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*Food Chemistry* **51** (1994) 227–229

0308-8146/94/$7.00

RESEARCH NOTE

# Sephadex LH-20 separation of pigments from shells of red sea urchin (*Strongylocentrotus franciscanus*)

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(Received 15 July 1993; revised version received and accepted 6 December 1993)

Extracts of shells of red sea urchin (*Strongylocentrotus franciscanus*) in diethyl ether were separated by Sephadex LH-20 column chromatography. Methanol was used as eluting solvent. Seven fractions were separated, the first four of which did not absorb in the visible range. The last three fractions, which were coloured, are attributed to spinochrome B, echinochrome A and spinochrome E.

## INTRODUCTION

The sea urchins are dominant macroscopic benthic animals along both the Atlantic and Pacific coasts of Canada. The urchin populations are concentrated in shallow waters, and their density can reach as high as 350 urchins per square metre (Himmelman, 1969). The gonads of sea urchins are of sufficient yield and sensory quality to justify their commercial exploitation. The edible portion of the body of the sea urchin has a high content of free amino acids, up to 10 g/100 g crude protein (Lee & Harada, 1982), which are important to the taste of 'uni', the salted unripe gonads of sea urchin (Komata *et al.*, 1962). Glycine is the dominant free amino acid, accounting for 35–41% of the total amount (Hirano *et al.*, 1978; Lee & Harada, 1982). Sea urchins are consumed primarily by Japanese, French, South Americans, and to a lesser extent, North Americans as raw or fermented products (Pleschner, 1985).

The shells are the major processing by-products of sea urchins and their value-added utilization is necessary. They may serve as a potential source of biologically active compounds. Spinochromes are present in the calcareous skeleton of sea urchins. These compounds are generally hydroxylated naphthoquinones (Natori, 1975); however, in one case, a benzoquinone derivative has also been identified. Utilization of extracted pigments from sea urchin shells in different applications needs to be explored. Figure 1 shows the chemical structures of naphthoquinones that are known to occur in sea urchin shells. The complex mixture of these pigments has been separated on a column of acid-treated, deactivated silica gel (Moore *et al.*, 1966; Singh *et al.*, 1968).

The degree of adsorption of phenolic compounds onto lipophilic Sephadex depends generally on the number of hydroxyl groups (Johnson *et al.*, 1968) present in the molecule. The objective of the present study was to examine the use of Sephadex LH-20 for the separation of naphthoquinone pigments of sea urchin shells. The presence of different numbers of hydroxyl groups in the molecules is expected to allow their adequate separation. Tentative identification of individual pigments so obtained was attempted.

## MATERIALS AND METHODS

Red sea urchins (*Strongylocentrotus franciscanus*) were harvested by divers in the Vancouver area. They were then frozen and kept at −20°C until use. After thawing, testes were cut with a knife, internal organs were removed and shells were washed with a stream of cold water at approximately 10°C and air-dried at 20°C for a 24-h period. Dried shells were ground in a Waring blender (Model 33 BL 73, Dynamics Corp., New Hartford, Connecticut, USA), vacuum-packed in polyethylene pouches and kept frozen at −20°C until use.

Ground shells (10 g) were dissolved in 40 ml 10%

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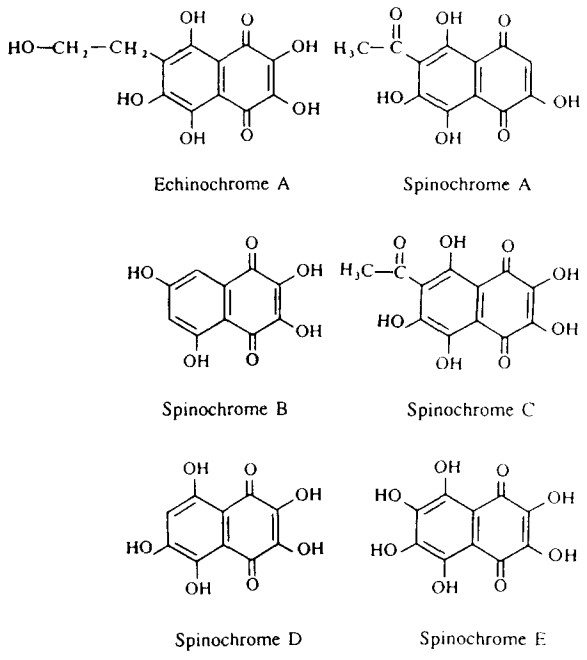


**Fig. 1.** Chemical structure of sea urchin pigments.

HCl at room temperature. The pigments were extracted into 50 ml of diethyl ether. The ether layer was then washed with small amounts of water until the acid was removed. The ether solution was dried over anhydrous sodium sulphate and placed into test tubes, and the solvent was removed with a stream of nitrogen. The residue was dissolved in 2 ml of methanol and applied on top of a 44 × 1·2 cm column packed with Sephadex LH-20. The eluent solvent was methanol. The fractions (5 ml) were collected using a fraction collector. The absorbance of methanolic solutions from each tube was measured at 270, 340 and 470 nm. According to their absorptions, eluates were then pooled into seven fractions, evaporated to dryness and weighed. The absorption spectra of each fraction in methanol were obtained

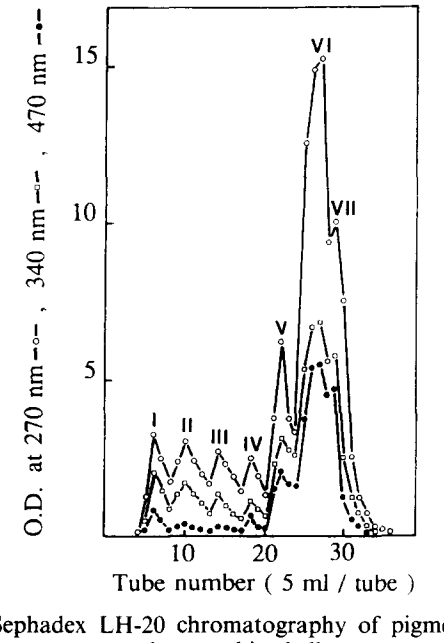


**Fig. 2.** Sephadex LH-20 chromatography of pigments from red sea urchin shells.

**Table 1. Composition of separated fractions (%) of sea urchin pigments and their absorption data ($\lambda_{max}$ and $\lambda_{sh}$, nm)**

| Fraction | Weight percentage | $\lambda_{max}$ | $\lambda_{sh}$ |
|---|---|---|---|
| I | 5·6 | 282 | 318 |
| II | 26·6 | 270 | 318 |
| III | 43·5 | 286 | 318 |
| IV | 7·5 | — | 240, 298 |
| V | 5·1 | 270, 324, 464 | 524 |
| VI | 4·7 | 264, 348, 476 | 524 |
| VII | 7·0 | 266, 346, 476 | 534 |

using a Hewlet–Packard 8452 A diode-array spectrophotometer.

## RESULTS AND DISCUSSION

Figure 2 shows the absorption characteristics of the seven fractions of diethyl ether extracts of sea-urchin pigments that were separated on a Sephadex LH-20 column, at 270, 340 and 470 nm. Whereas the first four fractions had negligible absorption at 470 nm, fractions V–VII showed intense absorptions at this wavelength. The absorption of fractions V–VII in the UV range was also much higher than that for fractions I–IV. However, the proportion of the first four fractions was considerably

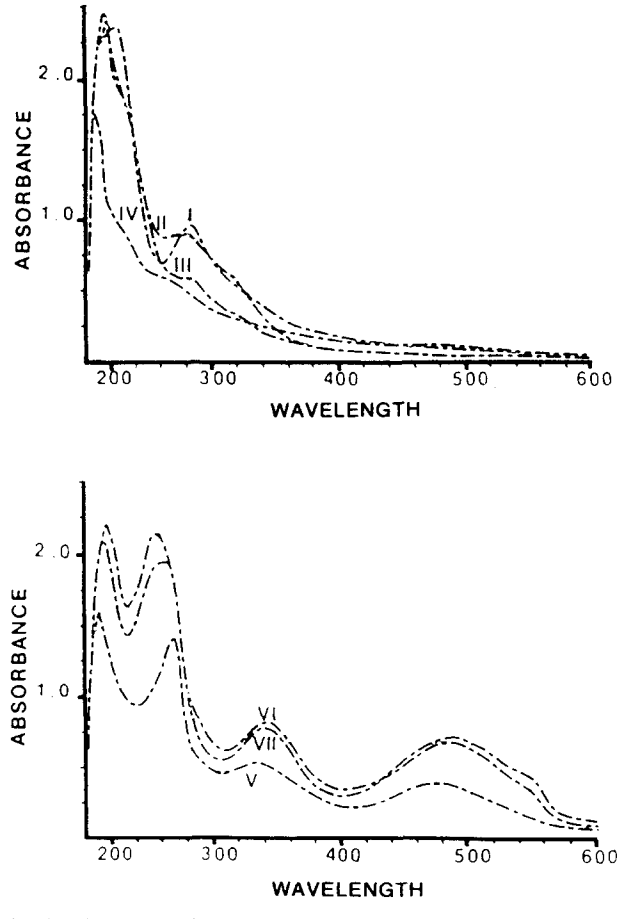


**Fig. 3.** Electronic absorption spectra of the fractions obtained after Sephadex LH-20 chromatography.

higher than that of the others. Fractions II and III contributed 26·6 and 43·5%, respectively, to the pigments present in the extracts from sea urchin shells (Table 1).

Electronic spectra of the first four fractions did not show any absorption in the visible range (Fig. 3, Table 1). Only fraction I exhibited a sharp absorption at 282 nm. The spectrum of fraction IV showed shoulders at 240 and 298 nm. Three absorption peaks were noticed for fractions V–VII. An examination of the spectral data indicated that fraction V may contain spinochrome B. According to Natori (1975), spinochrome B exhibits absorption maxima at 272, 323 and 480 nm, and a similar absorption pattern was noticed for fraction V. Echinochrome A was presumably the main compound present in fractions VI and VII. Both fractions showed absorption peaks at 260, 343 and 470 nm (Natori, 1975). Fraction VII also contained spinochrome E with absorption maxima at 270, 359 and 477 nm (Natori, 1975).

The results presented in this study are consistent with the theoretical assumption that adsorption of phenolic compounds onto Sephadex LH-20 depends on the number of hydroxyl groups present in the molecule (Johnson, 1968). Spinochrome B and echinochrome A possess five hydroxyl groups in their molecule, whereas spinochrome E has six hydroxyl substituents. The elution of these pigments was in the order of spinochrome B, echinochrome A and spinochrome E.

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