*Structure of Namakochrome. I**

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In a previous paper[1] of this series the isolation of namakochrome, which would be a new derivative of the polyhydroxynaphthoquinones, from *Polycheira rufescens* was reported.

Polycheira rufescens (in Japanese, Murasaki-kuruma-namako) is a sort of tiny sea cucumber, its body being dark purple in color. A crowd of the animals may be frequently found in the stony tidal zone. As preliminary examination assigned the purple coloration of the sea cucumber to a chromoprotein, some methods to isolate the prosthetic group of the protein had been undertaken, and namakochrome was obtained.

Twenty-eight thousand of the sea cucumbers were collected for material at a sea coast near the Amakusa Marine Laboratory, the Faculty of Science, Kyushu University. These animals were dipped into a dilute aqueous solution of acetic acid. The chromoprotein dissolved easily in the aqueous acid to give a red solution. To the red extract, diluted aqueous solution of sodium hydroxide was added till the color of the extract changed to dark purple. After the resulting opaque solution was allowed to stand overnight, a pale yellow supernatant liquid was decanted off and the remaining purple pasty precipitates were collected***. The precipitates were treated with concentrated hydrochloric acid, followed by extraction with ether. Two and thirty-six hundredths grams of crude, orange red pigment of the prosthetic group were obtained. Repeated recrystallization from glacial acetic acid afforded pure namakochrome, a pigment of crimson prisms, m. p. 218°C.

Namakochrome contains only three elements, namely, carbon, hydrogen and oxygen. Its molecular weight determination (by the micro-Rast method with camphorquinone) and elementary analysis yield the result which is in agreement with the molecular formula $C_{11}H_8O_8$. The ultraviolet (ethanol) and infrared (Nujol) absorption spectra are shown in Figs. 1 and 2, respectively. On heating, a greater part of namakochrome sublimes, leaving some carbonized matter. This pigment is easily soluble in solvents of high polarity. It is soluble in mineral acids, to which it is very stable; even in hot concentrated sulfuric acid a red color of the solution lasts for a considerably long time. It is unstable to the solution of weak alkali. With sodium bicarbonate solution it gives a light yellow color, changing gradually to colorless. On the other hand, it dissolves easily in

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* "Pigments of Marine Animals", IX. VIII of this series: M. Yamaguchi, This Bulletin, 32, 1171, (1959).

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[1] T. Mukai, *Mem. Fac. Sci., Kyushu Univ., Series C, Chem.*, 3, No. 2, 29 (1958).

*** The same precipitates could be obtained by the addition of methanol instead of aqueous sodium hydroxide, but this caused only imperfect precipitation, and its supernatant liquid still remained deep red.

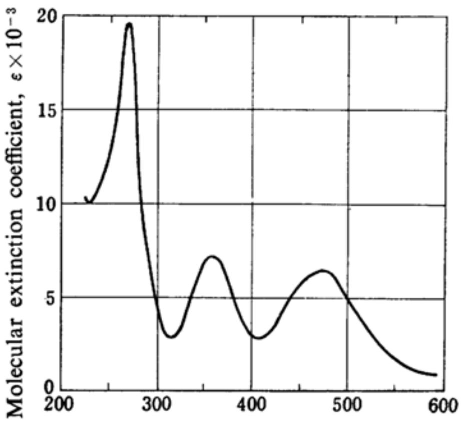


Fig. 1. Absorption curve of namakochrome: solvent, ethanol.

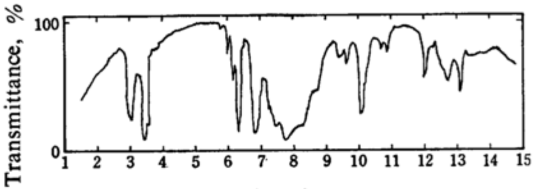


Fig. 2. Infrared spectrum of namakochrome.

dilute solution of strong alkali with orange red color, while with its more concentrated aqueous solution, it forms immediately purplish black salt, which is sparingly soluble in it. With heavy metals also, it gives the difficultly soluble black salts with purple luster. Namakochrome reduces silver nitrate, and gives a dark purple color with ferric chloride. It gives a purple precipitation with neutral lead acetate, a reddish purple precipitation with basic lead acetate, and a dark orange-yellow color with magnesium acetate. On adding zinc dust to a solution of namakochrome in an acetic anhydride pyridine mixture, an original red color soon changes to yellow, then the solution gradually turns colorless, and at last returns to yellow****. This yellow solution becomes red when warmed with concentrated sulfuric acid.

Now, the above-mentioned properties and the molecular formula would suggest that namakochrome is a derivative of polyhydroxynaphthoquinones. To obtain information on the basic structure of the molecular skeleton, zinc dust distillation of namakochrome was carried out, and naphthalene was isolated in a relatively good yield.

Brockmann[2] found that when most of quinone derivatives were hydrogenated in the presence of palladium catalyst on barium sulfate, in aqueous alkaline solution, only the quinone-system prevailed over the other reducible groups in the same molecule. He extended this selective hydrogenation method to a determination of a molecular weight of Actinorhodin, a new hydroxyquinone derivative, isolated from *Streptomyces coeli* color. This method was applied to catalytic hydrogenation of namakochrome, and the result showed that namakochrome shoud have a quinone-system in the molecule, as it absorbed one molecular equivalent of hydrogen. On coming in contact with the air, the resulting pale brown solution changed its color to original red.

In the presence of concentrated sulfuric acid as a catalyst, namakochrome reacts with acetic anhydride to yield a lemon yellow acetyl derivative, m. p. 158~163°C. The acetyl derivative is labile to alkali. When its ethereal solution is brought into contact with aqueous sodium bicarbonate solution even for a short time, decomposition of the acetate occurs and the original material, namakochrome, is isolated. In contrast with namakochrome, the acetate is easily soluble in benzene, sparingly soluble in cold ethanol, and recrystallization from ethanol is very easy. The analytical data[3] obtained for the acetate is in accordance with the formula, $C_{11}H_3O_8(COCH_3)_5$. The infrared absorption spectrum of the acetate is shown in Fig. 3.

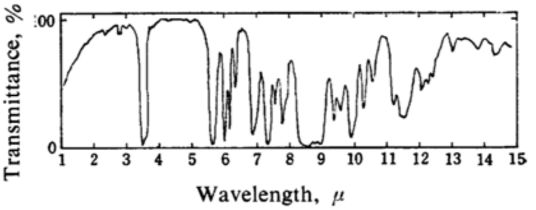


Fig. 3. Infrared spectrum of penta-acetyl namakochrome.

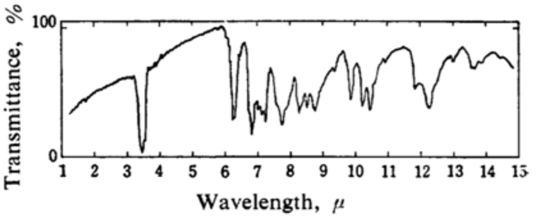


Fig. 4. Infrared spectrum of trimethyl ether of namakochrome.

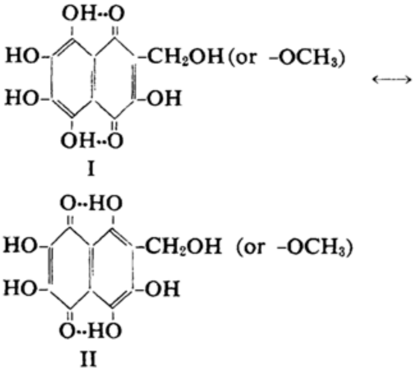
**** There must be a leuco-acetyl derivative of namakochrome in the resulting yellow solution, but no crystalline product is obtained as yet.

2) H. Brockmann et al., *Ber.*, **88**, 781 (1955).

3) In the estimation of acetyl groups of the acetate, 5 N NaOH was emloyed as a saponifying agent. Namakochrome was also treated under the same saponifying condition, because there remained doubt whether this agent degrades namakochrome to acidic volatile substances. It was shown that under these conditions namakochrome which was liberated from the acetate by the saponification, did not suffer an undesirable change.

Since the formation of the acetate evidently shows many hydroxyl groups to be present in the namakochrome molecule, namakochrome was strongly methylated by the Kuroda's method[4]. The resulting ether is of brownish red long needles, melting at 185~187°C. Analysis of the ether yields results which are in accordance with the formula $C_{11}H_5O_5(OCH_3)_3$. The infrared absorption spectrum of the ether is shown in Fig. 4. In the trimethyl ether, two of the hydroxyl groups at the α-positions of naphthalene nucleus would, as usual, not be methylated and remain free. Furthermore, the following properties of the ether indicated that the positions of the three methylated hydroxyl groups would be located in 3-, 6- and 7-positions of the naphthalene nucleus. Such properties were of the color reactions, which were introduced by Kuroda[5], and were taken up in the work of Kuhn[6] on the structure of Echinochrome A. That is, namakochrome trimethyl ether, like naphthazarin and β-alkyl naphthazarin, dissolved in an aqueous solution of sodium hydroxide to give a purplish blue color[7], and did not dissolve in an aqueous solution of sodium bicarbonate. On the contrary, the color reaction and the precipitation reaction of namakochrome itself, already described, could be recognized it as the analogue of naphthopurpurin or β-alkyl naphthopurpurin.

Although, there is not, as yet, the clue given to the nature of one of the eight oxygen atoms in the namakochrome molecule, this oxygen atom should form a group together with the other remaining one carbon atom and three hydrogen atoms. The unascertained group would, therefore, be either a hydroxymethyl or a methoxyl group, which should locate at the β-position of the naphthalene nucleus. Namakochrome may be, anyhow, a new derivative of the naphthoquinones, possessing the following structure (I ↔ II).



Experimental

Distillation of Namakochrome with Zinc Dust.—Essentially the method of Ochiai and Tsuda[8] was followed for the distillation of namakochrome with zinc dust. Namely, one end of a glass tube (9 mm. in diameter and about 50 mm. in length) was drawn to a capillary about 2 mm. in diameter. A plug of clean asbestos was inserted at the point where the capillary began. Zinc dust was introduced into the tube so that it covered a length of about 10 mm. from the plug, and a mixture of namakochrome and zinc dust was introduced into the tube to fill a length of about 10 mm. of the tube. Zinc dust was last placed in the tube, which was then sealed at the open end. The mixture of 370 mg. of namakochrome and 7 g. of zinc dust was treated with seventeen of the above-mentioned apparatus. The tube containing the materials was heated from the open side to the closed side with continuous rotation, and was finally heated red hot. The distillates were three separate fractions. The first distillate was a waxy substance having a blue fluorescence near the asbestos plug. At a short distance, there was found the second distillate, colorless liquid over which the third colorless leaflets and white solid was lapped. The portions of the crystals and the solid were cut into sections (about 10 mm.) and packed at a sealed end of a glass tube (about 15 mm. in diameter). The other end of the tube had been drawn out to form a capillary, whose length and inside diameter were 70 mm. and 1.5~2 mm., respectively. Then the apparatus was emptied to about 20 mmHg from the open end of the capillary, which was then sealed off. The portion of the larger tube was horizontally inserted in an aluminum block maintained at 95°C, and a wet filter paper condenser was attached at a half of the capillary portion from its closed end. The part of the capillary where the colorless oil separated perfectly, was cut off, and the remainder portions of the capillary were handled again in the same manner as described. Repeated operations afforded the solid which is slightly contaminated with the oil. The solid was then purified by crystallization from ethanol. About 4 mg. of crystals, m. p. 80°C, were obtained. This was naphthalene, whose melting point was unaffected by admixture with an authentic sample.

Catalytic Hydrogenation.—In 4 ml. of 1 N sodium hydroxide, 7.65 mg. of namakochrome were hydrogenated in the presence of about 50 mg. of palladium on barium sulfate catalyst.

Found: 1.02 mol. of hydrogen.

Penta-acetyl Namakochrome.—One drop of concentrated sulfuric acid was, at room temperature, added to a suspension of namakochrome (50 mg.) in acetic anhydride (1.5 g.), to give a lemon yellow solution. After setting aside for two hours at room temperature, the resulting solution was poured into

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[4] T. Kuroda, *Nat. Sci. Rep. Ochanomizu Univ., Tokyo*, **2**, 90 (1951).

[5] T. Kuroda et al., *Proc. Imp. Acad.*, **12**, 239 (1936); ibid., **13**, 158 (1938).

[6] R. Kuhn et al., *Ber.*, **72**, 1407 (1939).

[7] When the trimethyl ether was catalytically hydrogenated by the Brockmann's method described above, the purplish-blue color caused by the alkali rapidly changed into nearly colorless and again backed to purplish-blue on exposure to air. At this time the trimethyl ether absorbed one molecular equivalent of hydrogen.

[8] E. Ochiai and K. Tsuda, "Yuki Biryo Shyoryo Teiryo Bunsekiho", Nanzando, Tokyo (1940), p. 300.

ice water and extracted with benzene. The yellow extracts were concentrated to a small volume under the reduced pressure. The concentrate was filtered through a calcium carbonate column (3×16 cm.). Evaporation of the effluent under reduced pressure, and recrystallization of the resulting yellow residue from ethanol gave penta-acetyl namakochrome as lemon yellow needles (50 mg.), m. p. 158~163°C.

Found: C, 52.68; H, 3.93; AcO, 45.40. Calcd. for $C_{21}H_{18}O_{13}$: C, 52.73; H, 3.79; AcO, 44.99%.

Trimethyl Ether of Namakochrome.—A solution of diazomethane (prepared from 5 g. of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide) in ether (40 ml.) was added dropwise to crystalline namakochrome (300 mg.) spread on a large evaporating dish. When the violence of the reaction abated, and evaporation of the ether slowed, the solvent remaining in the evaporating dish was removed. Addition of the diazomethane solution was then continued. After the addition of the whole ethereal solution of diazomethane, the remaining solvent was evaporated as before. The resulting residue was dissolved in benzene, and filtered through a calcium carbonate column. The red effluent was evaporated to dryness under reduced pressure, and there remained brownish red, fine, long, needles, which, by recrystallization from a mixture of ethanol and benzene, gave trimethyl ether (279 mg.), m. p. 185~187°C.

Found: C, 54.17; H, 4.72. Calcd. for $C_{14}H_{14}O_8$: C, 54.20; H, 4.55%.

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