204. *Naturally Occurring Quinones. Part V.*[1] *Spinochromes E and N.*

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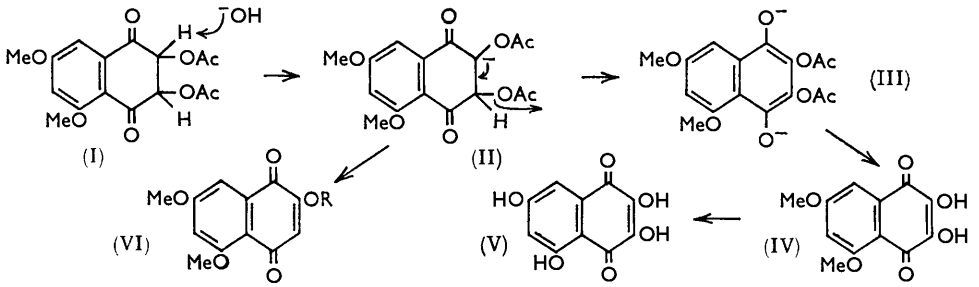
The structure of spinochrome N, 2,3,5,7-tetrahydroxy-1,4-naphthaquinone, is confirmed by synthesis. Spinochrome E is 2,3,5,6,7,8-hexahydroxy-1,4-naphthaquinone.

One of the many unusual features of sea urchins is the presence of polyhydroxynaphthaquinones (spinochromes) in the spines and test (shell) of many species. Although most work on the isolation of these pigments has been limited to the calcified regions of the animals, the occurrence of spinochromes in the internal organs and tissues is not excluded. In *Diadema antillarum* Philippi, for example, a naphthaquinone pigment is distributed generally throughout the animal.[2] This may be the case in other species and is probably significant. Although little is known of the biological function of these pigments some progress is now being made,[3] and it is desirable that their chemical structures should be more firmly established. About a dozen spinochromes are known,[4] but some of the structures put forward are not securely based, and none has been synthesised. In this paper we describe a synthesis of spinochrome N and put forward a structure for spinochrome E.

Spinochrome N.[5]—This pigment was isolated from the dark green spines of *Hemicentrotus pulcherrimus* (Ag.) [6] and the dark violet spines of *Anthocidaris crassispina* (Ag.) [7] by Kuroda and her co-workers, who proposed structure (V).[8] This has recently been supported [9] by oxidative degradation of its tetramethyl ether to 3,5-dimethoxyphthalic

[1] Part IV, Lovie and Thomson, *J.*, 1961, 485.
[2] Millott, *Proc. Zool. Soc. Lond.*, 1957, **129**, 263.
[3] Millott and Yoshida, *J. Exp. Biol.*, 1957, **34**, 394.
[4] Thomson, " Naturally Occurring Quinones," Butterworths, London, 1957.
[5] For nomenclature see ref. 4.
[6] Kuroda and Iwakura, *Proc. Imp. Acad.* (*Tokyo*), 1942, **18**, 74.
[7] Kuroda and Koyasu, *Proc. Imp. Acad.* (*Tokyo*), 1944, **20**, 23.
[8] A full account is given by Okajima, *Sci. Papers Inst. Phys. Chem. Res., Tokyo*, 1959, **53**, 356.
[9] Kuroda and Okajima, *Proc. Japan Acad.*, 1958, **34**, 616.

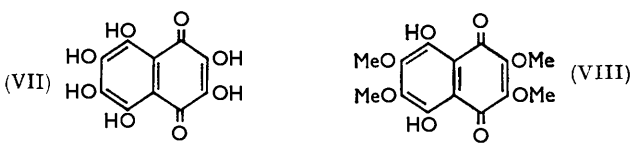
acid, and is now confirmed by synthesis. By the general procedure [10] for preparation of 2,3-dihydroxy-1,4-naphthaquinones, 5,7-dimethoxy-1,4-naphthaquinone was converted into 2,3-dihydroxy-5,7-dimethoxy-1,4-naphthaquinone (IV). Treatment of this with



diazomethane gave a tetramethoxyquinone identical with spinochrome N tetramethyl ether, and demethylation with aluminium chloride–sodium chloride afforded spinochrome N.

The formation of the dihydroxyquinone (IV) by aeration of the diacetate (I) in alkaline solution did not proceed smoothly and the yield was poor. Best results were obtained when the reaction was stopped before all the diacetate had disappeared, but even then a mixture was produced from which a monohydroxydimethoxyquinone was isolated. Larger amounts of the latter were isolated if either the reaction time or the alkali concentration was increased, probably owing to removal of the desired dihydroxyquinone by oxidative degradation. The monohydroxy-derivative was shown to have structure (VI; R = H) by treatment with methanolic hydrochloric acid which gave 2,5,7-trimethoxy-1,4-naphthaquinone (flaviolin trimethyl ether). The relative difficulty in obtaining the quinone (IV) from the diacetate (I), in comparison with previous examples,[10] may be attributed to the presence of two methoxyl groups in conjugation with the carbonyl group at position 4. This arrangement should assist initial proton abstraction from position 2 and subsequent elimination of acetate anion (see II), leading to the monohydroxyquinone (VI; R = H). The latter step competes with the formation of the dienol anion (III) which is required for the production of the dihydroxyquinone (IV).

Spinochrome E.[11]—This quinone occurs, with several others, in Mediterranean specimens of *Paracentrotus lividus* (Lam.) [12] and was recently isolated by Yoshida [13] from the test and spines of *Psammechinus miliaris* (Gmelin) (it is also present in the coelomic fluid of this species). In contrast to other spinochromes this one is insoluble in ether and has a very

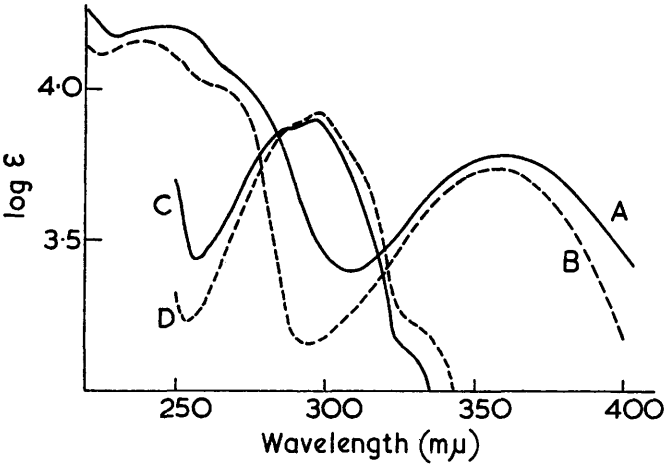


high fusion point (>350°),[11] but the usual polyhydroxynaphthaquinone structure is apparent from its alkali-solubility, reversible reduction with dithionite, and ultraviolet- and visible-light absorption.[11,12]

The sample of spinochrome E, kindly supplied by Dr. M. Yoshida, had been crystallised from acidified (HCl) methanol and contained chlorine (3·3%). Nitrogen (2·3%) was also present which may have originated from the pyridine used in the purification procedure or may have been associated with the pigment *in vivo* as the initial crude material is soluble

[10] Garden and Thomson, *J.*, 1957, 2483.
[11] Smith and Thomson, *Tetrahedron Letters*, 1960, No. 1, 10.
[12] Lederer, *Biochim. Biophys. Acta*, 1952, **9**, 92.
[13] Yoshida, *J. Mar. Biol. Assoc. U.K.*, 1959, **38**, 455.

in hydrochloric acid. After purification *via* the acetate the quinone gave satisfactory analyses for a formula $C_{10}H_6O_8$. Since it formed a hexa-acetate, which on reductive acetylation gave a leuco-octa-acetate, all the oxygen atoms are accounted for, and moreover the ultraviolet spectra of these two compounds are very similar to the corresponding derivatives of spinazarin (2,3,5,8-tetrahydroxy-1,4-naphthaquinone) (see Figure). Spinochrome E is thus the hexahydroxynaphthaquinone (VII). (Tautomeric structures are not



(A) *Spinochrome E hexa-acetate*, (B) *2,3,5,8-tetra-acetoxy-1,4-naphthaquinone, in methanol*; (C) *spinochrome E leuco-octa-acetate*; (D) *1,2,3,4,5,8-hexa-acetoxynaphthalene, in chloroform.*

ruled out on this evidence but are much less probable.) This structure is consistent with its colour reactions,[11] low solubility, and high melting point. On treatment with diazomethane it gave a tetramethyl ether which, from its colour reactions and the absence of hydroxyl absorption in the 3 μ region, contains no β-hydroxyl groups and therefore has structure (VIII).

Mukai [14] has shown recently that namakochrome, the prosthetic group of a purple chromoprotein present in the holothuroid *Polycheira rufescens* (Brandt), is a pentahydroxymonomethoxynaphthaquinone. It gives a trimethyl ether with diazomethane which we find to be identical with spinochrome E tetramethyl ether (VIII). Hence namakochrome is a mono-β-methyl ether of spinochrome E. The occurrence of this pigment in a holothuroid and the recent discovery [15] of polyhydroxyanthraquinones in crinoids indicate that polyhydroxyquinones may be fairly widely distributed in echinoderms, but their origin remains obscure. Their close structural relationship to acetate-derived fungal quinones (*e.g.*, flaviolin) suggests that they might be biosynthesised from C_2 units but there is no evidence, as yet, which shows that this metabolic pathway operates in animals.

Experimental

5,7-Dimethoxy-1,4-naphthaquinone.—A suspension of 5,7-dihydroxy-1,4-naphthaquinone [10] (1 g.) in chloroform (35 ml.) was vigorously shaken with silver oxide (5 g.) and methyl iodide (4 ml.). Two additions of silver oxide (2·5 g.) and methyl iodide (2 ml.) were made at intervals of 2 hr. and the shaking was continued overnight. The *dimethoxyquinone* crystallised from light petroleum (b. p. 100—120°) in light-yellow flakes, m. p. 146° (760 mg.) (Found: C, 65·9; H, 4·6. $C_{12}H_{10}O_4$ requires C, 66·1; H, 4·6%).

2,3-Dihydroxy-5,7-dimethoxy-1,4-naphthaquinone (IV).—30% Hydrogen peroxide (0·42 ml.) and 10% aqueous sodium carbonate (0·90 ml.) were added to a solution of 5,7-dimethoxy-1,4-naphthaquinone (400 mg.) in alcohol (10 ml.) at 47°. The temperature rose to 50° and after being stirred at this temperature for 5 min. the mixture was cooled, diluted with water, and set

[14] Mukai, *Mem. Fac. Sci. Kyushu Univ., Series C, Chem.*, 1958, **3**, No. 2, 29; *Bull. Chem. Soc. Japan*, 1960, **33**, 453, 1234; personal communication.
[15] Sutherland and Wells, *Chem. and Ind.*, 1959, 291.

aside on ice. The *epoxide*, which separated, crystallised from light petroleum (b. p. 100—120°) in straw-coloured needles, m. p. 136° (215 mg.) (Found: C, 61·4; H, 4·4. $C_{12}H_{10}O_5$ requires C, 61·5; H, 4·3%). When the epoxide (220 mg.) was stirred for 15 min. in ice-cold acetic anhydride (2·4 ml.) containing concentrated sulphuric acid (0·2 ml.) it gradually dissolved and then 2,3-*diacetoxy*-1,2,3,4-*tetrahydro*-5,7-*dimethoxy*-1,4-*dioxonaphthalene* (I) separated. Crystallised from a large volume of ethyl acetate (charcoal), it had m. p. 194—196° (decomp. >180°) (160 mg.) (Found: C, 56·8; H, 4·9. $C_{16}H_{16}O_8$ requires C, 57·1; H, 4·8%). The diacetate (230 mg.) was triturated with ice-cold N-sodium hydroxide (7 ml.), the initial transient grey colour changing rapidly through green to deep blue. After 2 min. the solution was filtered quickly and acidified (*ca.* 20 mg. of undissolved starting material was recovered), and then diluted with water and extracted with ether (3 × 10 ml.). Repeated extraction with chloroform then yielded the *dihydroxyquinone* (IV) which crystallised from methanol in long orange-red needles, m. p. 225° (40—60 mg.) (Found: C, 56·9; H, 4·2. $C_{12}H_{10}O_6$ requires C, 57·6; H, 4·0%), and gave a violet solution in aqueous sodium hydroxide. The *diacetate* crystallised from light petroleum (b. p. 100—120°) in yellow needles, m. p. 182° (Found: C, 57·2; H, 4·2. $C_{16}H_{14}O_8$ requires C, 57·4; H, 4·2%).

2,5,7-*Trimethoxy*-1,4-*naphthaquinone*.—The combined ethereal extracts from three of the above reactions were kept at room temperature until an orange solid was deposited; complete evaporation of the solvent left only a black residue. The orange material was crystallised once from methanol (charcoal), and a portion of the product (140 mg.) was boiled under reflux with 3% methanolic hydrogen chloride (5 ml.) for 15 min. The small yellow needles which separated on cooling were dissolved in benzene (charcoal), chromatographed on alumina according to Davies *et al.*,[16] and crystallised from benzene–light petroleum (b. p. 50—60°) to give golden needles of 2,5,7-trimethoxy-1,4-naphthaquinone, m. p. and mixed m. p. with tri-*O*-methylflaviolin, 187° (mixed m. p. with 3,5,7-trimethoxy-1,4-naphthaquinone, 170°) (42 mg.) (Calc. for $C_{13}H_{12}O_5$: C, 62·9; H, 4·9. Found: C, 62·8; H, 4·9%).

2,3,5,7-*Tetramethoxy*-1,4-*naphthaquinone*.—An ethereal solution of diazomethane (from 2 g. of nitrosomethylurea) was added to an ice-cold suspension of 2,3-dihydroxy-5,7-dimethoxy-1,4-naphthaquinone (150 mg.) in ether (15 ml.) and kept at 0° for 30 min. After 2 hr. at room temperature the flocculent precipitate of *tetramethyl ether*, which had replaced the starting material, was collected and crystallised from light petroleum (b. p. 100—120°) in long thin yellow needles, m. p. 131° (unaltered on admixture with spinochrome N tetramethyl ether, m. p. 130°) (110 mg.) (Found: C, 60·4; H, 4·8. $C_{14}H_{14}O_8$ requires C, 60·4; H, 5·1%). The infrared spectra (KBr disc) of the " natural " and the synthetic material were identical.[8]

2,3,5,7-*Tetrahydroxy*-1,4-*naphthaquinone*.—2,3-Dihydroxy-5,7-dimethoxy-1,4-naphthaquinone (230 mg.) was stirred in a melt of anhydrous aluminium chloride (8 g.) and sodium chloride (1·4 g.) at 180° for 2 min. After cooling somewhat, the molten mixture was poured into 5N-hydrochloric acid (120 ml.). A dark solid (with bright-red inclusions) separated and was extracted with chloroform; the aqueous solution was extracted with ether and the combined, dried ($MgSO_4$) extracts were evaporated. The residue sublimed at >130°/6 × 10^{-7} mm. and the sublimate was taken up in the minimum amount of ether. Addition of light petroleum (b. p. 50—60°) precipitated a bright red, amorphous material which was throughly washed with light petroleum (b. p. 50—60°) containing a little ether, and crystallised from methanol forming small, red needles (7—8 mg.) of 2,3,5,7-*tetrahydroxy*-1,4-*naphthaquinone* which gradually decomposed at >260° (Found: C, 54·2; H, 2·6. $C_{10}H_6O_6$ requires C, 54·05; H, 2·7%). The tetrahydroxyquinone gave a yellowish-green solution in aqueous sodium hydroxide, a green solution in aqueous sodium hydrogen carbonate, and a transient green ferric colour in methanol. Its infrared, ultraviolet, and visible absorption curves were identical with those of spinochrome N.[8]

Spinochrome E.—The sample, previously crystallised [13] from methanol containing hydrochloric acid, was acetylated and then hydrolysed with boiling methanolic sulphuric acid. It separated on dilution as brownish-red needles, which blackened without melting >300° (Found: C, 47·0; H, 2·5. $C_{10}H_6O_8$ requires C, 47·2; H, 2·4%). The infrared spectra of samples obtained from *Psammechinus miliaris* [13] and *Paracentrotus lividus* [12] were virtually identical and showed only minor discrepancies. The pure material showed $\nu_{max.}$ (in KBr) at 3520, 3150, 1652sh, 1629, 1586, 1490, 1456, 1344, 1274, 1181, 1062w, 1041w, 992, 839, 803, 766, 716 $cm.^{-1}$. The *hexa-acetate* was obtained by boiling the pigment (96 mg.) in acetic anhydride (2 ml.)

[16] Davies, King, and Roberts, *J.*, 1955, 2782.

containing one drop of concentrated sulphuric acid; it (160 mg.) crystallised from ethanol in yellow needles, m. p. 192° (Found: C, 52·6; H, 3·7; Ac, 50·2. $C_{22}H_{18}O_{14}$ requires C, 52·1; H, 3·6; Ac, 51·0%). The hexa-acetate (100 mg.) was boiled in acetic anhydride (2·3 ml.) with zinc dust (200 mg.) and a trace of triethylamine for 10 min. to give the *leuco-octa-acetate* which formed needles (from glacial acetic acid), m. p. 265° (decomp.) (53 mg.) [Found: C, 53·0; H, 4·0; Ac, 57·1%; *M* (ebullioscopic in benzene), 556. $C_{26}H_{24}O_{16}$ requires C, 52·7; H, 4·05; Ac, 58·1%; *M*, 592].

Spinochrome E Tetramethyl Ether (cf. Kuroda [17]).—Spinochrome E (32 mg.) was spread on six watch-glasses and treated dropwise with ethereal diazomethane until there was no further reaction. The residue was washed with small amounts of ether and crystallised from methanol, forming long, brown, needles, m. p. 185° (8 mg.) (Found: C, 54·1; H, 4·6. Calc. for $C_{14}H_{14}O_8$: C, 54·2; H, 4·5%). This ether gave a brown ferric reaction, a violet *solution* with methanolic lead acetate, and a blue-violet solution in aqueous sodium hydroxide, but was not extracted from ethereal solution by aqueous sodium hydrogen carbonate. On prolonged contact with this reagent a purple solution is obtained (spinazarin 2,3-dimethyl ether behaves similarly). The m. p. was not depressed on admixture with namakochrome trimethyl ether and the infra-red spectra of the specimens were identical.[14]

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[17] Kuroda, *Proc. Imp. Acad.* (*Tokyo*), 1942, **18**, 69.