Notes

Shoji Shibata and Michio Takido: Metabolic Products of Fungi. III.[1] The Coloring Matters of *Pachybasium candidum* Saccardo.

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Pachybasium candidum Saccardo is a species included in Moniliaceae. It was first described in 1885[2,3] but isolated for the first time in Japan by Kobayashi and Tsubaki.[4] The strain, on which chemical studies are being made, grows on malt-agar in light yellow or greenish yellow colonies with brownish yellow reverse, occasionally producing yellow crystals in agar or within the submerged hyphae.

A mixture of coloring matters produced by the strain of *Pachybasium candidum* grown on malt-peptone-glucose agar was obtained by extraction of the dried mycelia with ether. The separation of the pigments was carried out by chromatography as described in the experimental part of this paper.

The fractionation gave the following results:

i) From the aqueous bicarbonate-soluble portion, an anthraquinone was proved on the paper chromatogram; the Rf value did not correspond to that of any other known fungal anthraquinone-carboxylic acids.

ii) From the aqueous carbonate-soluble portion, four pigments were separated. Although none of them could be subjected to further study by their insufficient yields, two of these pigments strongly suggested alizarin derivatives according to their characteristic color reactions.[5]

iii) The coloring matter which is insoluble in aqueous carbonate but soluble in aqueous caustic alkali to form a red solution was fractionated into two portions by chromatography. One of them was identified as chrysophanol (4,5-dihydroxy-2-methylanthraquinone), m.p. 192~193°, and from the lower band a yellow crystalline pigment of m.p. 174.5~175° was isolated, with which the present paper chiefly deals.

The yellow pigment forms needles whose analytical figures correspond well to $C_{15}H_{10}O_3$. The color reactions, the ultraviolet (Fig. 1) and the infrared spectra (Fig. 2), as well as the formation of monoacetate, m.p. 153°, and a monomethyl ether, m.p. 187~188°, suggested that this pigment is an α-monohydroxy-methylanthraquinone.

A carboxylic acid, m.p. 276°, with a molecular formula, $C_{14}H_6O_2(OH)COOH$, was derived by the oxidation of the acetate of the pigment with chromic acid. On decarboxylation of the acid 1-hydroxyanthraquinone[6] (I) was obtained and was synthetically confirmed.

On the basis of the above result, we reached a conclusion that the yellow pigment should be 4-hydroxy-2-methylanthraquinone (II) which was already known in literature,[7~11]

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1) Part II: S. Shibata, S. Natori: This Bulletin, **1**, 160(1953).
2) P. A. Saccardo: Syll. Fung., 4, 150(1886); Rev. Mycol., **7**, 161(1885).
3) G. Lindau: Rabenhorst, Krypt. Fl. Pilze, 8, 310(1907).
4) Y. Kobayashi, K. Tsubaki: "Sci. Res. of the Ozegahara Moor" (Tokyo) 576(1954).
5) cf. S. Shibata, M. Takido, O. Tanaka: J. Am. Chem. Soc., **72**, 2789(1950).
6) A. Baeyer, H. Caro: Ber., **7**, 969(1874).
7) W. Bentley, H. Gardner, C. Weizmann: J. Chem. Soc., **91**, 1638(1907).
8) F. Ullmann, W. Schmidt: Ber., **52**, 2113(1919).
9) S. Keimatsu, I. Hirano: J. Pharm. Soc. Japan, **49**, 85(1929).
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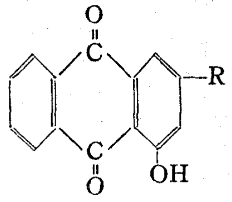
but has never been found in natural products.



(I) R : H, (II) R : CH_3, (III) R : COOH.

The confirmation was made by mixed fusion of the acetates of the fungal pigment and the synthetic 4-hydroxy-2-methylanthraquinone (II) prepared by Waldmann's method.[10] The melting point of the acetate of 4-hydroxyanthraquinone-2-carboxylic acid recorded by Mitter[12] is about 60° higher than that of the corresponding compound derived both from the fungal pigment and synthetically prepared.

A number of hydroxyanthraquinones ever found in the metabolic products of fungi possess, with an exception of boletol,[13] at least two of their hydroxyls in 4- and 5-positions of the anthraquinone nucleus.

4-Hydroxy-2-methylanthraquinone produced by *Pachybasium candidum* should be noted as the simplest fungal anthraquinone pigment having only one hydroxyl in the α-position.

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Experimental

Culture—The strain of *Pachybasium candidum* Saccardo used in this work was supplied by Dr. Y. Kobayashi and Mr. K. Tsubaki who originally isolated it from snow in Oze region, in May, 1952.

Cultural Condition—The constitutents of the medium are 5% malt extract, 4% D-glucose, 0.2% peptone, and 2% agar. The sterilized medium was poured into Petri dishes of 10-cm. diameter and inoculated with *Pachybasium candidum*. After 1~2 weeks' incubation at 25° the mycelia were harvested, when the surface of the mycelia gave reddish brown color becoming reddish violet at the end stage of growth.

Isolation of the Coloring Matters—The dried mycelia were extracted with ether in a Soxhlet apparatus. Some crystalline substance separated out during the extraction. The ethereal solution was successively shaken with 5% aq. $NaHCO_3$ and 5% aq. Na_2CO_3. The residual ethereal solution was evaporated. The yield of the ethereal extract was 400~500 mg. per 100 plates.

On acidification of the bicarbonate-soluble portion, a minute amount of anthraquinone pigment was obtained, Rf value of which did not correspond to any of the known fungal anthraquinone-carboxylic acids.

The coloring matter obtained from the carbonate-soluble portion was dissolved in acetone and fractionated by chromatography on MgO. Three well-distinguished bands, from the top to bottom, violet, red, and orange, appeared on the chromatogram.

From the lowest orange band, yellowish orange powder was obtained which gave an orange coloration with $(AcO)_2Mg$ in EtOH. From the red band, brown powder was isolated, which gave color reaction and Rf value similar to those of emodin.

The pigments separated from the violet band was subjected to the cellulose-powder partition chromatography using MeOH-saturated benzine as a developing solvent. A pigment which appeared in the first eluate formed red needles by sublimation. It gave a violet blue coloration in aq. Na_2CO_3 and ethanolic $(AcO)_2Mg$ solution, and showed an Rf value 0.5* on the paper chromatogram developed by MeOH-saturated benzine. From the second eluate, red needles, micro-m.p. 257~259°, were isolated by sublimation, which gave a violet coloration with aq. Na_2CO_3 solution and $(AcO)_2Mg$ reagent, and Rf value 0.2* on the paper chromatogram.

The residual ethereal extract free from the bicarbonate and carbonate-soluble pigments was fractionated by chromatographing a $CHCl_3$ solution on MgO.

From the first orange-colored eluate, yellow needles, m.p. 174.5~175°, were obtained by recrystallization from MeOH or glacial AcOH. Yield : 40~50 mg. from 100 mg. of the ethereal extract. From a red color layer, golden yellow plates, m.p. 192~193°, were separated, which was identified as chrysophanol by a mixed fusion with the authentic specimen. Yield : 10~20 mg. from 100 mg. of the ethereal

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12) P. C. Mitter : J. Indian Chem. Soc., **11**, 873(1934).

13) F. Kögl, W. B. Deijs : Ann., **515**, 10(1934).

* 3-Methylalizarin, Rf 0.5; Alizarin, Rf 0.2 (developed by MeOH-saturated benzine).

extract.

Properties of the Yellow Pigment, m.p. 175°—It is insoluble in 5% aq. Na_2CO_3 but soluble in 5% caustic alkali to give orange red solution. It exhibits orange red coloration in ethanolic $(AcO)_2Mg$ solution. It showed no melting point depression by a mixed fusion with synthetic 4-hydroxy-2-methylanthraquinone. *Anal.* Calcd. for $C_{15}H_{10}O_3$: C, 75.62; H 4.23, Fuond: C, 75.33; H, 4.03.

Acetate (4-Acetoxy-2-methylanthraquinone): Light yellow needles, m.p. 153~154°, from MeOH or glacial AcOH. It showed no melting point depression by a mixed fusion with synthetic acetate of 4-hydroxy-2-methylanthraquinone. *Anal.* Calcd. for $C_{17}H_{12}O_4$: C, 72.85; H, 4.32. Found: C, 72.71; H, 4.32.

Methyl Ether (4-Methoxy-2-methylanthraquinone): Prepared by boiling with Me_2SO_4 and anhyd. K_2CO_3 in acetone. Yellow needles, m.p. 187~188°, from glacial AcOH. *Anal.* Calcd. for $C_{16}H_{12}O_3$: C, 76.18; H, 4.80. Found: C, 76.21; H, 4.74.

Oxidation of the Acetate of the Yellow Pigment, m.p. 175° (Formation of 4-hydroxyanthraquinone-2-carboxylic acid)—The acetate of the pigment (300 mg.) was oxidized with CrO_3 by the usual method and the product was hydrolyzed to an anthraquinone-carboxylic acid as yellow feathery crystals, m.p. 276°. Yield: 200 mg. It was identified as 4-hydroxyanthraquinone-2-carboxylic acid by a mixed fusion with the synthetic specimen. *Anal.* calcd. for $C_{15}H_8O_5$: C, 67.17; H, 3.01. Found: C, 67.11; H, 3.37.

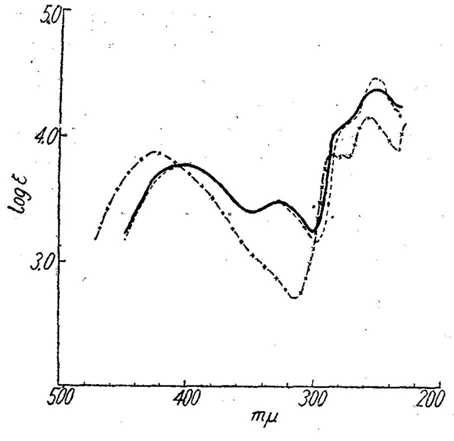
Acetate: Light yellow needles, m.p. 213~214°, alone or mixed with synthetic 4-acetoxyanthraquinone-2-carboxylic acid of the same melting point (Mitter gave m.p. 276° for this compound[12]).

Decarboxylation of the Acid, $C_{15}H_8O_5$—The acid (50 mg.), $C_{15}H_8O_5$, was decarboxylated by the action of Cu chromite in quinoline. Yield: 7 mg. Yellow needles, m.p. 189~190°, alone or mixed with the synthetic 1-hydroxyanthraquinone of the same melting point, which was prepared by the condensation of phthalic anhydride and phenol with conc. H_2SO_4[10].

Ultraviolet Absorption Spectrum (Fig. 1)—The pigment of *Pachybasium candidum*, 4-hydroxy-2-methylanthraquinone, and 1-hydroxyanthraquinone which have one free hydroxyl group in α-position of the anthraquinone nucleus show absorption maxima at 405 mμ ($\log \varepsilon = 3.75$) and 327 mμ ($\log \varepsilon = 3.48$), whereas chrysophanol (4,5-dihydroxy-2-methylanthraquinone) has a maximum at 425 mμ ($\log \varepsilon = 3.88$).

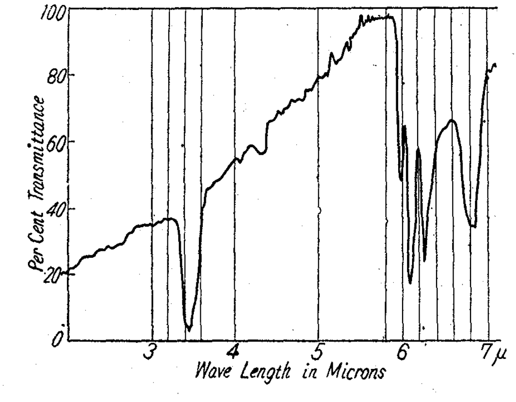
Infrared Absorption Spectrum (Fig. 2)—The pachybasium pigment gives a non-chelated carbonyl band at 6.00 μ and a chelated carbonyl band at 6.16 μ; band for phenolic hydroxyl in α-position disappears by the effect of chelation.[14]

Fig. 1.



——— Pigment of *Pachybasium candidum* (4-Hydroxy-2-methylanthraquinone)
·········· 1-Hydroxyanthraquinone
—×—×— Chrysophanol

Fig. 2.



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