The Constitution of the Pigments of Brevibacterium Crystalloiodinum, Sasaki, Yoshida et Sasaki

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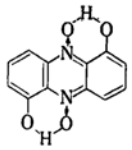
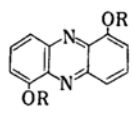
Sasaki, Yoshida and Sasaki[1] isolated a deep purple, antibiotic pigment and a yellow pigment from the culture medium of *Brevibacterium crystalloiodinum*, Sasaki, Yoshida et Sasaki*, and named them crystalloiodinine A and B, respectively. The name of crystalloiodinine was proposed on account of the purple pigment resembling to iodinine[2].

The present paper is concerned with the study on the structure of these pigments, which were sent by Professor Yuji Sasaki, to whom the authors' thanks are due. It has been established that crystalloiodinine A is identical with iodinine, *i.e.*, 1,6-dihydroxyphenazine di-*N*-oxide (I), and crystalloiodinine B is 1,6-dihydroxyphenazine (II: R=H).



(I)



(II)

The purple pigment, isolated from the culture medium, was purified by repeated recrystallizations from chloroform or dioxane; its homogeneity was indicated by the fact, that it gives a single blue band on alumina chromatogram. Crystalloiodinine A, $C_{12}H_8O_4N_2$, was thus obtained in deep purple prisms with coppery lustre, m. p. 230°C (decomp.). It dissolves in aqueous sodium hydroxide to a blue solution, and separates in purple crystals when the alkaline solution is acidified. It shows greenish coloration in ethanol with ferric chloride. The ultraviolet spectrum in chloroform shows maxima at 291 mμ ($\log \varepsilon = 5.1$) and 355 mμ ($\log \varepsilon = 3.9$) (Fig. 1); and the infrared spectrum exhibits absorptions due to a bonded hydroxyl group at 3400 cm^{-1} and that of *N*-oxide group at 1285, 1160 and 1025 cm^{-1} (Fig. 2). The infrared spectrum and certain chemical properties[1] indicated, that the pigment closely resembles iodinine[2-6]. Accordingly, crystalloiodinine A

1) Y. Sasaki, T. Yoshida and H. Sasaki, presented at the Meetings of the Society of Agricultural Chemistry of Japan, Sapporo, Nov. 21, 1957 and Tokyo, April 4, 1959.

2) G. R. Clemo and H. McIlwain, *J. Chem. Soc.*, **1938**, 479.

* This new species of *Brevibacterium* was isolated at the Faculty of Agritculture of Hokkaido University by Prof. Y. Sasaki and his collaborators[1].

3) G. R. Clemo and A. F. Daglish, *J. Chem. Soc.*, **1950**, 1481.

4) A. I. Kiprianov, S. B. Serebryanyi and V. P. Chernernetskii, *Doklady Akad. Nauk. S. S. S. R.*, **69**, 651 (1949); *Chem. Abstr.*, **46**, 4010 (1952).

5) S. B. Serebryanyi, V. P. Chernetskii and A. I. Kiprianov, ibid., **70**, 645 (1950); *Chem. Abstr.*, **45**, 4249 (1951).

6) I. Yoshioka and Y. Kidani, *J. Pharm. Soc. Japan*, **72**, 1301 (1952).

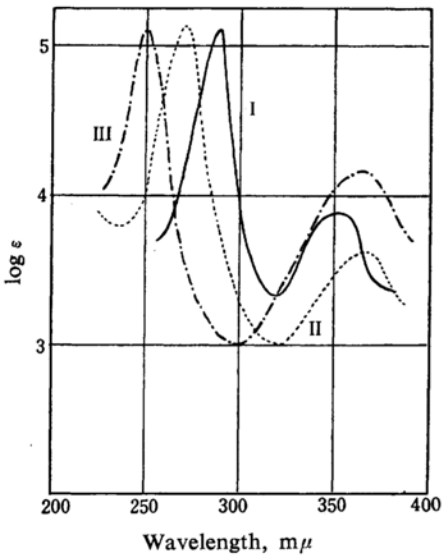


Fig. 1. Absorption spectra of:
I. crystalloiodinine A and 1, 6-dihydroxyphenazine di-*N*-oxide (in chloroform),
II. crystalloiodinine B (in ethanol),
III. acetate of crystalloiodinine B (in ethanol).

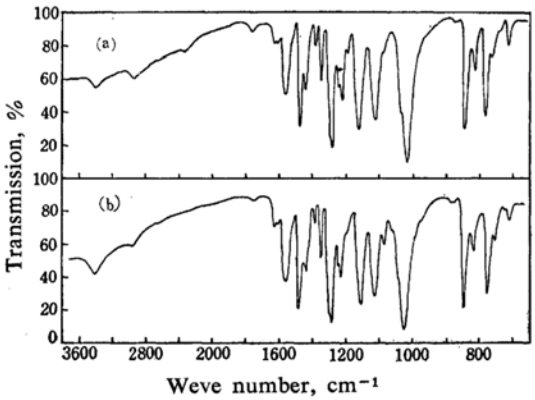


Fig. 2. IR spectra in KBr of (a) crystalloiodinine A and (b) 1, 6-dihydroxyphenazine di-*N*-oxide.

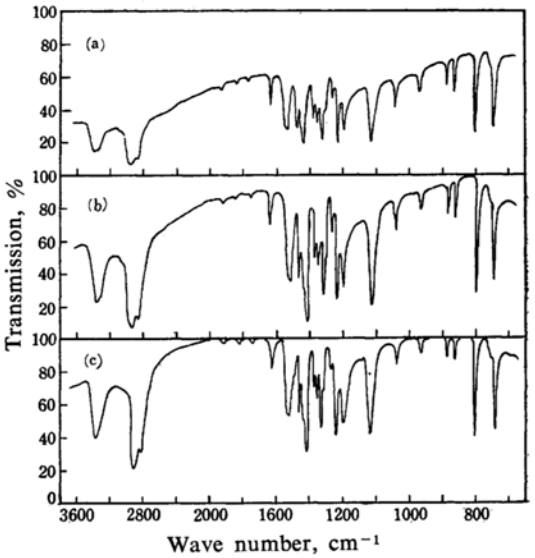


Fig. 3. IR spectra in nujol of (a) reduction product of crystalloiodinine A, (b) crystalloiodinine B, and (c) 1, 6-dihydroxyphenazine.

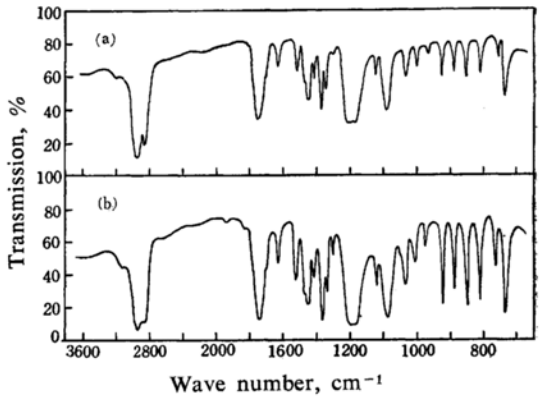


Fig. 4. IR spectra in nujol of (a) diacetate of crystalloiodinine B and (b) 1, 6-diacetoxyphenazine.

has been found to be identical with 1, 6-dihydroxyphenazine di-*N*-oxide, which was synthesized after Yoshioka and Kidani[6], by the mixed melting point method and by comparison of ultraviolet** and infrared spectra (Figs. 1 and 2).

On hydrogenating the pigment over Adams platinum catalyst, golden yellow plates, $C_{12}H_8O_2N_2$, m. p. 273~4°C (diacetate: pale yellow needles, m. p. 234°C) were yielded. This material has been identified as 1, 6-dihydroxyphenazine[7] as described below.

The yellow pigment, isolated from the culture medium, was also purified by the chromatographical method and recrystallization from benzene or sublimation under reduced pressure. Crystalloiodinine B, $C_{12}H_8O_2N_2$, was obtained in golden yellow plates, m. p. 273~4°C. It dissolves in aqueous sodium hydroxide to a purplish-red solution and separates in yellow crystals on acidification of the alkaline solution. It shows greenish coloration in ethanol with ferric chloride. The ultraviolet spectrum in ethanol shows maxima at 274 mμ ($\log \varepsilon = 5.1$) and 370 mμ ($\log \varepsilon = 3.6$) (Fig. 1); and the infrared spectrum exhibits absorption due to a

** The ultraviolet spectrum does not agree with that shown in the literature.[2]

7) I. Yoshioka and Y. Kidani, ibid., 72, 847 (1952).

hydroxyl group at 3390 cm^{-1}, but does not exhibit an *N*-oxide band (Fig. 3). Diacetate of this pigment, $C_{16}H_{12}O_4N_2$, was obtained in pale yellow needles, m. p. 234°C (the ultraviolet and infrared spectra are shown in Figs. 1 and 4, respectively). Thus, by the mixed melting point method and by comparison of the absorption spectra of the pigments and of their diacetates, it has been established that crystalloiodinine B is identical with the above reduction product of crystalloiodinine A and with 1, 6-dihydroxyphenazine.

It is possible to assume that 1, 6-dihydroxyphenazine, which has not hitherto been isolated from any natural source, plays the role of a precursor of iodinine[1].

Experimental

Isolation of the Pigments. (1) Purple Pigment, Crystalloiodinine A.—The bacteria were incubated in the broth containing 1% glycerine. The cultures were shaken at 30°C for 5 days, during which deep purple crystals developed. Water was added to the cultures and shaken, and the washings were allowed to stand. After 24 hr. the suspension of bacteria was decanted from precipitated pigment. Several treatments were sufficient to remove the pigment. The crude pigments were filtered and extracted in a Soxhlet apparatus with chloroform. On concentration, the pigment separated as purple prisms with coppery lustre; it was recrystallized from chloroform or dioxane, m. p. 230°C (decomp.). Qualitative tests showed the pigment to contain nitrogen; sulfur and metals were absent.

Found: C, 58.61; H, 3.43; N, 11.42. Calcd. for $C_{12}H_8O_4N_2$: C, 59.02; H, 3.30; N, 11.48%.

It was slightly soluble in chloroform, carbon disulfide, petroleum ether, glacial acetic acid, acetone, dioxane and benzene, but not soluble in water, ethenol, nor ether. It was soluble in concentrated sulfuric acid with blue-greenish coloration, and also in aqueous sodium hydroxide with blue coloration. It showed greenish coloration in ethanol with ferric chloride. It dissolved in glacial acetic acid to give a red solution, which on warming with the addition of 10% potassium iodide liberated iodine.

This compound was found to be identical with 1,6-dihydroxyphenazine di-*N*-oxide, which was synthesized after Yoshioka and Kidani[6], by the mixed melting point method and by comparison of their absorption spectra.

(2) Yellow Pigment, Crystalloiodinine B.—The water layer obtained by decantation to remove crystalloiodinine A, was acidified by hydrochloric acid and extracted with ether. After evaporation of the ether, black gruel was obtained. This was treated with a small amount of chloroform and filtered. Dark brown crystals were boiled with benzene and filtered. Evaporation of the solvent afforded yellow crystals, which were recrystallized from benzene or sublimated under reduced pressure. Golden yellow plates were obtained, m. p. 273~4°C.

Found: C, 67.98; H, 3.53; N, 13.12. Calcd. for $C_{12}H_8O_2N_2$: C, 67.97; H, 3.80; N, 13.28%.

This material was soluble in benzene, alcohol, and ether. It was soluble in concentrated sulfuric acid with greenish coloration and also in aqueous sodium hydroxide with purplish-red coloration. It showed greenish coloration in ethanol with ferric chloride.

This compound was identified as 1,6-dihydroxyphenazine[7] by the mixed melting point method and by comparison of absorption spectra with an authentic specimen.

Reduction of Crystalloiodinine A.—The pigment (0.6 g.), platinum oxide (0.6 g.) and absolute ethanol (50 cc.) were shaken in hydrogen (1 atm.) for 24 hr., during which the pigment completely dissolved. Catalysts were then filtered off. On being exposed to air, the color of the filtrate changed soon from pale orange to yellow. This meant, that the hydrogenation yielded the dihydrophenazine compound and it was oxidized by air to the corresponding phenazine derivative. After evaporation of the solvent under reduced pressure, dark brown crystals were obtained. On purification by recrystallization from benzene or sublimation under reduced pressure, golden yellow plates were yielded, m. p. 273~4°C. This material was found to be identical with 1,6-dihydroxyphenazine and crystalloiodinine B, by the mixed melting point method and by comparison of the absorption spectra.

Found: C, 67.48; H, 3.45; N, 13.08. Calcd. for $C_{12}H_8O_2N_2$: C, 67.97; H, 3.80; N, 13.28%.

Acetylations of the Reduction Product and Crystalloiodinine B.—The compound (50 mg.), pyridine (5 cc.), and acetic anhydride (1 cc.) were refluxed for 2 hr. The solvent was removed under reduced pressure and the residue was recrystallized from ethanol. Pale yellow needles were obtained, m. p. 234°C. This compound was found to be identical with an authentic specimen of 1,6-diacetoxyphenazine (II: $R=COCH_3$) by the mixed melting point method and by comparison of the absorption spectra.

Found: C, 64.58; H, 3.86; N, 9.50. Calcd. for $C_{16}H_{12}O_4N_2$: C, 64.86; H, 4.06; N, 9.46%.

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