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THE STRUCTURE OF SPINOCHROME B

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Spinochrome B (nomenclature of Goodwin, Lederer and Musajo[1]) was isolated from the spines of the sea urchin Paracentrotus lividus by Musajo and Minchilli[2] in 1942, being then designated spinochrome P_1. This red polyhydroxynaphthoquinone yielded analyses best fitting $C_{12}H_8O_7$ and was thus presumably related to other known echinoid pigments[3]. The quinone yielded some acetic acid in the C-Me estimation, a characteristic polymethyl ether with diazomethane and a leucoacetate (see column (i) in Table I). Structure I was suggested. Goodwin and Srisukh[4] in 1950 isolated what they considered to be the same pigment (see (ii) in Table I) but renamed it spinochrome B using a system later approved by Goodwin, Lederer and Musajo[1]. From spectral considerations Goodwin and Srisukh put forward structures II or III as typifying the structure of spinochrome B. Lederer[5] also isolated spinochrome B from P. lividus spines but obtained analytical figures not consistent with those of Musajo and Minchilli nor with those of Goodwin and Srisukh (see (iii) in Table I). Lederer suggested that the high melting point of spinochrome B might be the consequence of a structure having a 2,2' aliphatic bridge between two quinone nuclei.

TABLE I

	(i) Spinochrome P_1 Musajo et al[2]	(ii) Spinochrome B Goodwin et al[4]	(iii) Spinochrome B Lederer[5]
Source	spines of Paracentrotus lividus	spines of P. lividus and Echinus esculentus	spines of P. lividus
Melting point etc.	glistening red tablets from dioxan, mp 350-355°	red rods from MeOH, mp 283° decomp; later[1] mp < 300°	red prisms from dioxan, mp > 340°; sublimes at 230°.
Molecular formula	$C_{12}H_8O_7$ $(C_{10}H_6O_6 \cdot \frac{1}{2}C_4H_8O_2)$[a]	$C_{12}H_{12}O_8$[b] $(C_{10}H_6O_6 \cdot CH_3OH)$[a]	C, 53.42; H, 4.58[c] $(C_{10}H_6O_6 \cdot C_2H_5OH)$[a]
Spectrum (λ max in mu.)	as for (ii)?[d]	272, 320, 388 and 480 in MeOH	270, 320, 387 and 480 in MeOH
Colour reactions	green zone on $CaCO_3$	olive green on $CaCO_3$	green on $CaCO_3$
Leuco-acetate	mp 235-236°	mp 226-230° decomp.	-
Trimethyl ether (CH_2N_2)	mp 111-112°	-	-
Tetra-methyl ether	-	-	-
Tetra-acetate	-	-	-

NOTES

a. Calculated C and H values for this solvate correspond satisfactorily to published data.

b. Calculated composition figures given in paper actually correspond to $C_{12}H_{11}O_8$.

c. Solvent used for crystallization of analytical sample not specifically stated in paper.

TABLE I (continued).

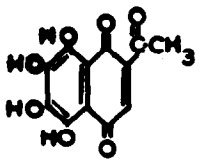
(iv) Spinochrome B_1 (earlier B) Kuroda et al[7,8]	(v) Spinochrome M_2 Kuroda et al[7,9]	(vi) Spinochrome N Smith and Thomson[13]	(vii) Present authors
spines of *Strongylocentrotus pulcherrimus*	spines of *Anthocidaris* (*Heliocidaris*) *crassispina*	synthetic 2,3,5,7 tetrahydroxy-naphthoquinone	spines of *Salmacis sphaeroides*
as for M_2	bright red crystals from MeOH; sublimes without melting over 200°	red needles; decomp. > 260°	dark red needles from MeOH, mp 325-330° decomp; light red after drying.
as for M_2	$C_{10}H_6O_6.H_2O$	$C_{10}H_6O_6$	$C_{10}H_6O_6.CH_3OH$ but $C_{10}H_6O_6$ after drying at 150°.
324, 383, 467, 484 and 512 in MeOH [e]	325, 385, 476, 516 and 537 in MeOH [e]	"absorption curves identical with those of spinochrome N"	271, 322, 388 and 477 in EtOH.
as for M_2	yellowish green in NaOH solution	yellowish green in NaOH; green in $NaHCO_3$.	olive green on $CaCO_3$; yellow green in $NaHCO_3$.
as for M_2	mp 233-234°	-	mp 242° decomp.
as for M_2	orange needles mp 110.5-111°	-	orange crystals mp 102° ⟶ red crystals mp 112°.
as for M_2	mp 132-132.5° (Me_2SO_4/K_2CO_3/ acetone)	mp 131° [f]	mp 130-130.5° (CH_2N_2/acetone)
as for M_2	mp 154.5-155°	-	mp 157°

NOTES (continued)

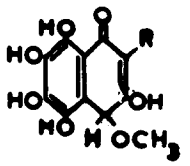
d. Not sighted by us but used by Goodwin and Srisukh[4] as a basis of identity between (i) and (ii).

e. Read from a small graph.

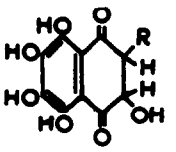
f. Identical with spinochrome N (i.e. $B_1 = M_2$) tetramethyl ether, mp. 130° by mixed melting point test.



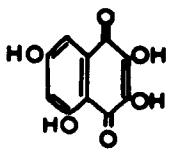
I



II



III



IV

When investigating the pigments of the spines and test of the echinoid, Salmacis sphaeroides, we found one pigment [(vii) in Table I] of the six present, which was consistent in the U.V. and visible spectra, in the melting points of the trimethyl ether and leucoacetate and in the zone colour on calcium carbonate columns, with the pigment of the European workers. This hydroxynaphthoquinone was readily isolated since it alone was not adsorbed from ether solutions by columns of B.D.H. chromatography grade calcium carbonate. On certain other more alkaline calcium carbonate preparations, e.g. May and Baker's B.P., the quinone was strongly adsorbed as an olive-green zone as noted by Lederer[5]. We have found however, that the adsorption of this spinochrome is also affected by the solvent flow rate. Samples dried at 150°C/$<$10 mm. or sublimed, analyse correctly for $C_{10}H_6O_6$. Samples crystallised from methanol and dried at room temperature, analyse for $C_{10}H_6O_6.CH_3OH$.

The N.M.R. spectrum of the trimethyl ether in $CDCl_3$ shows nine protons in three methoxyl groups, two more as doublets in the aromatic region (2.84 and 3.38 τ, J = 2.5 cps) and one unsplit proton at -2.08 τ. This last proton is not seen in the presence of D_2O and is interpreted as a strongly hydrogen-bonded hydroxyl proton while the two aromatic protons are assumed meta from their coupling constant. In the C-Me estimation, some acetic acid (0.23 moles) was obtained as noted by Musajo and Minchilli[2],

a result reminiscent of the behaviour of resorcinol (0.05 moles). Accordingly the pigment has structure IV.

This structure IV has already been assigned to spinochrome N however, which designation was used by Thomson[6] for the urchin pigment which had been isolated by Kuroda et al[7,8,9] from two Japanese echinoids and designated by her as spinochrome B_1, (originally B) when isolated from Strongylocentrotus pulcherrimus (Japanese name, bafun-uni) or spinochrome M_2 (see (iv) and (v) of Table I) when isolated from Anthocidaris (Heliocidaris) crassispina (Japanese name, murasaki-uni). Kuroda et al[7] in 1944 deduced the structure IV for spinochrome B_1 ($\equiv M_2$) by degradation but made no reference in later papers[10] to the possible identity of the pigment with the spinochrome B of Goodwin, Lederer and Musajo. Nor apparently were Goodwin, Lederer and Musajo aware of Kuroda's designation of a spinochrome B since her 1942 paper[8] has not yet appeared in Chemical Abstracts and her 1944 paper[7] was not abstracted until 1955.

The identity of Kuroda's spinochrome B_1 ($\equiv M_2$) with the spinochrome B of Goodwin, Lederer and Musajo was perhaps obscured to Kuroda and to Okajima[11] as also to Smith and Thomson[6,12] and initially to ourselves by the incorrect molecular formulae deduced by the European workers[2,4,5], the difficulty[1] of distinguishing an actual melting or decomposition temperature for this highly coloured quinone, the inadequate spectra of B_1 and M_2 (both probably impure) published by Kuroda and Okajima[13] in 1954 and the very brief description (see (vi) of Table I) given by Smith and Thomson[12] of their synthetic spinochrome N.

While the identity of certain pairs (eg. (iii) and (vi)) considered in isolation from Table I, might possibly be questioned on the evidence presented, it is clear that all substances (i) to (vii) in Table I are in fact identical and the designations spinochrome P_1, spinochrome B (Goodwin, Lederer and Musajo), spinochrome B (Kuroda), spinochrome B_1, spinochrome M_2

and spinochrome N are synonomous - a Gilbertian situation indeed. The most appropriate trivial name for this pigment, if one is needed, would seem to be spinochrome B, which is the designation agreed upon by Goodwin, Lederer and Musajo and also the original[14] designation used by Kuroda.

We wish to thank General Motors - Holden's P/L. Melbourne, for the award of a Research Fellowship to one of us (J.G.).

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14. The change from B to B_1 followed the isolation of a second pigment (B_2) from the same source as B_1 but since B_2 was subsequently considered to be identical with M_1 (see C. Kuroda and M. Okajima, Proc. Japan Acad. 29, 27 (1953)) and as M_2 is identical with B_1, only two letters B and M used as in ref. (1) are required to describe all four isolates.

Dr. R.H. Thomson (Aberdeen) has kindly provided us with a copy of the paper by Musajo and Minchilli (ref. 2). The carbon, hydrogen and methoxyl analytical data shown therein for the leucoacetate and polymethyl ether are in close agreement with those calculated for 2,3,5,7-tetrahydroxy-naphthoquinone derivatives. The reported C methyl values (0.19 and 0.21) and the visible spectrum in methanol (broad λ_{max} 480 mu, λ_{min} 443 mu, intensity ratio 1.16; of present work - 477 mu, 442 mu and 1.16 respectively) closely parallel our observations. Inspection of the paper shows that of the four recorded analyses of the spinochrome only one was for material crystallised from dioxan (see Column 1 of Table 1). The three other analyses, on material crystallised from acetic acid, give very similar values (0.3 to 0.7 high in hydrogen compared with the unsolvated naphthoquinone which cannot be rationalised on the basis of solvation by acetic acid and/or water.

We wish also to thank Dr. Thomson for his prompt action in obtaining and supplying us with specimens of Kuroda and Okajima's spinochrome M_2, Musajo and Minchilli's spinochrome P_1 and Lederer's spinochrome B. Paper chromatography of all these pigments in acidic and basic systems, yielded R_F values identical with those shown by 2,3,5,7-tetrahydroxynaphthoquinone from Salmacis sphaeroides.

Analyses of the compounds prepared by the present authors are all in satisfactory agreement with the proposed structures.