The quinoid pigments of the test and needles of the sea urchin Strongylocentrotus nudus (Agas) consist mainly of spinochromes A, B, and C and echinochrome A [2]. We have established that, in addition to the components mentioned, these animals produce a number of minor quinones of the same type. The chromatographic mobilities of the pigments are given below (the $R_f$ values were determined in a nonfixed layer of KSK-ShK silica gel in system 1):

<table>
<thead>
<tr>
<th>Pigment</th>
<th>$R_f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigment I</td>
<td>0.67</td>
</tr>
<tr>
<td>Pigment II</td>
<td>0.60</td>
</tr>
<tr>
<td>Spinochrome A</td>
<td>0.56</td>
</tr>
<tr>
<td>Spinochrome C</td>
<td>0.42</td>
</tr>
<tr>
<td>Pigment III</td>
<td>0.40</td>
</tr>
<tr>
<td>Echinochrome A</td>
<td>0.38</td>
</tr>
<tr>
<td>Pigment IV</td>
<td>0.37</td>
</tr>
<tr>
<td>Spinochrome B</td>
<td>0.34</td>
</tr>
<tr>
<td>Pigment V</td>
<td>0.30</td>
</tr>
<tr>
<td>Pigment VI</td>
<td>0.25</td>
</tr>
<tr>
<td>Pigment VII</td>
<td>0.24</td>
</tr>
</tbody>
</table>

The least polar minor component I, with a violet color, has an absorption spectrum that is typical for naphthazarin derivatives [3]. The mass spectrum contains the peaks of the molecular ion $m/e$ 278 and of fragmentary ions with $m/e$ 236 (M-42) and 235 (M-43). These fragmentation pathways are confirmed by the presence in the spectrum of the peaks of metastable ions at 200.3 and 198.5 and show the presence of an acetyl group in the molecule of this fragment [4]. Since the splitting out of CO from the molecular ion precedes the loss of the methyl radical of the acetyl group, the latter is most probably located in the quinoid part of the molecule. The ion (M-28) also splits out water, which is confirmed by a metastable ion at 215.5, showing the adjacent positions of the acetyl and hydroxy groups [4]. The known quinoid pigments of sea urchins are either unsubstituted polyhydroxynaphthoquinones or compounds of this type containing one two-carbon substituent, and therefore the observation in the fragment under investigation of, in addition to the acetyl group, another carbon-containing substituent, a methyl group, was all the more unexpected and interesting. Its presence is shown by an ion with $m/e$ 263 (M-15) in the mass spectrum, and by a signal at 2.17 ppm (ar. CH$_3$) in the PMR signal.

On methylation with diazomethane, pigment I formed a dimethyl ether, which was accompanied by an increase in the molecular weight by 14 x 2 units and by the appearance in the PMR spectrum of a signal at 4.08 ppm (6H, 2 ar. CH$_3$O). The signals of the protons of the two peri-hydroxyls of the naphthazarin system at 12.70 and 13.04 ppm remained unchanged.
On the basis of these results it may be assumed that the pigment is 3-acetyl-2,7-dihydroxy-6-methyl-
naphthazarin, or 2-acetyl-3,7-dihydroxy-6-methylnaphthazarin. The C-methylation of natural spinochrome A
with acetyl peroxide according to Fieser gave 3-acetyl-2,7-dihydroxy-6-methylnaphthazarin. The chromatog-
ographic behavior, melting point, and absorption and mass spectra of the substance synthesized were identical
with those of the natural pigment, and this has permitted us to assign the following structure to it:

\[
\begin{array}{c}
\text{OH} \\
\text{H} \\
\text{OH} \\
\text{OH} \\
\text{CH}_2 \\
\text{OH} \\
\text{O} \\
\end{array}
\]

Another minor violet pigment, IV, and Rf values very close to those of echinochrome A. Absorption and
mass spectra and the melting points of the pigment and its dimethyl ether agree well with those given in the
literature for moppain (2,7-dihydroxynaphthazarin) and its dimethyl ether, respectively [5]. A confirmation of
this structure is the complete coincidence of the chromatographic behavior, melting point, and absorption spec-
tra of pigment IV and the product obtained by the deacetylation of natural spinochrome A.

In individual years, considerable, sometimes predominating, amounts of the highly polar pigment VII ap-
pear among the total pigments of the sea urchin, this substance corresponding in all its indices to the spino-
chrome E described in the literature [6]. The product of its methylation with diazomethane likewise corresponds
to spinochrome E tetramethyl ether. Consequently, pigment VII is spinochrome E.

The other minor pigments were isolated in amounts not exceeding 1-1.5 mg. From the nature of their
absorption spectra, three of them (II, V, and VI) are naphthazarin derivatives [3]. They were methylated with
diazomethane, forming methyl ethers the absorption spectra of which were characteristic for the spectra of
methyl ethers of hydroxynaphthazarins [3].

The PMR spectrum of the methyl ether of pigment V contains signals at 12.92 and 13.03 ppm showing the
presence of two peri-hydroxyls, and at 3.95, 4.07, and 4.15 ppm, confirming the presence of three methoxyls in
the ether molecule. However, neither the initial pigment or its methyl ether showed under electronic impact
the fragmentation characteristic for the naphthazarin derivatives usually found in sea urchins and the methyl
esters of these compounds. A similar uncertain picture was found in the case of the mass spectrum of pigment
II and its methyl ether, and therefore further investigations are necessary to establish the complete structure
of these pigments.

Pigment VI has a mass spectrum coinciding, in the main, with that of the binaphthoquinone isolated from
Strongylocentrotus intermedius and Strongylocentrotus dreebachensis, but possesses greater mobility on TLC
and a lower melting point of 221-222°C (for the binaphthoquinone mentioned, Rf = 0.22, system 1, mp > 310°C). Pigment VI and its methyl ether have absorption spectra close to those of the binaphthoquinone and its hexa-
methyl ether, respectively. Furthermore, the ether of VI has mp 66-68°C, which is close to the melting point
of the hexamethyl ether of the binaphthoquinone isolated from Strongylocentrotus dreebachensis (71-73). The
mass spectrum of the methyl ether of pigment VI differs not only from the spectrum of the methyl ether of the
binaphthoquinone but also from the spectra of the methyl ethers of monomeric polyhydroxynaphthoquinones.

Another pigment, III, present in trace amounts, has been found in this species of sea urchin. On KSK
silica gel in all solvent systems used it has Rf values very close to those of spinochrome C. It was possible
to separate it on silica gel LCh of Czech origin containing 15% of 1 N oxalic acid solution. Pigment III is yellow
but on storage in the crystalline state and in solution a substance with the Rf values and absorption and mass
spectra of spinochrome C appears. The mass spectrum of pigment II is also identical with that of spino-
chrome C [2].

When a methanolic solution of spinochrome C was stored for a long time, again the appearance of a yellow
pigment chromatographically indistinguishable from pigment III took place. We have also observed such mutual
transitions of one colored form of a minor pigment into another in the case of other species of sea urchin (for
example, in Scaphechinus mirabilis. It is possible that the mutual conversions of these pigments are connected
with the tautomerism of the naphthalene system upon which they are based.

**EXPERIMENTAL**

The pigments were separated first by column chromatography and then by repeated preparative TLC in a
nonfixed layer of KSK silica gel containing 15% of a 1 N aqueous solution of oxalic acid (KSK-ShchK), using the