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A REVISED STRUCTURE OF TYRIVERDIN

THE PRECURSOR OF TYRIAN PURPLE[1]

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Abstract—The structure of tyriverdin, a precursor of Tyrian purple, has been revised by comparison of data obtained from synthetic and naturally derived samples. Model studies with indigotin, the debromoanalogue, are described and it is shown that the light induced transformations of precursors to pigments are chain reactions.

The art of dyeing with Tyrian purple dates back several thousand years.[2] Although numerous papers dealing with the occurrence, cultural history and chemistry of this pigment have appeared, there are still several questions that need clarification. Not even the ethymology of the word purple is fully clarified.[3]†

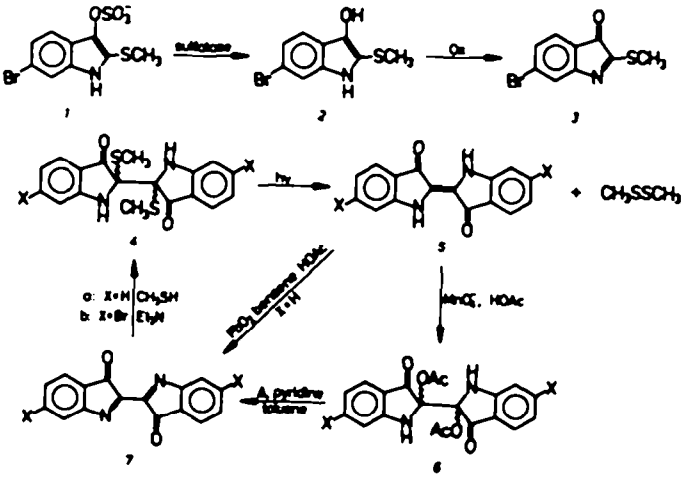
Until recently the occurrence of Tyrian purple was entirely connected to the genera *Murex* of the families *Muricidae* and *Thaisidae*, since some of the members of these gastropod molluscs concentrate precursors of Tyrian purple in their hypobranchial glands. It is thus of considerable interest that Tyrian purple has now been identified in a hemichordate *Phychodera flava laysanica* Spengel.[4]

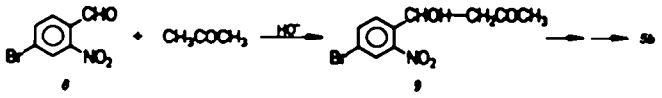
The chemical investigation of the generation of Tyrian purple was given a firm basis about 70 years ago by *Friedländer's proof of the structure* being 6,6'-dibromoindigotin[5] (we are at present investigating the stereochemistry, which has never been clarified). The next break-through came with the work of Baker *et al.*[2,6] who identified the precursor present in the hypobranchial glands of *Dicathais orbita* Gmelin as the anion of tyrindoxyl sulfate[6] (1). The counter cation was identified in *D. orbita* and *Mancinella keineri* Deshayes as choline and choline esters.[7] An enzyme catalyzed reaction gives rise to tyrindoxyl (2), which in turn was believed to be oxidized to the corresponding indoline-one (3). Compounds 2 and 3 were believed to form a "quinhydrone" type complex, i.e. tyriverdin, the immediate precursor of Tyrian purple.[2]

In a previous communication[1] we proposed the structure of tyriverdin to be 4b.[8] Our preference for 4b was based mainly on model experiments with indigotin derivatives.

In the case of the indigotin derivatives, the anhydrocompound 7a could be obtained directly from commercial indigotin (5a) by PbO_2 oxidation.[9] Base catalyzed addition of methanethiol gave a fair yield of the unstable green compound 4a. Tyrian purple (5b) is not commercially available, but was synthesized from 4-bromo-2-nitrobenzaldehyde (8) by a modified literature proce-

†English purple is derived from Latin purpura, which in turn is derived from Greek πορφύρα (porphýra was pronounced porphúra in the oldest times). The derivation of πορφύρα is unknown, it may not even be related to πορφύρω (porphýro: to be in a state of unrest: used about the sea), since the meaning of the two words is different. The two words have, however, interacted so that the meaning of πορφύρω in later times becomes "to be purple coloured" in analogy with the meaning of πορφύρα: purple-mollusc, purple coloured. It has been suggested that the Greek word originates from the Indo-European bharbhur; this word is not recorded in Sanskrit but could be a misinterpretation of jarbhur in jahrbhuriti जर्भुरीति (to move violently or rapidly, used about fire). The word dhumra धूम्र means smoke coloured, reddish, purple coloured, but this word could not have evolved to πορφύρα. Thus no relation to Sanskrit can at present be established. (Personal communication from Prof. H. Headrikaca, The Institute of Indian Philology, University of Copenhagen).





dure.[10] We found the aldol condensation product **9** as an isolable intermediate in the synthesis of **5b**. Oxidation of **5b** with PbO_2 gave virtually no yield of **7b**, whereas in the presence of acetic acid compound **6b** could be obtained by PbO_2 oxidation of **5b** but only in low yield. Finally, a fair yield of **6b** was obtained by $KMnO_4$ oxidation of **5b**.[9] The anhydro compound **7b** was smoothly obtained by pyridine catalyzed elimination from **6b**. As in the case of the indigotin model, **7b** could be induced (triethyl amine catalyzed, solvent chloroform) to add methanethiol, thereby forming the green coloured **4b**. These reactions, however, are solvent dependent, i.e. changing the solvent from chloroform to ethyl ether gave rise to a mixture of at least two compounds (**4a**, **4a'**, **4b**, **4b'**), one of which is the same as the one formed in chloroform (**4a**, **4b**). The products are very closely related judging from spectral evidence (Table 1), but also from the fact that both of them on irradiation form purple (or indigotin). The most straightforward explanation is that since **4** possesses two chiral centers, three stereoisomers may be formed, namely a *meso* form and a *d,l*-pair.

The main reason for the rejection of the formulation **4b** by Baker was the absence of molecular ions in the mass spectrum of tyriverdin.[8] We have shown, however, that field desorption/field ionization mass spectrometry produces molecular ions of **4b** as well as of **4a**, which is not the case with conventional electron impact mass spectra. From Table 1 it is seen that the ^{1}HMR spectrum of **4b** is virtually identical with that of an authentic sample of tyriverdin[11] and we thus conclude that **4b** is the structure of tyriverdin. Whether tyriverdin is a single stereoisomer or a mixture of stereoisomers has to await further clarification.

As pointed out earlier,[1] the light-induced transformation of **4a** to indigotin is a very efficient process. We have measured the quantum efficiency and have found a value of 4.95 ± 0.25 in deoxygenated chloroform. Due to the low solubility of **4b** and **5b** we were not able to perform similar experiments with this compound, but judging from qualitative observations of the reaction we believe that the quantum efficiency is at least as high or higher in the latter reaction. This high quantum yield is indicative of a chain reaction.

All available evidence indicates that Tyrian purple produced from molluscs is an artefact formed from precursors in the hypobranchial glands. However, Tyrian purple from *P. flava laysanica* Spengel is apparently not an artefact.[4] It is interesting that only animals from certain locations contain the pigment.[12] The latter observation still leaves a lot of unanswered questions about the biochemical and ecological significance of this type of compound.

EXPERIMENTAL

IR spectra were recorded using a Perkin Elmer Infrared Spectrophotometer 580. ^{1}H NMR spectra were obtained from a Bruker HX 270 and Varian T-60A NMR Spectrometer. Microanalyses were carried out by Mr. P. Hansen and his staff.

2,2'-Bis(methylthio)indigotin (**4a**)

Treatment of a slurry of **7a** (200 mg) in $CHCl_3$ (5 ml) containing two drops triethylamine with an excess gaseous methanethiol at room temp. led to immediate reaction. The mixture was left for 15 min at room temp. followed by filtration. The remanence was treated with diethylether to yield 73% (200 mg) **4a**. (Found: C, 60.05; H, 4.33; N, 7.83; S, 17.14. Calc. for $C_{18}H_{16}N_2O_2S_2$: C, 60.64; H, 4.53; N, 7.86; S, 17.99%). Because of instability of the product this preparation was used without further purification.

^{1}H-NMR data are presented in Table 1.

If the $CHCl_3$ was replaced by diethylether as reaction medium **4a** was formed in addition to a product of similar chemical composition and reactivity (**4a'**).

(d,l)4-Hydroxy-4-(4-bromo-2-nitrophenyl)-butan-2-one

To a stirred soln of 4-bromo-2-nitrobenzaldehyde (2 g) in acetone (20 ml) and water (10 ml) was added 2N NaOH (1 ml). Traces of **5b** formed on stirring for 24 hr were removed by filtration. Filtration of the diluted mixture (100 ml water added) left 40% (1 g) of pale brown crystals, recrystallization from water yielded **9** as colourless crystals, m.p. 104–5°. Dilute NaOH aq converted **9** to **5b**. (Found: C, 41.76; H, 3.67; N, 4.85; Br, 27.55. Calc. for $C_{10}H_{10}O_4Br$: C, 41.70; H, 3.51; N, 4.86; Br, 27.75%).

^{1}H NMR ($CDCl_3$, 60 MHz): δ 2.1 (3H, s), 2.8 (1 H, d J 9 Hz), 2.9 (1 H, d J 3 Hz), 4.2 (1 H, s broad), 5.6 (1 H, q J_1 9 Hz J_2 3 Hz). 7.3–8.1 (3 H, m. aromatic protons). EI: MS showed no molecular ion but M^+-18 (m/e 270) could be recognized.

6,6'-Dibromoindigotin

To a soln of 4-bromo-2-nitrobenzaldehyde (20.0 g) in water (1000 ml) and acetone (900 ml) was added 2N NaOH (2 ml portions) at such a rate that pH was kept >10 (in total 50 ml). The mixture was kept overnight at room temp. followed by filtration yielding 7.5 g (42%) violet crystals. Washing with water followed by acetone gave pure **5b**. (Found: C, 45.65; H, 1.84; N, 6.43. Calc. for $C_{16}H_8Br_2N_2O_2$: C, 45.74; H, 1.92; N, 6.67%).

2,2'-Diacetoxy-6,6'-dibromoindigotin (**6b**)

To a stirred slurry of **5b** (1.6 g) in glacial AcOH (20 ml) was added, at 80°, $KMnO_4$ (0.4 g). Stirring for 2 hr at 80° gave a yellow slurry (in some cases brown-yellow). Filtration followed by washing with glacial AcOH (20 ml) and water (100 ml) gave rise to a yield of 68% (1.4 g) **6b**. The product consisting of yellow crystals darkens at 200°, m.p. 355° (dec). (Found: C, 44.52; H, 2.93; N, 5.01. Calc. for $C_{20}H_{14}Br_2N_2O_6$ C, 44.65; H, 2.63; N, 5.21%).

Anhydro-6,6'-dibromoindigotin (**7b**)

2,2'-diacetoxy-6,6'-dibromoindigotin (1.0 g) was added to a mixture of pyridine (3 ml) and toluene (5 ml). Refluxing for 5 min produced a red-brown soln, which upon cooling in ice precipitated red-brown crystals. Filtration followed by treatment with diethyl ether yielded 77% (0.6 g), m.p. *ca.* 310° (dec.). (Found C, 45.95; H, 1.62; N, 6.37; Br, 36.40. Calc. for $C_{16}H_6Br_2N_2O_2$, C, 45.95; H, 1.20; N, 6.70; Br, 38.23%).

2,2'-Bis(methylthio)-6,6'-dibromoindigotin (**4b**)

Treatment of a slurry of **7b** (100 mg) in $CHCl_3$ (5 ml) containing 2 drops of EtN_3 with an excess gaseous methanethiol at room temp. led to immediate reaction. The mixture was left for 15 min at room temp. followed by filtration. The filtrate was discarded and the remanence extracted several times with $CHCl_3$ (in total 300 ml), which on evaporation left a yield of 48% (60 mg) light green solid. (Found: C, 42.00; H, 2.78; N, 5.64; S, 12.54; Br, 31.95. Calc. for $C_{18}H_{14}Br_2N_2O_2S_2$: C, 42.04; H, 2.74; N, 5.45; S, 12.48; Br, 30.80%). The values obtained for authentic tyriverdin were: C, 41.5; H, 2.8; N, 5.4; S, 12.2; Br, 30.8. As the product is light sensitive, the above mentioned procedure should preferably be carried out in the dark.

Table 1. ^{1}H NMR (270 MHz)

Compound	Solvent	δ-SCH_3	δ-NH	aromatic protons δ-H_4	δ-H_5	δ-H_7
authentic tyriverdin	$(CD_3)_2SO$	1.88(s)	8.20(s)	7.47(d)J_{45}8Hz	6.96(q)J_{54}8Hz J_{57}1.5Hz	7.28(d)J_{75}1.5Hz
4b	$(CD_3)_2SO$	1.89(s)	8.23(s)	7.48(d)J_{45}8Hz	6.98(q)J_{54}8Hz J_{57}1.5Hz	7.29(d)J_{75}1.5Hz
4b'	$(CD_3)_2SO$	1.92(s)	8.05(s)	7.33(d)J_{45}8Hz	6.90(q)J_{54}8Hz J_{57}1.5Hz	7.07 J_{75}1.5Hz
4a	$CDCl_3$	1.90(s)	6.36(s)	7.68(1H.d) 7.54(1H.t)	6.99(1H.d) 6.89(1H.t)	
					Total 4 protons	
4a'	$CDCl_3$	1.92(s)	5.74(s)	7.49(1H.t) 7.35(1H.d)	6.96(1H.d) 6.78(1H.t)	
					Total 4 protons	

^{1}H NMR data are presented in Table 1.

UV in $CHCl_3$, λ_{max} 255 nm, 280 nm, 366 nm, 400 nm, 595 nm, λ_{min} 268 nm, 340 nm, 490 nm.

IR in KBr, ν_{max} cm^{-1}: 3380 (s), 3080 (w), 2910 (w), 1680 (s), 1600 (s), 1570 (m), 1450 (s), 1365 (w), 1310 (s), 1280 (m), 1240 (m), 1190 (w), 1150 (w), 1105 (m), 1085 (w), 1050 (m), 1030 (m), 960 (w), 900 (m), 850 (w), 820 (w), 780 (w), 715 (w), 680 (w), 600 (m), 580 (m), 555 (m), 425 (m), 390 (w), 340 (w).

If the chloroform was replaced by diethylether as reaction medium 4b was formed in addition to a product of similar chemical composition and reactivity (4b').

Photochemical conversion of 4b *to* 5b

The reaction was followed by IR spectroscopic analyses using a KBr-disc (300 mg KBr) containing 4b (0.73 mg). On exposition to light (SP 200), the initial spectrum of 4b underwent a fast transformation to a spectrum superimposable with that of analytically pure 5b except for the absorptions at 2910 cm^{-1} assigned to dimethyl disulfide. Within the accuracy of the intensity measurements (NH stretch and CO stretch at 3380 and 1620 cm^{-1} respectively) the yield was quantitative.

Quantum yield determination in the reaction 4a→5a

The experiments were carried out using a Perkin Elmer Fluorescence Spectrophotometer MPF-3 producing light of wavelength 400 ± 4 nm. As standard the ferric oxalate method was used, measuring the phenanthroline complex (510 nm), with a recorded quantum efficiency of 1.14.[13]

A soln of 4a (10 mg) in deoxygenated $CHCl_3$ (50 ml) was irradiated for 1.5 min and the concentration of indigotin determined, using the absorption at 605 nm (ϵ = 12300). It was secured that the soln permitted no light transmission at 400 nm. As indigotin does not absorb at 400 nm there was no need for correction for an inner filter effect. Indigotin is soluble under the mentioned experimental conditions allowing the quantum efficiency to be calculated from experiments like these. Values of 4.95 ± 0.25 were obtained.

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