CH_2CH_3, 1.12 (triplet, J = 7.5 cps); C-7 methoxyl, 3.88; C-8 hydrogen, 7.25.

Synthesis of 2-Hydroxy-3-ethyljuglone (**14**).—Propionyl peroxide (64 mg) was added to a solution of 80 mg of 2-hydroxyjuglone in 20 ml of acetic acid and the mixture was heated at *ca.* 90° for 2.5 hr. After dilution with water, the product was extracted into ether, the ether layer was washed with water and evaporated, and the residual solid was chromatographed on a 45 × 2 cm column of acid-treated, deactivated silica gel. Elution of the major orange-yellow band was achieved with benzene and after recrystallization from benzene a 55% yield of 2-hydroxy 3-ethyljuglone, mp 185–186°, was obtained.

Anal. Calcd for $C_{12}H_{10}O_4$: C, 66.1; H, 4.4; Found: C, 66.0; H, 4.0.

Synthesis of 2-Hydroxy-3-ethylnaphthazarin (**7**) **and 2-Hydroxy-6-ethylnaphthazarin** (**8**).—A suspension of 570 mg of ethylnaphthazarin in 7 ml of acetic acid was treated with 1.5 g of lead tetraacetate. When the solution had become yellow-brown, 250 ml of benzene was added and the mixture was filtered through a short column of magnesium sulfate. The filtrate was evaporated *in vacuo* at room temperature and the residue was treated with 10 ml of acetic anhydride and 0.5–1 ml of concentrated sulfuric acid. After the mixture had stood for 3 hr, it was poured into ethanol and concentrated hydrochloric acid and heated on the steam bath for 0.5 hr. After dilution with water the mixture of ethylnaphthopurpurins was extracted with benzene–ether and chromatographed on an 80 × 5 cm column of acid-treated, deactivated silica gel. Continuous elution with carbon tetrachloride removed a yellow-orange band [90 mg of 2-hydroxy-3-ethylnaphthazarin (**7**, 15%), dark red plates benzene, mp 190.5–191.5°] followed by two orange bands which travelled very closely. The faster moving material crystallized from benzene to give 210 mg of 2-hydroxy-6-ethylnaphthazarin (**8**, 35%) as brown, fernlike crystals, mp 204–204.5°.[23,25] Crystallization of the material in the latter band gave 210 mg of 2-hydroxy-7-ethylnaphthazarin (35%) brown, fernlike crystals from benzene, mp 195.5–196°.[23,25]

Synthesis of 2,7-Dihydroxy-3-ethylnaphthazarin (**4**).—A solution of 100 mg of 2-methoxy-6-ethylnaphthazarin (from methylation of 2-hydroxy-6-ethylnaphthazarin with diazomethane) in 150 ml of benzene was shaken with 250 mg of lead tetraacetate. The yellow solution was filtered and evaporated *in vacuo* and the residue was treated with 10 ml of acetic anhydride and 0.5–1 ml of concentrated sulfuric acid. After standing for 15 min the mixture was decomposed in 6 *N* hydrochloric acid. The crude Thiele product was extracted into ether and subjected to a 24-hr reflux in 25 ml of ethanol–12 *N* hydrochloric acid (1:1) in a nitrogen atmosphere. The hydrolyzed material gave after chromatography a considerable amount of 2-hydroxy-6-ethylnaphthazarin and 28 mg of 2,7-dihydroxy-3-ethylnaphthazarin (**4**, 25%), mp 190–192° (lit.[24,26] mp 192°).

2-Hydroxy-7-ethylnaphthazarin could be converted to 2,6-dihydroxy-3-ethylnaphthazarin in a similar manner in 20% yield as greenish black plates from chloroform, mp 248–249°. The ultraviolet spectrum showed λ_{max} 318 mμ (ε 7530), sh 466 (4550), 490 (5830), sh 517 (4020), 525 (4150); λ_{min} 366 mμ (ε *ca.* 500), 511 mμ (ε 3980). Nmr spectrum of 2,6-dimethoxy-7-ethylnaphthazarin (mp 144–145°, long, orange needles from isooctane) in $CDCl_3$ gave C-6 methoxyl, δ 4.14; C-7 methylene, 2.75 (quartet, J = 7.5 cps); C-7 CH_2CH_3, 1.18 (triplet, J = 7.5 cps); C-5 hydroxyl, 13.23; C-2 methoxyl, 3.94; C-3 hydrogen, 6.26; C-8 hydroxyl, 13.14.

(24) K. Wallenfels, *Chem. Ber.*, **75**, 785 (1942).

(25) In ref 23 is reported the synthesis of the mixture of **8** and 2-hydroxy-7-ethylnaphthazarin, mp 196°. We found that the mixture had mp 194–195°.

(26) In ref 24 Wallenfels does not assign structure **4** to his synthetic compound as he could not exclude the 2,6-dihydroxy possibility.

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.