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PIGMENTS OF MARINE ANIMALS

VIII. PRECURSORS OF 6,6'-DIBROMOINDIGOTIN (TYRIAN PURPLE) FROM THE MOLLUSC DICATHAIS ORBITA GMELIN

J. T. Baker

Department of Chemistry, University College of

Townsville, Townsville, Australia

and

M. D. Sutherland

Department of Chemistry, University of

Queensland, Brisbane, Australia
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Friedländer (1) characterised the major constituent of the ancient dye Tyrian purple from Murex brandaris as the known 6,6'-dibromoindigotin (2,3) and later identified the same compound in the purple dyes from M. trunculus (4), Purpura aperta and P. lapillus (5).

Sunlight irradiation of the pale yellow-green chloroform extract of autolysed hypobranchial glands of the Australian mollusc Dicathais orbita Gmelin produced an insoluble violet precipitate ($C_{16}H_8Br_2N_2O_2$)* which was recrystallised from o-dichlorobenzene and then showed characteristics identical with those of synthetic 6,6'-dibromoindigotin. The natural product showed a molecular ion of m/e 418 (Br = 79,79) in the mass spectrum and was characterized as an N,N'-diacetyl derivative, m.p. 306° ($C_{20}H_{12}Br_2N_2O_4$).

Tyrian purple does not exist as such in the hypobranchial glands of live gastropod molluscs of the family Muricidae but is formed by the action of sunlight on smears of the gland from live specimens, the transformation through pale yellow, green and blue to purple being described by several investigators (6a-h).

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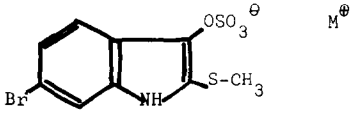
* All molecular formulae are supported by satisfactory analyses for all elements present and by acetyl and methoxyl analyses where relevant.

A neutralised aqueous solution of the residue from an ethanol extract of the hypobranchial glands from live *D. orbita* when chromatographed on Amberlite CG-50 (H form) yields, amongst others, an eluate fraction (7,8) of pH 3-4 which when treated at pH 6.5 with aqueous silver nitrate yields I, M = Ag (silver 'tyrindoxyl' sulphate) as pale yellow-orange leaflets, d. 118-120° ($C_9H_7BrNO_4S_2Ag.H_2O$). Silver tyrindoxyl sulphate gives quantitative yields of silver chloride (with hydrochloric acid) and barium sulphate (after acid hydrolysis and treatment with barium chloride solution).

The silver salt was converted by potassium chloride solution to the potassium salt ($C_9H_7BrNO_4S_2K$) which gave a p.m.r. spectrum in water showing a three proton singlet ($-S-CH_3$) at 2.42 p.p.m. a one proton singlet (NH) at 9.76 p.p.m. (eliminated in deuterium oxide solution) and an ABC system of three aromatic protons at 7.14 p.p.m.

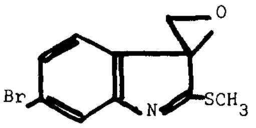
I



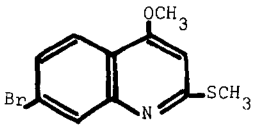
The structure I was confirmed by hydrogenolytic desulphurization and debromination of the sodium salt (I, M = Na) with Raney nickel at room temperature to produce sodium indoxyl sulphate which showed analytical data, physical properties, infrared, ultraviolet and p.m.r. spectra and a *p*-toluidine salt identical with those of an authentic sample.

Aqueous extraction of the glandular residue remaining after ethanolic extraction gave a solution from which an enzyme concentrate could be precipitated by the addition of absolute ethanol. Sodium tyrindoxyl sulphate (I, M = Na) was chromatographed on Whatman No. 1 paper at 25°C with the solvent system butanol, acetic acid, water (78:5:17) (7,8), and the dried paper was sprayed with an aqueous solution of the enzyme concentrate and then exposed to sunlight. A purple colouration developed to reveal a spot of R_f 0.56, which was previously invisible in daylight but purple under ultraviolet light. The crude ethanol extract of hypobranchial glands of live *D. orbita* chromatographed and developed similarly showed only one purple spot also at R_f 0.56. The purple colour development could alternatively be effected by spraying the paper with N hydrochloric acid and heating at 100°C. In buffered solutions of sodium tryindoxyl sulphate, the enzyme showed maximum sulphatase activity at pH 5.6-6.0. Whereas enzymatic hydrolysis of sodium tryindoxyl sulphate, followed by irradiation of the product in sunlight produced only one purple compound, 6,6'-dibromoindigotin, hydrolysis by hot hydrochloric acid also produced small amounts of a red ether-soluble substance, not yet examined.

Grinding freshly excised glands of *D. orbita* with sand in the presence of moist ether yielded an ether extract which deposited tyriverdin (see below) on standing but which when treated immediately with excess diazomethane at 0°, yielded II as pale yellow crystals ($C_{10}H_8NOSBr$) of m.p. 109.5-110.5°



II



III

and III as colorless needles ($C_{11}H_{10}NOSBr$) of m.p. 117-118°. These structures have been deduced from infrared, ultraviolet, proton magnetic and mass spectra and are as yet not otherwise confirmed.

Extraction of the hypobranchial glands of autolysing *D. orbita* with various organic solvents yields a pale yellow-green solid ($C_{36}H_{30}Br_4N_4O_5S_4$) (tyriverdin) which darkens above 60° and yields a blue sublimate at 220° without melting. Crystals of tyriverdin were also slowly deposited in the dark from an ether extract of the reaction mixture of sodium tyrindoxyl sulphate with cold hydrochloric acid. Tyriverdin on irradiation with sunlight in chloroform solution, gives 6,6'-dibromoindigotin, dimethyl disulphide and traces of 6-bromoisatin (9), m.p. 273-274°, the yield of the latter being much enhanced by the presence of air during the irradiation. The chemistry of tyriverdin is obscured by its very limited solubility in organic solvents and by its ready conversion to dibromoindigotin but the mass spectrum strongly suggests that it is a "quinhydrone" of tyrindoxyl and a dehydrotyrindoxyl ($C_9H_8BrNOS.C_9H_6BrNOS.\frac{1}{2}H_2O$). Acetylation ($Ac_2O/HClO_4$ at 0°) of tyriverdin furnishes dibromoindigotin (ca. 1% yield) a low yield of a sublimable sulphur-containing product, about 10% of N-acetyldibromoindigotin and as the principal products, NN'-diacetyldibromoindigotin and 6-bromoisatin.

Our observations have thus clarified the nature of the yellow light-insensitive crystals observed by Schunk (6d) in 1879 and by Letellier (6f) in 1889, as being probably 6-bromoisatin. The insoluble apple-green photosensitive component observed by Letellier (6f) was undoubtedly tyriverdin. The pale yellow water-soluble crystals obtained by Bouchilloux and Roche (7,8) seem now likely to have been a salt of the 3-O-sulphate of 6-bromo-2-methylthioindoxyl and the actual tissue component yielding the Tyrian purple dye of antiquity. Further work is proceeding.

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