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QUINOID PIGMENTS FROM THE SEA URCHIN *Toxopneustes pileolus*

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Many studies of the sea urchin *Toxopneustes pileolus* (Lamarck) are dedicated to toxic compounds released from various organs, especially the pedicellariae [1]. However, its pigments have not yet been studied. Quinoid pigments of the spinochrome are a distinguishing feature of this class of echinoderms and impart color to the testae and spines as the Ca and Mg salts [2]. The set of spinochromes for a certain species of sea urchin is not random but is determined by the maternal side [3]. Nevertheless, the color is variable and can differ not only in different populations [4] but also over several days in an individual [5] or from the effect of various factors [6]. These facts have not yet been fully explained. Although these compounds exhibit antimicrobial [7] and anti-oxidant [8] properties, their functions are apparently not limited to these.

We investigated pigments of *T. pileolus* collected near Nya Chang Bay of the South China Sea during the 30th expedition of the SRS Academic Oparin. The color of the sea urchins varied from pale-blue to brownish-green although the pedicellariae of all were pale-rose. We selected one representative from each of three differently colored groups of sea urchins, pale-blue, green, and brownish-green. The remaining specimens (20) were combined to obtain a total pigment extract by collecting from them beforehand air-dried pedicellariae (~3 g).

Before pigments were extracted the testae with spines were first washed with EtOH and *n*-BuOH. Spinochromes were extracted exhaustively with *n*-BuOH, decomposing the material with dilute HCl. The resulting extract was washed with water to remove HCl, evaporated, and chromatographed several times over KSK silica gel with elution by benzene with a gradient of increasing acetone content. Pure compounds were purified further over Sephadex LH-20 using $CHCl_3$:EtOH (4:1) to afford **1** (12 mg), **2** (2 mg), and **3** (52 mg). These were identified by comparing their UV, mass, and NMR spectra with the literature [2] and with those of known spinochrome samples that were isolated by us earlier from *Strongylocentrotus nudus* [9] as spinochromes A, C, and B, respectively.

Only spinochrome A was observed in pedicellariae of this species of sea urchin.

The composition and ratio of spinochromes in representatives of the various color groups were determined in extracts obtained as described above except for the use of Et_2O as the extractant. Spinochromes were separated from the resulting extracts by saturated $NaHCO_3$ solution and were again transferred to an Et_2O solution by acidifying the extract with HCl. The extract was washed with H_2O to remove HCl, dried over Na_2SO_4, evaporated, and analyzed by TLC and HPLC using spinochromes isolated during this work as references.

Differently colored testae and spines contained all three spinochromes observed in the total extract from this species. Spinochrome B dominated (>97 and 86.4%) in green and brownish-green specimens, respectively. Spinochromes A and C made up 0.5% for the green specimens. The brownish tone was imparted to the animals by higher contents of these compounds, 6.7 and 4.2%, respectively. The main component in extracts from pale-blue specimens was spinochrome A. Spinochromes B and C were present in trace quantities.

These results and those from a single study in the literature [4] indicate that the pigment compositions of testae and spines of sea urchins are characteristic of a species. However, color variability in individuals is determined by the quantitative ratio of spinochromes.

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