真珠の黄色色素 -I.

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Yellow Pigments of Pearl—I. Carotenoid Pigment in Yellow Nacre

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Yellow pearl which is found relatively high frequency in cultured pearl oyster in Japan is generally rather iridescent though, it is of little commercial value because of its undesirable color.

The color of pearl has been considered due both to the constructive interference of light reflected from pearl layer and chemical constituents such as heavy metals[1], porphyrins[2], and other organic pigments[3] in nacre.

It have been reported that porphyrins[2] and iron complex of peptide[4] may be responsible for the coloration of yellow pearl. However, the iron complex of peptide reported by SAWADA[4] seems to be more important.

In the course of the present investigation which was carried out to throw more light on the SAWADA's pigment, a small amount of carotenoid pigment was found in the nacre of yellow pearl, of which existence had not been reported before.

The present paper deals with extraction, separation, and absorption properties of the pigment.

Experimental

Extraction of acetone soluble pigments. An outline of extraction procedure is shown in Fig. 1. A lot of yellow pearls were crushed with hammer and separated into nacre layer and nucleus. Those nacres having brown or black stains were discarded and only stainless nacres were used for extraction of the pigments. The nacres were reduced into 60 mesh powder in a ball mill and thrown into a large volume of 5% acetic acid for the purpose of decalcification. After the mixture was continuously stirred for one hour, colorless supernatant liquid was decanted off. The sediment was again treated with newly added 5% acetic acid. This procedure was repeated further several times until the nacres were completely decalcified. Crude conchiolin, completely decalcified nacres, was washed with water and then treated with 90% acetone for extraction of the pigments. The extract of the pigments was concentrated in vacuo to a small volume and shaken with added water and ether. By this treatment the yellow pigments migrated from aqueous layer into ether layer. The etherial solution was dried on anhydrous sodium sulfate followed by filtra-

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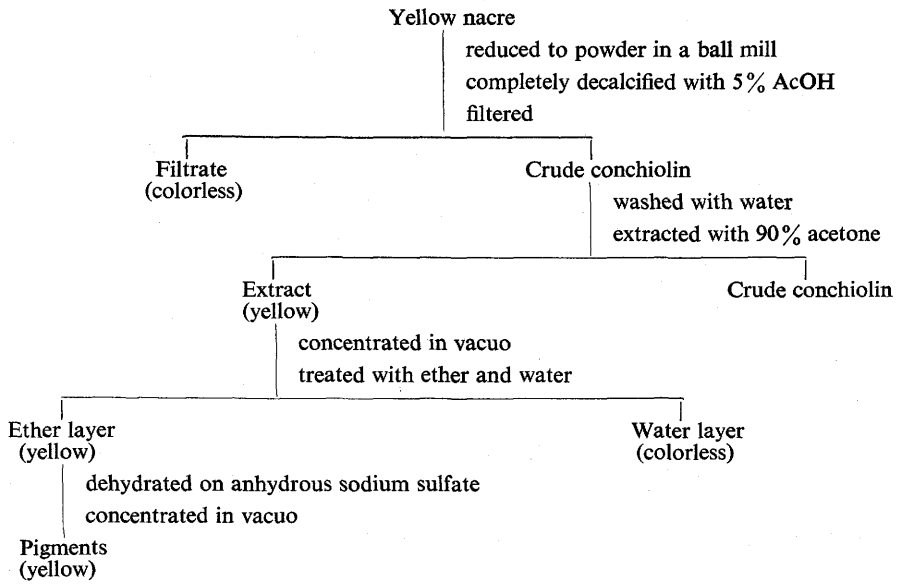

Fig. 1. Extraction of acetone soluble pigments from the nacre of yellow pearl.

tion and concentration in vacuo. A small amount of yellow oily residue was obtained.

Thin-layer chromatography. Thin-layer chromatography was carried out using silica gel G layer and benzene-n-hexane mixture (1:1) as solvent. Resulting chromatogram was at first inspected under ultraviolet light and then after application of antimony trichloride reagent[5].

Absorption spectra. Absorption spectra were recorded between 350 mμ and 650 mμ with Shimazu Multipurpose Recording Spectrophotometer MPS-model 50L.

Results and Discussion

Two visible spots were noticed on the thin-layer chromatogram. One of them was yellow (Rf 0.54) and the other orange (at origin) in color, as shown in Fig. 2. Under ultraviolet light they appeared as dark brown absorption spots. When the chromatogram was sprayed with antimony trichloride reagent and heated, the yellow and orange spots turned their color into purple and brown-purple, respectively.

Besides these two spots three weak fluorescent ones were also noticed under ultraviolet light and these spots, with antimony trichloride reagent, colored to violet or purple. By application of the antimony trichloride reagent, another purple spot was newly noticed which, without application of the reagent, had been detected neither under ultraviolet light nor under day-light.

These results suggest that the extract of yellow pearl contains carotenoids or their related compounds.

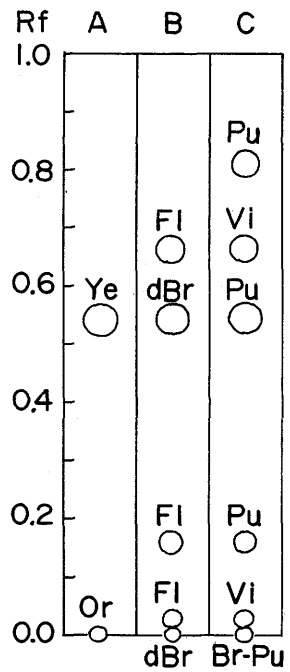

Fig. 2. Diagrammatic thin-layer chromatograms of the acetone soluble pigments. Inspected (A) under day-light, (B) under ultraviolet light, and (C) after application of antimony trichloride reagent. Ye: Yellow, Or: Orange, Fl: Fluorescent, dBr: Dark brown, Pu: Purple, Vi: Violet, Br-Pu: Brown-purple.

One of the yellow pigments was eluted from the major spot (Rf 0.54) on the thin-layer chromatogram and subjected to determination of absorption spectra in several sorts of solvents. The spectra and the absorption properties of the pigment are shown in Fig. 3 and table 1, respectively. The pigment exhibited the absorption spectra having a major band and a weak one at 460 mμ and 432 mμ in chloroform, at 446 mμ and 418 mμ

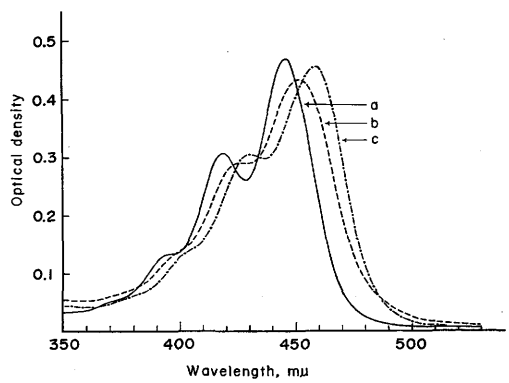

Fig. 3. Absorption spectra of an acetone soluble yellow pigment separated by thin-layer chromatography.
a: in n-hexane, b: in ethanol, c: in benzene.

Table 1. Comparison of absorption properties of an acetone soluble yellow pigment of pearl with those of flavoxanthin[6].

Solvents	Absorption maxima (mμ)			
	Yellow pigment		Flavoxanthin	
Carbon disulfide	473	455*	479	449
Chloroform	460	432	459	430
n-Hexane	446	418	—	—
Ethanol	453	426	448	421
Benzene	459	430	—	—

* Absorption shoulder

in n-hexane, at 453 mμ and 426 mμ in ethanol, and at 459 mμ and 430 mμ in benzene, respectively. In carbon disulfide the major band shifted to 473 mμ and an absorption shoulder appeared at 445 mμ. In the region between 500 and 650 mμ no absorption band was noticed.

These absorption properties of the pigment resemble those of carotenoids, especially flavoxanthin. In the table, the data for flavoxanthin are cited for comparison[6]. The pigment possesses similar absorption properties to the flavoxanthin, but is slightly different from the carotenoid in shape of the spectrum[7], i.e. the flavoxanthin gives two absorption maxima of almost equal intensity in the region between 400 and 500 mμ.

STAIN[8] reported that a few kinds of carotenoids such as violaxanthin yielded yellow flavoxanthin-like pigments when treated with acetic acid. When the yellow nacres were submitted to prolonged treatment with 10% or more concentrated acetic acid, less pigment was yielded. And the resulting pigment exhibited an absorption spectrum with two weak bands in 400–500 mμ region, of which shape resembled that of flavoxanthin. These properties almost coincide with STAIN's observations on the behavior of certain carotenoids to acetic acid.

A few kinds of minor carotenoid-like pigment seemed to be contained in the nacre, since one orange spot and three ones showing fluorescence under ultraviolet light which gave purpole or violet color with antimony trichloride reagent appeared on the thin-layer chromatogram. These components, however, remain unexamined because of their extremely small contents in the nacre.

According to the microscopic observation[9], nacres are constructed alternately piled many colorless thin laminae and yellow colored ones, and yellow pearl, especially gold one, contains thick yellow laminae or deep yellow colored laminae, compared with pink pearl. SAWADA[10] has pointed out that the reflectance spectra of pearl reveal three specific absorptions at 460 mμ, 430 mμ, and 407 mμ regardless of the color of pearl such as pink, yellow or green. The wavelength locations of these specific absorptions at 460 mμ and 430 mμ are identical with those of the absorptions of the yellow pigment of under consideration in benzene and chloroform. These findings seem to indicate that the yellow carotenoid having flavoxanthin-like spectrum distributes not only in yellow pearl but also in another color ones such as pink or green pearl.

Therefore, the carotenoid pigments may contribute some extent to the coloration of yellow pearl though, other yellow pigments seem to play an important role in the coloration of the yellow pearl.

Summary

As one of the yellow pigments of pearl, acetone soluble pigment was obtained from the decalcified nacre of yellow pearl and purified by thin-layer chromatography. The pigment revealed purple color with antimony trichloride and gave an absorption spectrum having two absorption bands in the region between 400 and 500 mμ, of which character resembled that of flavoxanthin.

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