30. **The Studies on the Derivatives of Naphthoquinone. Part IX.**

The Pigments from the Sea-Urchins. IV.

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In the previous papers\(^1\)\(^2\)\(^3\) for the Spinochrome Aka, for the pigment from the spines of sea-urchin *Pseudocentrotus depressus* the formula \([I]\) was given, with its derivatives \([I'], [I'']\), etc., which also were obtained, and the analytical values for all of them, and the results of methoxy and acetyl determination were shown to be all in agreement with calculation.

For the Spinochrome B, for the pigment from spines of sea-urchin *Strongylocentrotus pulcherrimum*, formula \([II]\)\(^4\) was given for the following facts. (1) Analytical results of Spinochrome B, its acetyl derivative, and its methyl ether, and the acetyl derivative of dihydrospinochrome B; (2) the values of the acetyl and methoxy determination; (3) the properties of Spinochrome B and its derivatives, namely behaviors for solvents; colour reaction with alkali or FeCl₃ etc.; (4) oxidation products from methylether of B with O₃\(^5\).

When the above pigments were compared with the following synthetic derivatives of hydroxy naphthoquinone etc., important

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\begin{align*}
[I] & \\
[I'] & \\
[I''] & \\
[II] & \\
[III] & \\
[IV] & \\
[V] & \\
[VI] & \\
[VII] & \\
\end{align*}
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\(^1\) C. Kuroda & H. Ohshima: Proc., 16 (1940), 214.
properties were found common for all of the natural and the synthetic compounds as follows:

1. For methylation with diazo-methane a special procedure by Kuroda\(^6\) was necessary.

2. Demethylation with dil. caustic alkali. When the following compounds III', IV', V', VI', & VII' were treated with hot dil. (2%) caustic alkali the demethylation from CH\(_3\)O in quinone ring took place slowly in about one hour or more whereas the reactions were instantaneous in the case of 2:3 dimethoxynaphthoquinone and 2 methoxynaphthoquinone.

In another part\(^7\) a very interesting note has been recorded that in the course of careful isolation of pigments from the spines of several kinds of sea-urchins, new pigments were found in each kind of them, namely from the spines of Strongylocentrotus pulcher-rimun, another pigment B\(_2\) along with B; from Anthocidaris crassispina, another pigment M\(_2\) along with M etc. for which interesting relations have been anticipated. Yet it was impossible to study more about them, because the material was so poor in quantity. Recently, however, some Anthocidaris crassispina\(^8\) was obtained from Hokkaido to continue this work, and this material was fortunately very convenient for the isolation of two kinds of pigment. A very interesting result was shown as follows.

The pigment from the spines of sea-urchins was extracted by aid of HCl and organic solvent as previously. In this case, however, two* kinds of organic solvent were applied while ether alone was used in the previous work; namely at first with benzene*, then after finishing the extraction with the above solvent, it was extracted with ether*. From each fraction by the above two solvents Mg salt of each pigment was precipitated with bicarbonate of Mg. A free pigment M\(_1\) (M itself in the previous reports is named M\(_1\) in this work), obtained from Mg salt from benzene fraction by means of HCl aq., was purified with acetic acid as in the case of M itself which had already been mentioned.

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8) This material was kindly sent from Miss Sada Yamanishi.
Another new pigment M₂ obtained from Mg salt from ether fraction by means of HCl was purified with methylalcohol. M₂ resembles Spinochrome B in appearance giving namely, bright red crystal which sublimes without melting over 200° C; it turns yellowish green with aq. caustic alkali and dark green with FeCl₃.

Moreover as the results of the following experiments, M₂ was proved to be quite identical with Spinochrome B.

1. Analytical values found: C 50.07, H 3.68%. \( \text{C}_{10} \text{H}_6 \text{O}_6 + \text{H}_2\text{O} \) requires C 50.00, H 3.33%.

2. Acetylderivative in yellow needles mp 154.5–155° (no change in mixed sample with authentic sample). C 55.64, H 3.58%. \( \text{C}_{10} \text{H}_2 \text{O}_2 (\text{OAc})_4 \) requires C 55.38, H 3.55%. It was produced when the pigment was treated with acetic anhydride and conc. \( \text{H}_2\text{SO}_4 \).

3. The methylether in orange needles mp 110.5–111° (no change in mixed sample with authentic sample). Found: C 59.31, H 4.71%. \( \text{C}_{10} \text{H}_3 \text{O}_6 (\text{CH}_3)_3 \) requires C 59.09, H 4.71%. It was obtained when the pigment was carried out with diazo-methane in the special procedure suggested by Kuroda previously.9)

4. Demethylation with caustic alkali.

The etheral solution of the above methylether was shaken with cold 2 N NaOH aq. for a few minutes; when the aqueous alkaline layer which contains the pigment was acidified, an orange yellow crystalline substance was produced.

This compound when purified from methylalcohol dissolved in sod. bicarbonate aq. with red colouration. This is therefore considered to be demethylated from \( \text{CH}_3\text{O} \) group in quinone ring (see p. (6) 34, and analytical values also proved the above relation as follows.

Found: C 57.24, H 4.09%. \( \text{C}_{10} \text{H}_9 \text{O}_6 \) requires C 57.61, H 4.00%.

From the spine of sea-urchin Anthocidaris from Kagoshima, pigment M₂ which is quite identical with Spinochrome B also was likewise isolated in small yield.

In conclusion, it is very interesting to note that the colour of the above material, the spines of sea-urchin from Hokkaido, is lighter than that from the same kind of sea-urchin from Kagoshima above mentioned.

The difference in the colour is considered as result of difference in the proportion of M₁ (deeper coloured pigment than M₂) and M₂, namely yield of M₂ from the above spines produced in Hokkaido is very rich (M₂ 50 mg from spine 600 g) while that of M₁ is very poor. On the contrary, in deeper coloured spines produced in Kagoshima, the yield of M₁ isolated is rich while that of M₂ is poor.

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The above interesting relation seems also to show some important meaning in biochemistry.

The presence of Spinochrome B₁ along with B₂ in the spines of *Strongylocentrotus pulcherrimum* also gives an interesting and important anticipation. More confirmatory experiments are in progress.

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