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SPINOCHROME SYNTHESIS*

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Abstract– Of the five widely distributed structural pigments of the sea urchins (echinoids) one (spinochrome B) is a derivative of juglone, while four (spinochromes A, C, D, and E) are derivatives of naphthazarin. In addition to the two *peri* OH groups of naphthazarin these compounds bear one or more β-hydroxyls and/or an acetyl side chain. The synthesis of these four naphthazarin derivatives is described. The naphthalene skeleton was constructed by condensing 1,2-dihydroxy-3,4-dimethoxybenzene with either chloro- or dichloromaleic anhydride in an aluminum sodium chloride melt. Further oxygen functions were introduced by nucleophilic substitution of chlorine by methoxide ion or by the Thiele method. An acetyl side chain was attached to the polyhydroxynaphthoquinone by preparing the leucoacetate and treating it with acetic anhydride boron trifluoride. The naphthazarin system was regenerated after hydrolysis and oxidation during work-up.

SPINOCHROMES are the pigments which occur in the calcareous skeleta of sea urchins (echinoids). These compounds are, with the exception of a single derivative of benzoquinone,[1] hydroxylated naphthoquinones. Among the unique features of these compounds is their high degree of hydroxylation coupled with the occurrence of ethyl and acetyl side chains. Although these pigments possess inherently simple structures, variants of a naphthalene or β-ethylnaphthalene framework, progress in the elucidation of their structures has until recently been slow for a number of reasons. Isolation of the pigments from the natural sources by the traditional calcium carbonate chromatography rarely yields pure substances; combustion data of these highly oxygenated compounds are often ambiguous; and good synthetic methods for this class of compounds are lacking.

We developed[2,1] efficient column and TLC procedures based on strongly deactivated silica gel, which allowed us to isolate pure compounds and to monitor their homogeneity. We no longer relied on dubious combustion data, but rather made extensive use of NMR[3] and mass spectrometric methods[4] to reinforce postulated structures. We felt nevertheless that rigorous synthetic structure proof of the key pigments was necessary in view of the multiplicity of proposed structures.[5,6] We pointed out above that good synthetic methods are lacking for highly hydroxylated naphthoquinones. The various approaches which have been used over the years were recently reviewed by Fariña.[7] From a review of the pertinent literature it becomes evident that for the synthesis of substituted naphthazarins the tetralone route and the hydroquinone-maleic anhydride condensation possess the greatest versatility although one approach suffers from its multi-step nature and the other from generally low

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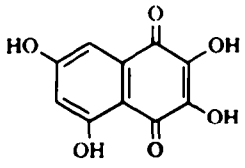
yields and the necessity for separation of by-products. For the problem at hand, the synthesis of several key pigments, we chose the hydroquinone-maleic anhydride approach since we had successfully solved the separation problem.

As a clear picture of the spinochrome structures began to unfold in recent years, spinochromes A, B, C, D and E emerged as the most widely distributed echinoid pigments. Spinochrome B (**1**) is a juglone derivative. Its structural tangle was unravelled by Sutherland[8] and its synthesis was accomplished (at that time known as spinochrome N) by Thomson.[9] Thomson[10] also synthesized spinochrome D (**2**), but A (**3**), C (**4**), and E (**5**) had not been synthesized. In a preliminary communications[11]



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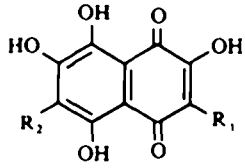
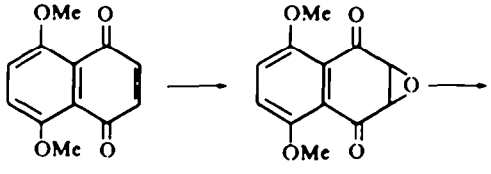


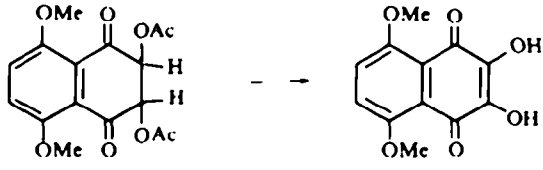
2: R_1 = OH; R_2 = H
3: R_1 = $COCH_3$; R_2 = H
4: R_1 = $COCH_3$; R_2 = OH
5: R_1 = OH; R_2 = OH

we reported a new synthesis of spinochrome D (**2**) and syntheses of A (**3**), C (**4**), and E (**5**). We now wish to report the full details of this investigation.

Spinochrome D (2,3,6-*trihydroxynaphthazarin*, **2**). While our work was in progress, Thomson *et al.*[10] published a synthesis of spinochrome D by the tetralone route. Thereby they established that the published[12] structure of D, 2,3,6-trihydroxy-7-methylnaphthazarin, was incorrect. We also had reached that conclusion as had Sasse[13] in Australia. We felt that a simple synthesis of D could be achieved by further hydroxylation of the known 2,3-dihydroxynaphthazarin. Thomson[14] had described a synthesis of a convenient precursor **9** by the following route. We followed this scheme and arrived at Thomson's **9**, m.p. 211–213° (dec). When this compound was refluxed



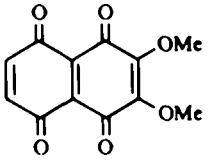
6 **7**



8 **9**

for 2 hr with conc hydrobromic acid, presumably leading to the desired 2,3-dihydroxynaphthazarin, TLC of the product showed a two-component mixture of 2,6- and 2,7-dihydroxynaphthazarins with the 2,6-isomer predominating. We could obtain the same mixture of isomers more simply and in better yield by direct hydrobromic acid treatment of "**8**", followed by air oxidation during work-up. Examination of the reported intermediates led us to conclude that the yellow epoxide **7** did not form the reported glycol diacetate **8** with acetic anhydride-sulfuric acid. The NMR spectrum in deuteriochloroform of the intermediate believed to have structure **8** reveals that **8** in fact is a mixture of 80% 1,2,4,6-tetraacetoxy-5,8-dimethoxynaphthalene and 20% 1,2,4,7-tetraacetoxy-5,8-dimethoxynaphthalene. Apparently opening of the epoxide is followed by formation of a hydroxy- or acetoxyquinone, which undergoes acetylation at either of the free aromatic positions. The quinone is then aromatized and the remaining hydroxy groups are acetylated.

For another attempt at further hydroxylation of 2,3-dihydroxynaphthazarin we prepared the starting material by condensing 1,2-dihydroxy-3,4-dimethoxybenzene with maleic anhydride in an aluminium chloride–sodium chloride melt. This reaction was introduced by Zahn and Ochwat[15] and was used by Wallenfels and Gauhe[16] for their successful synthesis of echinochrome A. The resulting 2,3-dihydroxynaphthazarin was converted to its 2,3-dimethyl ether with diazomethane, and the diether was oxidized with lead tetraacetate to the diquinone **10**. When **10** was treated with



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acetic anhydride in the presence of sulfuric acid, followed by hydrolysis, only 2,3-dihydroxynaphthazarin resulted. Apparently diquinone **10** is not sufficiently reactive for a successful Thiele reaction. This contrasts with the behavior of the monomethoxy compound, which can be hydroxylated under these conditions (*vide infra*).

Since nucleophilic substitution of chlorine by methoxide in a quinone system is a feasible reaction, we next proceeded to prepare 6-chloro-2,3-dihydroxynaphthazarin from chloromaleic anhydride and 1,2-dihydroxy-3,4-dimethoxybenzene. We obtained in fact a mixture of three compounds which after treatment with diazomethane could be separated by preparative TLC on silica gel. The major product (50% of the mixture) was the desired 2,3-dimethoxy-6-chloronaphthazarin, m.p. 134–135°. The other two products, also 50% of the mixture and present in equal amounts, were the result of disproportionation: 2,3-dimethoxynaphthazarin, m.p. 136–137°, and 2,3-dimethoxy-6,7-dichloronaphthazarin, m.p. 204–205°. 2,3-Dimethoxy-6-chloronaphthazarin was refluxed for two days with sodium methoxide in methanol and thus converted to 2,3,6-trimethoxynaphthazarin, the trimethyl ether of spinochrome D, m.p. 161–162°, in addition to small amounts of the 6-hydroxy derivative and unreacted starting material. The trimethyl ether was refluxed in conc hydro-

bromic acid for an hour and converted to spinochrome D, subl. 280–290° without melting, identical in all respects with an authentic sample.*

Spinochrome E (2,3,6,7-*tetrahydroxynaphthazarin*, **5**). 2,3-Dimethoxy-6,7-dichloronaphthazarin, which was an undesired by-product in the spinochrome D synthesis, was the starting material for the preparation of spinochrome E. Alternately, this compound could be made exclusively and in good yield by the condensation of dichloromaleic anhydride with 1,2-dihydroxy-3,4-dimethoxybenzene to 2,3-dihydroxy-6,7-dichloronaphthazarin, m.p. 256–257°. Methylation with diazomethane led to 2,3-dimethoxy-6,7-dichloronaphthazarin. Treatment of this compound with sodium methoxide in methanol led to a mixture which was separated by chromatography on deactivated silica gel. Band **1** was 2,3,6-trimethoxy-7-chloronaphthazarin, m.p. 140–141°; band **2** was 2,3-dimethoxy-6-chloro-7-hydroxy-naphthazarin, m.p. 210–211°; and band **3** was the desired 2,3,6-trimethoxy-7-hydroxynaphthazarin, m.p. 134–135°. It was converted with diazomethane to the tetramethyl ether of spinochrome E, m.p. 185–186°. When the tetramethyl ether was refluxed with conc hydrobromic acid, it was transformed into spinochrome E, subl 300–320° without melting, and identical in all respects with the natural pigment.

Acetyl derivatives of naphthazarins. Since spinochrome A (**3**) and C (**4**) bear acetyl side chains, we first investigated the problem of constructing an acetylnaphthalene skeleton in general terms. Spruit[17] successfully introduced acetyl side chains into hydroxynaphthalenes with acetic anhydride and zinc chloride in glacial acetic acid *via* a Fries rearrangement. We repeated Spruit's work and introduced β-acetyl side chains into 1,5-, 1,7-, and 1,8-dihydroxynaphthalene. We discovered reaction conditions, glacial acetic acid and boron trifluoride etherate or acetic anhydride and boron trifluoride gas, which afforded a cleaner product and more favorable yields than did Spruit's[17] original experiments.

In order to extend this reaction to the preparation of acetylnaphthazarin, naphthazarin had to be reductively acetylated to 1,4,5,8-tetraacetoxynaphthalene. This transformation has been customarily performed with zinc and acetic anhydride. We accomplished this reaction in much improved yields by replacing zinc with hydrogen and 10% palladium-on-charcoal. An acetyl group was then introduced into the leucoacetate and the naphthazarin system was regenerated. When we applied this reaction sequence to 1,4,5,8-tetraacetoxynaphthalene and introduced the acetyl group with acetic anhydride and boron trifluoride, the product was 1-hydroxy-2-acetyl-4,5,8-triacetoxynaphthalene. This compound in turn was hydrolyzed by brief base treatment, followed by exposure to air, and acidification to furnish 6-acetylnaphthazarin. When, on the other hand, the acetyl group was introduced with glacial acetic acid and boron trifluoride, the same starting material yielded 2,6-diacetyl-1,4,5,8-tetrahydroxynaphthalene, from which 2,6-diacetylnaphthazarin was regenerated with lead tetraacetate. Cort and Rodriguez[18] using similar reaction conditions and working on a multigram scale obtained two novel furanonaphthoquinones. We then applied this reaction sequence to 1,2,4,5,8-pentaacetoxynaphthalene (the leucoacetate of naphthopurpurin). Treatment with acetic anhydride-boron trifluoride, followed by alkaline hydrolysis and acidification, led, in addition to some recovered starting material, to 3-acetyl and 6-acetylnaphthopurpurin, which were

* We are grateful to Drs. C. Kuroda and M. Okajima for a sample of spinochrome D.

readily separable by preparative TLC on deactivated silica gel. 3-Acetyl and 6-acetyl-naphthopurpurin proved to be identical with naturally occurring pigments isolated from the genus *Echinothrix*.[5]

Spinochrome A, 3. For the synthesis of spinochrome A (3) we treated chloronaphthazarin with sodium methoxide in methanol, which yielded methoxynaphthazarin in addition to small amounts of a number of other compounds. The mixture was separated on a column of deactivated silica gel and the desired compound was recrystallized from absolute methanol, m.p. 195–196°. The corresponding 2-methoxy-1,4,5,8-diquinone was produced with lead tetraacetate. A Thiele reaction on the diquinone, acetic anhydride and conc sulfuric acid, yielded 2-hydroxy-6-methoxy-naphthazarin as the minor product and the desired 2-hydroxy-7-methoxy-naphthazarin m.p. 240–241° as the major product, readily separable on a column of deactivated silica gel. The leucoacetate, 1,2,4,5,8-pentaacetoxy-2-methoxy-naphthalene, m.p. 222–223°, was prepared from the 2,7-isomer by our improved reductive acetylation technique. For the introduction of the acetyl group we chose the reaction conditions for bisacetylation; we planned to remove subsequently one acetyl group selectively by the procedure which we developed in the course of the structure determination of spinochrome A.[5] Consequently, we treated 1,2,4,5,8-pentaacetoxy-7-methoxynaphthalene with glacial acetic acid and boron trifluoride, followed by base treatment and reacidification. The resulting product was after purification by chromatography in the usual manner the expected 2,7-dihydroxy-3,6-diacetylnaphthazarin, m.p. 237–238°. By boiling this compound for 10 min in methanolic hydrochloric acid and monitoring the progress of the reaction by TLC we obtained a 50% yield of spinochrome A, which we identified in all respects with the natural product. The two minor products were unreacted starting materials and the fully deacetylated 2,7-dihydroxynaphthazarin.

Spinochrome C (4). For the synthesis of spinochrome C (4) we chose as the starting material the leucoacetate of spinochrome D, 1,2,3,4,5,6,8-heptaacetoxynaphthalene, which was available to us through our synthesis of spinochrome D. Treatment with acetic acid-boron trifluoride, followed, without isolation of the intermediate, by hydrolysis with *ethanolic* hydrochloric acid, lead smoothly to spinochrome C, identical with the natural product in all respects. It is interesting to note that methanolic acid leads to deacetylation, while ethanolic acid achieves only normal hydrolysis.

EXPERIMENTAL

M.ps taken on a Fisher–Johns block and uncorrected. UV visible spectra on a Cary 14 recording spectrophotometer. NMR spectra on a Varian A-60 instrument. Signals are reported in δ units with TMS = 0 ppm as the internal standard. Ultra-microanalyses by Berkeley Analytical Laboratory, Berkeley, California.

Preparation of the adsorbent for chromatography

Silica gel (Baker and Adamson, 80–200 mesh, chromatography grade) was washed with 0·5N HCl, filtered and suction-dried. The resulting deactivated silica gel (DSG) was spread on trays and air-dried at room temp. All column parts were washed with dil acid prior to use since glass wool and sintered glass are sufficiently basic to adsorb appreciable amounts of hydroxynaphthoquinones.

For TLC a mixture of 30 g silica gel G (E. Merck, Germany) and 60 ml 0·5N HCl was stirred in a mortar and applied to the plates with a standard Desaga/Brinkmann applicator. The plates were allowed to dry at room temp for at least 6 hr before use.

Attempted preparation of 2,3-dihydroxynaphthazarin by Thomson's[14] procedure

Starting with **6** Thomson's procedure[14] was followed and led to a compound, m.p. 211–213°, as reported and believed to have structure **9**. This compound was refluxed for 2 hr with conc HBr. TLC of the reaction mixture revealed that it contained two components, which were identified as 2,6-dihydroxy- and 2,7-dihydroxynaphthazarin by comparison with authentic samples.[9] The same mixture of isomers was obtained in 20% yield by HBr treatment of the precursor **8**, followed by air oxidation during work-up.

NMR analysis (in $CDCl_3$) of the colorless compound presumed to be **8** showed it to be a mixture of 80% 1,2,4,6-tetraacetoxy-5,8,-dimethoxynaphthalene and 20% 1,2,4,7-tetraacetoxy-5,8-dimethoxynaphthalene: OAc, δ 2·29, 2·32, 2·33 (singlets, rel intensity 3:6:3), OMe, 3·77, 3·85, 3·88 (singlets, rel intensity 3:2·4:0·6); C_3-H, 7·07; C_6-H, 6·77 (singlets, rel intensity 0·2); C_7-H, 6·65 (singlet, rel intensity 0·8).

2,3-*Dihydroxynaphthazarin (spinazarin)*

This compound was prepared by an adaptation of Kuroda's published procedure.[19] To a fused mixture of NaCl (20 g) and anhyd $AlCl_3$ (100 g) at 180° was added a molten mixture of maleic anhydride (10 g) and 1,2,3,4-tetramethoxybenzene (20 g) with constant stirring. The reaction was conducted in N_2 atmosphere and maintained at 180° until the evolution of HCl had ceased. After cooling, the mixture was decomposed with dil HCl. The solid was removed, dried *in vacuo*, and extracted with $CHCl_3$ in a soxhlet. The $CHCl_3$ extract was chromatographed on DSG. Less polar by-products were eluted with $CHCl_3$ and the product with EtOAc–$CHCl_3$ (25:75); yield, 30%, m.p. 265° as reported.[19]

An improved yield (40%) was obtained by using 1,2-dihydroxy-3,4-dimethoxybenzene at 200°.

2,3-*Dimethoxynaphthazarin*

Careful addition of diazomethane to a soln of 2,3-dihydroxynaphthazarin in MeOH furnished a 90% yield of 2,3-dimethoxynaphthazarin, m.p. 136–137°, reported[12] m.p. 133·5°.

2,3-*Dimethoxy-6-chloronaphthazarin*

A 100 ml 3-necked flask was fitted with a stirrer, an air condenser protected by a $CaCl_2$ tube, and a dropping funnel. A finely powdered mixture of 52 g anhyd $AlCl_3$ and 12 g NaCl was heated to 130° by a Wood's metal bath. A mixture of 2 g 1,2-dihydroxy-3,4-dimethoxybenzene and 0·8 g chloromaleic anhydride was slowly released from the funnel while the temp was raised to 180–190° for 6 min when HCl evolution ceased. To the cooled mixture was added 20 ml of ice-cold HCl. After 6 hr standing the solid was filtered and extracted with $CHCl_3$ in a soxhlet. The $CHCl_3$ extract when spotted on TLC plates showed 3 compounds to be present. The crude mixture was dissolved in a minimum amount of MeOH to which an ethereal soln of diazomethane was slowly added. The reaction was followed by TLC. When all starting material had disappeared, MeOH was evaporated, and the residue was taken up in $CHCl_3$. The mixture was applied to thick-layer plates and developed with benzene. Three bands were removed, washed with chloroform and characterized.

Band **1** crystallized from MeOH, brownish-yellow needles, m.p. 204–205°: 2,3-dimethoxy-6,7-dichloronaphthazarin. NMR spectrum in $CDCl_3$, C_2-OMe and C_3-OMe, δ 4·16; C_5-OH and C_8-OH, 12·97. UV spectrum, $\lambda_{max}^{CHCl_3}$ 260, 325, 482 sh, 485, 515, 525 mμ (Found: C, 45·3; H, 2·8. $C_{12}H_8O_6Cl_2$ requires: C, 45·2; H, 2·5%).

Band **2** from MeOH, brownish plates, m.p. 134–135°: 2,3-dimethoxy-6-chloronaphthazarin. NMR spectrum in $CDCl_3$: C_2-OMe, δ 4·16; C_3-OMe, 4·12; C_7-H, 7·35; C_5-OH, 12·60 or 12·78; C_8-OH, 12·60 or 12·78. UV spectrum: $\lambda_{max}^{CHCl_3}$: 305, 470, 498, 535 sh mμ (Found: C, 50·2; H, 3·5. $C_{12}H_9O_6Cl$ requires: C, 50·6; H, 2·2%).

Band **3** from MeOH, green needles, m.p. 136–137°, reported[12] m.p. 133·5°. It was identical with a sample of 2,3-dimethoxynaphthazarin.

Spinochrome D

A saturated soln of NaOMe was prepared from MeOH (300 ml) and Na. The solution was kept under N_2 and 2,3-dimethoxy-6-chloronaphthazarin (30 mg) in MeOH was added slowly. The mixture was refluxed for 2 d under N_2 and the color changed from dark blue to purple. MeOH was removed under vacuum and the residue was acidified with ice-cold HCl. The acidic soln was extracted with $CHCl_3$; the extract was dried over Na_2SO_4 and stripped. The residue was dissolved in benzene and chromatographed over DSG. Elution with benzene yielded orange-red fraction **1**, red fraction **2**, and brick-red fraction **3**. Fraction **1** was unreacted starting material. Fraction **2** crystallized from MeOH, brown needles, m.p.

161–162°. It was shown to be a 42% yield of 2,3,6-trimethoxynaphthazarin by mixture m.p. and comparison of UV, IR, and NMR spectra with those of an authentic sample of spinochrome D trimethyl ether. Fraction 3 was shown to be 2,3-dimethoxy-6-hydroxynaphthazarin ($\lambda_{max}^{CHCl_3}$ 325, 465, 492, 530 mμ) by its conversion to spinochrome D trimethyl ether on treatment with diazomethane in MeOH.

2,3,6-Trimethoxynaphthazarin (10 mg) was suspended in 10 ml 48% HBr and refluxed for 1 hr under N_2. The soln was cooled in an ice bath and filtered. The residue (4 mg) was sublimed at 150° *in vacuo*. The sublimed product, subl 280–290°, was identical in all respects with an authentic sample of spinochrome D.*

2,3-Dimethoxy-6,7-dichloronaphthazarin

1,2-Dihydroxy-3,4-dimethoxybenzene was condensed with dichloromaleic anhydride in $AlCl_3$–NaCl and yielded 75% of 2,3-dihydroxy-6,7-dichloronaphthazarin, m.p. 256–257°. UV spectrum: $\lambda_{max}^{CHCl_3}$: 260, 325, 462 sh, 485, 515 mμ. On treatment with diazomethane in MeOH it was converted quantitatively to 2,3-dimethoxy-6,7-dichloronaphthazarin.

Spinochrome E

2,3-Dimethoxy-6,7-dichloronaphthazarin (80 mg) was dissolved in MeOH and dropped into a saturated soln of NaOMe in MeOH (800 ml). The mixture was refluxed for 2d under N_2. Solvent was removed *in vacuo* and the residue acidified with ice-cold HCl. The acidic soln was extracted with $CHCl_3$ and the $CHCl_3$ extract was chromatographed on DSG.

Band 1, light brown needles from MeOH, m.p. 140–141°: 2,3,6-trimethoxy-7-chloronaphthazarin. NMR spectrum in $CDCl_3$: C_2-OMe, δ 4·11; C_3-OMe, 4·15; C_6-OMe, 4·18; C_5-OH, C_8-OH, 12·58 and 12·84. UV spectrum, $\lambda_{max}^{CHCl_3}$ 240, 324, 478, 500, 530 sh mμ (Found: C, 49·3; H, 4·0. $C_{13}H_{11}O_5Cl$ requires: C, 49·6; H, 3·5%).

Band 2, orange-red needles from petroleum ether, m.p. 210–211°: 2,3-dimethoxy-6-hydroxy-7-chloronaphthazarin. NMR spectrum in $CDCl_3$: C_2-OMe, δ 4·10 C_3-OMe, 4·16; C_5-OH, C_8-OH, 12·16 and 13·20. UV spectrum, $\lambda_{max}^{CHCl_3}$ 241, 335, 475, 500, 535 mμ (Found: C, 47·9; H, 3·0. $C_{12}H_9O_5Cl$ requires: C, 48·0; H, 3·0%). Diazomethane in MeOH converted this compound smoothly into 2,3,6-trimethoxy-7-chloronaphthazarin.

Band 3, garnet red needles from chloroform, m.p. 134–135°: 2-hydroxy-3,6,7-trimethoxynaphthazarin (34% yield). NMR spectrum in $CDCl_3$: C_3-OMe, δ 4·16; C_6-OMe, 4·08; C_7-OMe, 4·04; C_2-OH, 6·90 (broad); C_5-OH, C_8-OH, 12·16 and 13·30. UV spectrum, $\lambda_{max}^{CHCl_3}$ 333, 460, 490, 522 mμ.

Treatment with diazomethane in MeOH converted this compound to the tetramethyl ether of spinochrome E, m.p. 185–186°; reported[20] m.p. 185°.

2,3,6-Trimethoxy-7-hydroxynaphthazarin (6 mg) was refluxed in 48% HBr for 2 hr. On cooling dark red needles, subl 300–320°, of spinochrome E could be collected. Reported [20] m.p. 320°. Direct comparison with a sample isolated from natural sources proved its identity.

1,4,5,8-Tetraacetoxynaphthalene

Method A. A mixture of 500 mg naphthazarin, 6 ml Ac_2O, 50 mg Zn dust, and 20 mg of KOAc was refluxed until the red color had been discharged (ca. 30 min). After filtration while still hot, the inorganic material was washed with hot HOAc and the combined filtrate and washings were evaporated *in vacuo*. The residue was washed with water and crystallized twice from HOAc to afford 200 mg of 1,4,5,8-tetraacetoxynaphthalene (21%) as white needles, m.p. 280–281° (lit.[21] m.p. 277–279°) NMR spectrum in $CDCl_3$: C_2-, C_3-, C_6-, and C_7-H, δ 7·21; acetoxyl, 2·36

Method B. A mixture of 200 mg naphthazarin, 20 mg anhyd KOAc and 10 mg 10% Pd–C in 10 ml Ac_2O was hydrogenated under ordinary conditions. After complete discharge of the red color (ca. 1·5 hr), the mixture was refluxed for 30 min under N_2, filtered, diluted with water, and cooled for several hr. The white precipitate was collected and crystallized from MeOH to give 240 mg of 1,4,5,8-tetraacetoxynaphthalene (63%).

2-Acetyl-4,5,8-triacetoxyl-1-naphthol

A soln of 100 mg 1,4,5,8-tetraacetoxynaphthalene in 5 ml Ac_2O was treated with BF_3 until the soln had become dark yellow and the temp had risen to 110°. After cooling and standing for 1 hr at room temp, Ac_2O was removed *in vacuo* and the residual solid was washed with water. The yellow product (90 mg)

* See footnote * on page 2972

was chromatographed on a 25 × 1·5 cm column of DSG. Elution with benzene removed 5 mg of 2,6-diacetyl-1,4,5,8-tetrahydroxynaphthalene (see below). The major product was removed with ethyl acetate, vacuum-sublimed, and crystallized from EtOAc to give 50 mg of 2-acetyl-4,5,8-triacetoxyl-1-naphthol (50%) as yellow needles, m.p. 209–210°. (Found: C, 60·0; H, 4·4. $C_{18}H_{16}O_8$ requires: C, 60·0; H, 4·4%). NMR spectrum in $CDCl_3$: C_1-OH, δ 14·56; C_2-acetyl, 2·58; C_3-H, 7·30; C_4-, C_5-, and C_8-acetoxyls, 2·36 (relative intensity acetoxyl: acetyl, C_6- and C_7-H, 3:1); 7·18 (partially resolved quartet).

Acetylnaphthazarin

Method A. A soln of 25 mg 2-acetyl-4,5,8-triacetoxyl-1-naphthol in 5 ml of 1Naq NaOH (prepared under N_2) was exposed to air for a few min until the initial red-orange color had changed to green and was then acidified with ice-cold, dil HCl. The product was extracted immediately with $CHCl_3$, the extract dried over Na_2SO_4, and the solvent removed *in vacuo* at 35°. Upon chromatography on DSG two red bands separated with benzene elution. The faster-moving material was naphthazarin, but the second band afforded after crystallization from petroleum ether 5 mg of acetylnaphthazarin (32%) as purple-black needles, m.p. 106°. (Found: C, 61·5; H, 3·9. Calc. for $C_{12}H_8O_5$: C, 62·1; H, 3·4%).

Method B. A soln of 25 mg 2-acetyl-4,5,8-triacetoxyl-1-naphthol in 5 ml MeOH and 5 ml of 6N HCl was heated at 70–75° for 30 min and then evaporated *in vacuo*. The residue was chromatographed on DSG as above. Naphthazarin (2·5 mg) was removed first with benzene, followed by 2 mg of acetylnaphthazarin (13%). Unhydrolyzed starting material (6 mg) was recovered when the column was eluted with EtOAc.

2,6-*Diacetyl*-1,4,5,8-*tetrahydroxynaphthalene*

A suspension of 100 mg 1,4,5,8-tetraacetoxynaphthalene in 6 ml HOAc was treated with BF_3 gas until the acetate had completely dissolved. The resulting orange-red soln was concentrated *in vacuo* and treated with water. The orange ppt was collected and chromatographed on DSG. Elution with benzene removed 50 mg 2,6-diacetyl-1,4,5,8-tetrahydroxynaphthalene (65%), small orange prisms from EtOH, m.p. 255–256°. NMR spectrum in $CDCl_3$: C_1- and C_4- *or* C_5- and C_8-OH, δ 13·10; C_2- and C_6- Acetyls, 2·70; C_3- and C_7-H, 7·20. (Found: C, 65·1; H, 4·0. Calc. for $C_{14}H_{12}O_6$: C, 64·8; H, 3·9%).

2,6-*Diacetylnaphthazarin*

Lead tetraacetate was added slowly to a soln of 5 mg 1,4,5,8-tetrahydroxy-2,6-diacetylnaphthalene in 5 ml benzene until the soln became light yellow. This soln was filtered and the filtrate was evaporated. To the residue MeOH was added and it was heated for 10 min. The concentrated soln was put on a column of DSG and eluted with $CHCl_3$. The purple soln was concentrated and chromatographed again, but eluted with benzene. Benzene was removed and the residue was crystallized from MeOH, purple needles, 2 mg, m.p. 250–251°. (Found: C, 58·9; H, 4·7. Calc. for $C_{14}H_{10}O_6 \cdot MeOH$ C, 58·8; H, 4·5%).

1,2,4,5,8-*Pentaacetoxynaphthalene*

2,5,8-Triacetoxy-1,4-naphthoquinone (50 mg) was taken up in 10 ml Ac_2O and 5 mg 10% Pd-C was added. On hydrogenation at room temp the dark red soln changed to light yellow. This soln was refluxed for 15 min under N_2 and filtered through sintered glass. The filtrate was evaporated on a water bath under reduced press. Water was added to the residue and the mixture was cooled. The resulting white ppt was filtered and crystallized from aq EtOH, white needles, m.p. 179° as reported.[15] NMR spectrum in $CDCl_3$: C_3-H, δ 7·33; C_6- and C_7-H, 7·22; C_2-OAc, 2·30; C_1-, C_4-, C_5-, and C_8-OAc, 2·40.

3-*Acetyl- and* 6-*acetylnaphthopurpurin*

1,2,4,5,8-Pentaacetoxynaphthalene (50 mg) was taken up in 5 ml Ac_2O. BF_3 was passed through the soln until it turned dark yellow. Some solvent (ca. 3 ml) was removed under vacuum and H_2O was added. The soln was cooled; 5 ml N NaOH was added; and the basic soln was allowed to stand for 10 min. The soln was acidified with ice-cold HCl and extracted with $CHCl_3$. The $CHCl_3$ extract was dried over Na_2SO_4 for 3 hr. The dried soln was filtered and concentrated to ca. 3 ml. This soln was applied to DSG plates to which 0·5 mm layers of adsorbent had been applied. $CHCl_3$ developed 4 bands which were removed, extracted with $CHCl_3$, and worked up as usual.

Band 1, dark purple needles from isooctane, m.p. 160–161°. It was identical in all respects with 2-hydroxy-3-acetylnaphthazarin isolated from sea urchin spines of the genus *Echinothrix*.[5]

Band 2 was naphthopurpurin.

Band 3, purple needles from isooctane, m.p. 179–180°. Direct comparison with a naturally occurring sample[5] proved it to be 2-hydroxy-6-acetylnaphthazarin.

Band 4, red crystals from isooctane, m.p. not determined, was not completely characterized. NMR spectrum in $CDCl_3$: C_7-Ac, δ 2·70.

2-Chloronaphthazarin

A powdered mixture of 10 g maleic anhydride and 14·5 chloroquinol was added to a mixture of 20 g NaCl and 100 g anhyd $AlCl_3$ at 180° under N_2. After HCl evolution had ceased, the mixture was cooled and treated with dil HCl. The resulting ppt was filtered and triturated with several portions of benzene. The combined benzene solns and benzene extracts of the filtrate were stripped *in vacuo*. The residue was chromatographed on a column of DSG and afforded 3·8 g (27%) chloronaphthazarin, m.p. 177–178°, reported[22] m.p. 179°. The product was not homogeneous when spotted on a plate. Impurities of dichloronaphthazarin (faster-moving spot) and naphthazarin (slower-moving spot) were observed indicating that disproportionation occurred during the Zahn–Ochwat reaction.

2-Methoxynaphthazarin

A saturated soln of crude chloronaphthazarin (1 g) in MeOH was added very slowly dropwise to 1 l. of a saturated soln of NaOMe in MeOH (from Na) at reflux and under N_2. The mixture was refluxed for 2–3 d and then evaporated *in vacuo*. The residual solid was treated carefully with dil HCl and the product was extracted into benzene. Chromatography on an 80 × 5 cm column of DSG using benzene as the eluant separated the crude product into a small amount of naphthazarin and recovered chloronaphthazarin (red band), methoxynaphthazarin containing a small amount of naphthopurpurin and chloromethoxynaphthazarins (orange band), and a small amount of 2,6-dimethoxynaphthazarin and 2,7-dimethoxynaphthazarin (red-orange band). The main orange band gave after two crystallizations from abs MeOH an 82% yield of 2-methoxynaphthazarin, black needles, m.p. 195–196°; reported[23] m.p. 178°.

2-Hydroxy-6- (and 7)-methoxynaphthazarins

A soln of 1 g 2-methoxynaphthazarin in 1 l. benzene was shaken with 2·5 g $Pb(OAc)_4$. The yellow soln was filtered and evaporated *in vacuo* and the residue was treated with 25 ml Ac_2O and 2–3 ml of conc H_2SO_4. After standing 1 hr the mixture was decomposed in EtOH and HCl and the resulting soln was warmed to 50° for 5–10 min. The products were removed by benzene–ether extraction. Chromatography on an 80 × 5 cm column of DSG achieved separation into an orange band (small amount of methoxynaphthazarin), an orange-yellow band [205 mg of 2-hydroxy-6-methoxynaphthazarin (20%), small brick-red needles from benzene–chloroform, m.p. 265–267°], and a red band [600 mg of 2-hydroxy-7-methoxynaphthazarin (55%) small purple needles from $CHCl_3$, m.p. 240–241°].

2,6-Dihydroxy- and *2,6-dimethoxynaphthazarin*

Hydrolysis of 2-hydroxy-6-methoxynaphthazarin with conc HBr (1 hr reflux under N_2) afforded a 50% yield of 2,6-dihydroxynaphthazarin, brick-red crystals after vacuum sublimation and crystallization from $CHCl_3$, subl 270–285° without melting. Methylation with diazomethane gave 2,6-dimethoxynaphthazarin, dark red needles from acetone, m.p. 295–296°, identical in all respects with the product obtained via the Charrier and Tocco reaction on 1,5-dinitro-2,6-dimethoxynaphthalene.[5]

2,7-Dihydroxy- and 2,7-dimethoxynaphthazarin

Hydrolysis of 2-hydroxy-7-methoxynaphthazarin with 1:1 EtOH–12N HCl (48 hr reflux under nitrogen) or with conc HBr (2 hr reflux under N_2) gave a 93% or 50% yield, respectively, of 2,7-dihydroxy-naphthazarin, dark red crystals after vacuum sublimation and crystallization from $CHCl_3$, subl 265–275° without melting, identical in every respect with the natural pigment and the acid hydrolysis product of spinochrome A (2 hr reflux in conc HBr under N_2). Methylation of 2-hydroxy-7-methoxynaphthazarin with diazomethane produced 2,7-dimethoxynaphthazarin, red needles from isooctane, m.p. 273–275° with some sublimation, identical in every respect with the product obtained when spinochrome A is treated with methanolic HCl.[9]

1,2,4,5,8-Pentaacetoxy-7-methoxynaphthalene

The leucoacetate was prepared in 72% yield from 2-hydroxy-7-methoxynaphthazarin using Method B above for the preparation of 1,4,5,8-tetraacetoxynaphthalene. The product crystallized from HOAc as

white needles, m.p. 222–223°. NMR spectrum in $CDCl_3$: C_1-, C_2-, C_4-, C_5-, and C_8-acetoxyls, δ 2·27, 2·36, and 2·38 (singlets, relative intensities 1:3:1); C_3- and C_6-H, 7·00; C_7-OMe, 3·86. (Found: C, 56·1; H, 4·2. $C_{21}H_{20}O_{11}$ requires: C, 56·2; H, 4·5%).

2,7-Dihydroxy-3,6-diacetylnaphthazarin

A soln of 36 mg 1,2,4,5,8-pentaacetoxy-7-methoxynaphthalene in 5 ml HOAc was treated with BF_3 until it had turned dark yellow. The solvent was removed *in vacuo* and the residual solid was treated with cold dil HCl or NaOH solution followed by acidification with ice-cold HCl. The product was extracted with $CHCl_3$, chromatographed on DSG, and crystallized from isooctane-$CHCl_3$ to give 5 mg of 2,7-dihydroxy-3,6-diacetylnaphthazarin (20%) as purple needles, m.p. 237–238°. (Found: C, 55·3; H, 4·1. $C_{14}H_{10}O_8$ requires: C, 54·9; H, 3·3%).

Spinochrome A

To a soln of 5 mg 2,7-dihydroxy-3,6-diacetylnaphthazarin in MeOH was added 10 ml dil HCl. This soln was boiled; an aliquot was removed every 2 min, extracted with $CHCl_3$ and spotted on a plate. Three products were isolated. Band 1 was starting material. Band 2 was spinochrome A (50% yield) identical in all respects with the natural pigment.[5,6] Band 3 was identical with an authentic sample of 2,7-dihydroxynaphthazarin.

1,2,3,4,5,6,8-Heptaacetoxynaphthalene

Spinochrome D was reductively acetylated as described above leading to the leucoacetate, m.p. 212–213°, reported[24] m.p. 210°.

Spinochrome C

1,2,3,4,5,6,8-Heptaacetoxynaphthalene (10 mg) in 2 ml HOAc was treated with BF_3. Most of the HOAc was removed *in vacuo*. To the residue was added 1 ml dil HCl and 2 ml EtOH and the mixture was heated at 80° for 20 min. Solvent was removed *in vacuo*. Following chromatography spinochrome C was obtained in 5% yield, identical in all respects with the natural product.[6]

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