382. *Naturally Occurring Quinones. Part VI.*[1] *Spinochrome* D.[2,3]

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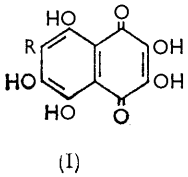
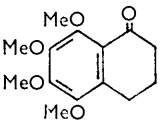
It is established, by synthesis, that spinochrome D is 2,3,5,6,8-pentahydroxy-1,4-naphthaquinone (I; R = H) and not the methyl homologue (I; R = Me).

Spinochrome D[2] is one of the pigments found in the spines of the sea urchin *Pseudocentrotus depressus* (Ag.) According to Kuroda and Ohshima[4] it has the molecular formula $C_{11}H_8O_7$, and forms a trimethyl ether[4,5] (with diazomethane), a penta-acetate[4,5] and a leuco-hepta-acetate.[6] From these data, the absence of methoxyl groups, and the general similarity of spinochrome D to spinazarin (2,3,5,8-tetrahydroxy-1,4-naphthaquinone) and 6-methylspinazarin, the structure (I; R = Me) was proposed.[4–6] As this relies heavily on analytical data, which are not above suspicion in this series (cf. ref. 7), synthetic confirmation seemed desirable although previous attempts (see ref. 3) were not encouraging.

6-Methylspinazarin is readily accessible but all efforts to introduce an additional oxygen function at position 7 failed. We therefore resorted to a step-wise synthesis initially from


(I)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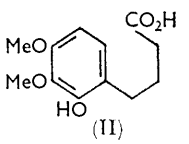
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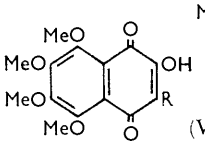
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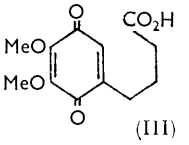
(III)

γ-(2,3,4,5-tetramethoxyphenyl)butyric acid, obtained in low yield *via* Friedel–Crafts reaction of 1,2,3,4-tetramethoxybenzene and succinic anhydride, and then, more conveniently, from the phenolic acid (II).[8] This, on oxidation with Fremy's salt, gave the quinone (III) from which the tetralone (IV) was obtained by standard procedures, but conversion of the latter into the hydroxy-quinone (V; R = H) by condensation with dimethyl-*p*-nitrosoaniline and hydrolysis of the dianil was very poor. The difficulty was surmounted by autoxidation of the tetralone in the presence of potassium t-butoxide. Two mol. of oxygen were rapidly absorbed to give the desired hydroxy-quinone (V; R = H) in 28% yield and the reaction promises to be a useful alternative to the dianil method. The quinone (V; R = H) thus obtained was finally *C*-methylated with acetyl peroxide and de-*O*-methylated with hydrogen bromide to give the compound (I; R = Me). The structure was confirmed by a parallel *C*-ethylation of the quinone (V; R = H) with propionyl peroxide to give compound (V; R = Et) which yielded, after demethylation, the pentahydroxy-quinone (I; R = Et), identical with natural echinochrome A.

Although the synthetic quinone (I; R = Me) and its derivatives appeared to agree in physical properties with the published data for spinochrome D, direct comparison with the natural pigment and its penta-acetate showed that there were small but definite differences in their R_F values, *X*-ray powder photographs, and ultraviolet spectra, greater

[1] Part V, J. Smith and R. H. Thomson, *J.*, 1961, 1008.
[2] For nomenclature see R. H. Thomson, "Naturally Occurring Quinones," Butterworths, London, 1957.
[3] A preliminary account has been written: H. A. Anderson, J. Smith, R. H. Thomson, and J. W. Wells, *Proc. Indian Acad. Sci.*, in the press.
[4] C. Kuroda and H. Ohshima, *Proc. Imp. Acad.* (*Tokyo*), 1940, **16**, 214.
[5] C. Kuroda and K. Koyasu, *Proc. Imp. Acad.* (*Tokyo*), 1944, **20**, 23.
[6] C. Kuroda and H. Iwakura, *Proc. Imp. Acad.* (*Tokyo*), 1942, **18**, 74.
[7] J. Gough and M. D. Sutherland, *Tetrahedron Letters*, 1964, 269.
[8] P. C. Mitter and S. De, *J. Indian Chem. Soc.*, 1939, **16**, 35.

discrepancies in their infrared spectra, and the m. p. of the natural acetate was depressed by the synthetic material. Examination of the n.m.r. spectrum (in deuterochloroform) of the " natural " penta-acetate revealed that only sixteen protons were present of which fifteen belonged to the acetate groups and one was an aromatic proton (τ 2·22). It appeared that spinochrome D was the pentahydroxy-quinone (I; R = H) and this was confirmed by demethylation of the intermediate (V; R = H) which gave a product indistinguishable from the natural material. Evidently Kuroda and her co-workers were misled by their anaytical data and we, too, have failed to obtain satisfactory analyses for the synthetic compound.

Experimental

All melting points were recorded on a Kofler hot-stage apparatus.

γ-(5,6-Dimethoxy-1,4-benzoquinon-2-yl)butyric Acid (III).—γ-(2-Hydroxy-3,4-dimethoxyphenyl)butyric acid [8] (6·46 g.) dissolved in acetone (10 ml.) was poured into a stirred solution of Fremy's salt (20·2 g.) in water (1 l.). After 24 hr. the *quinone* was collected and the filtrate was extracted with ether. The combined products crystallised from light petroleum (b. p. 80—100°) in orange needles, m. p. 129° (5·9 g., 87%) (Found: C, 56·5; H, 5·4; OMe, 24·7. $C_{12}H_{14}O_6$ requires C, 56·7; H, 5·5; OMe, 24·4%).

γ-(2,3,4,5-Tetramethoxyphenyl)butyric Acid.—The above quinone (5·8 g.) was hydrogenated in methanol (65 ml.) over Adams catalyst. After filtration and evaporation. the residual oily quinol was dissolved in water (30 ml.) containing sodium hydroxide (3 g.) and sodium dithionite (3 g.); dimethyl sulphate (9 g.) was added, and the mixture was stirred under reflux for 2 hr. Cooling and acidification gave an oil which was taken into ether, dried ($MgSO_4$), evaporated, and re-methylated by refluxing in acetone (50 ml.) for 18 hr. with dimethyl sulphate (8·8 g.) and anhydrous potassium carbonate (9·7 g.). Working up gave the *methyl ester* as a yellow oil, b. p. 125—130° (bath) 0·02 mm. (5·47 g., 79%) (Found: C, 60·4; H, 7·5; OMe, 50·5. $C_{15}H_{22}O_6$ requires C, 60·4; H, 7·4; OMe, 52·0%). This ester (4·41 g.) was refluxed with potassium hydroxide (0·9 g.) in water (20 ml.) for 3 hr. Acidification and ether extraction gave the *acid* as a viscous oil which solidified, and had m. p. 49—51° (from light petroleum) (3·86 g., 92%) (Found: C, 59·2; H, 7·0; OMe, 44·4. $C_{14}H_{20}O_6$ requires C, 59·2; H, 7·1; OMe, 44·4%). The crude acid was also obtained by reduction of the corresponding γ-keto-acid and converted directly to the tetralone (see below *b*).

5,6,7,8-Tetramethoxy-1-tetralone (IV).—(*a*) The tetramethoxy-acid (3·23 g.) in 85% sulphuric acid (14 ml.) was heated on the steam-bath for 20 min., poured on to ice and extracted with ether. Evaporation left a dark oil which was refluxed for 2 hr. with dimethyl sulphate (1·8 g.), sodium hydroxide (3·6 g.), and water (20 ml.). Working up yielded the *tetramethoxytetralone* as a yellow oil, b. p. 120—125° (bath)/0·05 mm. (1·65 g., 69·5%) (Found: C, 62·8; H, 7·0; OMe, 46·4. $C_{14}H_{18}O_5$ requires C, 63·2; H, 6·8; OMe, 46·6%). The *2,4-dinitrophenylhydrazone* crystallised from ethyl acetate–light petroleum (b. p. 80—100°) in orange-red needles, m. p. 168—169° (Found: C, 53·9; H, 5·2; N, 12·8. $C_{20}H_{22}N_4O_8$ requires C, 53·8; H, 4·95; N, 12·55%). The *semicarbazone* formed needles, m. p. 197—200° (from aqueous ethanol) (Found: C, 55·9; H, 6·5; N, 12·8. $C_{15}H_{21}N_3O_5$ requires C, 55·7; H, 6·5; N, 13·0%).

(*b*) Anhydrous aluminium chloride (27 g.) was added gradually (40 min.) to a stirred mixture of 1,2,3,4-tetramethoxybenzene (20 g.), succinic anhydride (10 g.), and tetrachloroethane (120 ml.) at 0°. After 1½ hr., the mixture was left at 7° for 3 days and then allowed to reach room temperature gradually and poured into ice (100 g.) and concentrated hydrochloric acid (70 ml.). The solvent was removed by steam-distillation. When solid distillate appeared the mixture was neutralised with solid sodium carbonate and steam-distillation continued to recover tetramethoxybenzene (9—10 g.). The residual liquor was acidified and filtered hot, and the precipitate extracted twice with boiling water. *β-(2,3,4,5-Tetramethoxybenzoyl)propionic acid* separated out when the combined filtrates were cooled; it formed needles, m. p. 115—116° (from ethanol) (3 g.) (Found: C, 56·2; H, 5·8. $C_{14}H_{18}O_7$ requires C, 56·3; H, 6·1%). This acid (1 g.) was heated with hydrazine hydrate (1 ml., 100%) and potassium hydroxide pellets (0·67 g.) until the temperature reached 195° (1½ hr.). It was then boiled under reflux for 3 hr., cooled, poured on to ice and hydrochloric acid, and extracted with ether and chloroform. The combined extracts were dried and evaporated leaving a brown oil the infrared spectrum of which showed

strong hydroxyl absorption. After methylation, by heating on the steam-bath in aqueous methanolic potassium hydroxide with dimethyl sulphate, the resulting oily ester was cyclised by heating with sulphuric acid (6 ml., 80%) for 10 min. on the steam-bath. Working up in the usual way gave the tetralone (420 mg.), identified by its semicarbazone, m. p. and mixed m. p. 197—200°.

(*c*) To a solution of 5-hydroxy-6,7-dimethoxy-1-tetralone [8] (0·55 g.) in aqueous sodium hydroxide (5 ml., 10%) stirred in an ice-bath, potassium persulphate (0·675 g.) in water (28 ml.) was added slowly (3½ hr.). Next day the solution was made acid to Congo Red and extracted with ether. Concentrated hydrochloric acid (5 ml.) was then added, the solution warmed on the steam-bath for 30 min., cooled, and extracted with ether. Evaporation of the dried extract left a residue which crystallised from light petroleum (b. p. 100—120°) in yellow needles, m. p. 161—162° (*ca.* 50 mg.). Methylation of this quinol with dimethyl sulphate–potassium carbonate–acetone gave the tetramethoxytetralone identified by its semicarbazone, m. p. and mixed m. p. 197—200°.

2-*Hydroxy*-5,6,7,8-*tetramethoxy*-1,4-*naphthaquinone* (V; R = H).—The above tetralone (1 g.) in t-butyl alcohol (5 ml.) was added to a solution of potassium t-butoxide in t-butyl alcohol (50 ml. N) under an atmosphere of oxygen. The solution became red immediately and when it was shaken 2 mol. of oxygen were absorbed in *ca.* 10 min. and the colour changed to deep brown. It was then poured into a large volume of dilute hydrochloric acid, extracted with ether, and the extract shaken with aqueous potassium hydrogen carbonate until the aqueous phase was colourless. Acidification gave an oil which, after being washed, and dried in ether, was chromatographed in benzene on a column of silicic acid. Elution of the main zone gave the *hydroxy-quinone* which crystallised from benzene in orange needles, m. p. 110—111° (308 mg., 28%) (Found: C, 57·3; H, 4·7; OMe, 42·7. $C_{14}H_{14}O_7$ requires C, 57·1; H, 4·8; OMe, 42·2%). The *methyl ether*, obtained by refluxing the quinone for 15 min. in 3% methanolic hydrogen chloride and concentrating to small bulk, formed yellow needles, m. p. 100—101° [from light petroleum (b. p. 80—90°)] (Found: C, 58·4; H, 5·4; OMe, 50·0. $C_{15}H_{16}O_7$ requires C, 58·4; H, 5·2; OMe, 50·3%). Methylation with diazomethane gave the same product. The yellow *acetate* had m. p. 85° [from ether–light petroleum (b. p. 40—60°)] (Found: C, 57·3; H, 4·8. $C_{16}H_{16}O_8$ requires C, 57·1; H, 4·8%). The acetate was also prepared from the tetralone as follows: to a solution of 5,6,7,8-tetramethoxy-1-tetralone (0·8 g.) and dimethyl-*p*-nitrosoaniline (1·25 g.) in ethanol (50 ml.) was added 2N-sodium hydroxide (2 ml.). After 4 days, concentrated sulphuric acid (8 ml.) in water (30 ml.) was added and the mixture was refluxed for 1 hr. Most of the solvent was removed *in vacuo*, the solution was diluted with water and extracted with ether and chloroform, and the combined extracts were shaken with aqueous potassium hydrogen carbonate. The alkaline solution was acidified and extracted with ether to give, on working up, a red oil which was chromatographed, in benzene, on AnalaR calcium carbonate, yielding the hydroxy-quinone as a brown oil which crystallised. Acetylation with acetic anhydride and a trace of sulphuric acid gave another brown oil which, after chromatography in benzene on silicic acid, gave a yellow oil which solidified. Crystallisation from light petroleum (b. p. 60—80°) afforded the acetate as yellow needles, m. p. and mixed m. p. 85°.

2-*Hydroxy*-5,6,7,8-*tetramethoxy*-3-*methyl*-1,4-*naphthaquinone* (V; R = Me).—A solution of acetyl peroxide (0·46 g.) in acetic acid (4·5 ml.) was added to one of 2-hydroxy-5,6,7,8-tetramethoxy-1,4-naphthaquinone (0·9 g.) in the same solvent. The mixture was heated over a flame until effervescence began and then on a steam-bath for 1¼ hr. The solvent was removed on a rotatory evaporator, then benzene was added, and re-evaporated. This was repeated three times and the remaining oil was chromatographed, in benzene, on silicic acid. Elution of the fast-moving orange band yielded the *methyl-quinone* which crystallised from ether–light petroleum (b. p. 40—60°) in long, silky, yellow needles, m. p. 110° (0·38 g., 39%) (Found: C, 58·6; H, 5·5. $C_{15}H_{16}O_7$ requires C, 58·4; H, 5·2%). The *acetate* distilled at 150—160° (bath)/0·05 mm., and was further purified by chromatography in benzene, on silicic acid, for analysis (Found: C, 58·0; H, 5·4. $C_{17}H_{18}O_8$ requires C, 58·3; H, 5·2%).

2,3,5,6,8-*Pentahydroxy*-7-*methyl*-1,4-*naphthaquinone* (I; R = Me).—The above tetramethoxyquinone (210 mg.) was refluxed with hydrobromic acid (35 ml.; 48%) for 1¾ hr. A copious precipitate, which appeared after a few minutes, redissolved on further heating. After being cooled in ice, the *product* (150 mg.) was collected, washed well with water, dried *in vacuo*, and sublimed at 140—150°/0·05 mm. forming red needles, m. p. 279°; $\nu_{max.}$ (in KBr) 3333, 1653, 1613, 1563, 1460, 1408, 1269, 1178, 1143, 1075, 1006, 948, 823, 785, 763, 741, 727 cm.$^{-1}$;

$\lambda_{max.}$ (in EtOH) 257, 343, 465, 490, 525 mμ (log ε 4·17, 3·49, 3·45, 3·44, 3·33), R_F 0·59 (90% formic acid on Whatman No. 1 paper) (Found: C, 52·2; H, 3·4. $C_{11}H_8O_2$ requires C, 52·4; H, 3·4%). The quinone gave a transient violet colour in aqueous sodium hydroxide which became brown, and a yellow solution in aqueous potassium hydrogen carbonate. A methanolic solution gave a violet precipitate with lead acetate and a dark brown coloration with ferric chloride. The *penta-acetate* crystallised (with difficulty) from benzene–light petroleum (b. p. 40—60°) and from methanol in yellow needles, m. p. 178—179° (Found: C, 54·7; H, 4·2. $C_{21}H_{18}O_{12}$ requires C, 54·8; H, 3·9%). The *leucohepta-acetate*, obtained by treatment with zinc dust-acetic anhydride and a trace of triethylamine, formed needles, m. p. 209° (from acetic acid) (Found: C, 54·9; H, 4·5; Ac, 54·8. $C_{25}H_{24}O_{14}$ requires C, 54·7; H, 4·3; Ac, 54·2%). The 2,3,6-*trimethyl ether*, prepared by treatment with ethereal diazomethane using Kuroda's method,[9] crystallised from methanol in brown needles, m. p. 158° (Found: C, 57·1; H, 5·0; OMe, 33·3. $C_{14}H_{14}O_7$ requires C, 57·1; H, 4·8; OMe, 31·6%).

3-*Ethyl*-2-*hydroxy*-5,6,7,8-*tetramethoxy*-1,4-*naphthaquinone* (V; R = Et).—The quinone (V; R = H) (257 mg.) in acetic acid (10 ml.) was treated with propionyl peroxide (1·1 mol.) in acetic acid. The solution was heated for 1 hr. on the steam-bath and then taken to dryness. The residual orange oil was chromatographed in benzene on a column of silicic acid, the major yellow zone yielding the *ethylquinone* which crystallised from ether–light petroleum (b. p. 40—60°) in yellow plates, m. p. 98—99° (100 mg., 37%) (Found: C, 59·5; H, 5·6; OMe, 38·5. $C_{16}H_{18}O_7$ requires C, 59·6; H, 5·6; OMe, 38·5%). The *acetate* is a yellow oil, b. p. 130—140° (bath)/0·05 mm. (Found: C, 59·1; H, 5·8. $C_{18}H_{20}O_8$ requires C, 59·2; H, 5·75%).

Echinochrome A (I; R = Et).—The above quinone (50 mg.) was heated under reflux in hydrobromic acid (10 ml., 48%) for 2 hr., and the product collected and dried. Sublimation at 130—150°/0·05 mm. gave 7-*ethyl*-2,3,5,6,8-*pentahydroxy*-1,4-*naphthaquinone* as red-purple micro-needles (which darkened to purple-brown on exposure to air), m. p. 220—221°, mixed m. p. with echinochrome A 220°, (20 mg., 47%) (Found: C, 52·3; H, 4·0. $C_{12}H_{10}O_7$ requires C, 52·2; H, 3·7%). Both natural and synthetic quinones had R_F 0·62 in 90% formic acid (Whatman No. 1 paper) and identical ultraviolet and infrared spectra.

2,3,5,6,8-*Pentahydroxy*-1,4-*naphthaquinone* (*spinochrome D*) (I; R = H).—2-Hydroxy-5,6,7,8-tetramethoxy-1,4-naphthaquinone (87 mg.) was refluxed with hydrobromic acid (20 ml., 48%) for 2 hr. and then cooled. The precipitate (58 mg.) was sublimed at 170—185°/0·05 mm. giving red micro-needles which sublime without melting at 285—290°; $\nu_{max.}$ (in KBr) 3333, 1637, 1590, 1490, 1471, 1443, 1351sh, 1282, 1230, 1183, 1093, 1026, 971, 917w, 874, 859w, 808, 778, 741w, 705 cm.$^{-1}$; $\lambda_{max.}$ (in EtOH) 263, 331, 463, 486, 528 mμ (log ε 4·11, 3·44, 3·41, 3·41, 3·34). The natural material showed $\nu_{max.}$ (in KBr) 3333, 1634, 1592, 1481, 1466, 1439, 1350sh, 1282, 1227, 1188, 1093, 1029, 972, 918w, 872, 858, 808, 769, 738w, 707 cm.$^{-1}$; $\lambda_{max.}$ (in EtOH) 264, 328, 463, 485, 530 mμ (log ε 4·10, 3·44, 3·41, 3·41, 3·26) (Found: C, 50·8; H, 3·1. $C_{10}H_6O_7$ requires C, 50·4; H, 2·5%). Both natural and synthetic quinones had R_F 0·57 in 90% formic acid (Whatman No. 1 paper) and gave a transient violet colour in aqueous sodium hydroxide becoming brown, and then yellow, and a greenish yellow solution in aqueous potassium hydrogen carbonate. In methanolic solution they gave a violet precipitate with lead acetate and a dark brown colour with ferric chloride. The synthetic quinone formed a *penta-acetate*, yellow needles (from methanol), m. p. and mixed m. p. with "natural" penta-acetate, 179—180°; $\nu_{max.}$ (in KBr) 1799, 1689, 1600, 1466, 1439, 1377, 1351, 1282, 1250, 1183, 1156sh, 1122, 1096, 1044, 1015, 966, 940, 913, 874sh, 867 cm.$^{-1}$; $\lambda_{max.}$ (in EtOH) 252, 274, 357 mμ (log ε 3·12, 3·13, 2·36). The "natural" penta-acetate showed $\nu_{max.}$ (in KBr) 1803, 1692, 1605, 1471, 1439, 1377, 1351, 1282, 1250, 1183, 1156sh, 1122, 1096, 1044, 1015, 966, 940, 913, 874sh, 867; $\lambda_{max.}$ (in EtOH) 252, 274, 357 mμ (log ε 3·11, 3·09, 2·38) (Found: C, 53·6; H, 3·6; Ac, 46·2. $C_{20}H_{16}O_{12}$ requires C, 53·6; H, 3·6; Ac, 47·8%).

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[9] C. Kuroda, *J. Sci. Res. Inst., Tokyo*, 1952, **46**, 188.