Studies on the Metabolic Products of *Macrosporium porri* Elliott

Part I. Isolation and General Properties of Macrosporin, A New Pigment

By Rikisaku SUEMITSU, Yukio MATSUI, and Makoto HIURA

Institute of Dairy Chemistry, Rakuno College

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A strain of *Macrosporium porri* Elliott, when grown at 25° on 2% glucose solution of stone-leek decoction, gives rise to a hitherto undescribed pigment to which the name "macrosporin" is proposed. Optimum cultural condition for the production of the pigment and the method of its isolation are described here. Macrosporin, $C_{16}H_{12}O_5$, crystallizes as orange-yellow rhombic and melts at 300–302° with decomposition. General properties of macrosporin, its diacetate, m.p. 209–210°, and trimethylether, m.p. 260–261°, are described. Macrosporin is recognized to be a hydroxyanthraquinone derivative from the results of elementary analysis, molecular weight determination, and ultra-violet and infra-red spectra.

In the previous literature, there are many reports concerning the pigments produced by species of *Penicillium*, *Helminthosporium*, *Aspergillus*, and *Fusarium*. However, nothing has been reported, either on the pigments, or on the metabolic products of *Macrosporium*. Two crystalline pigments were isolated from the mycelia of *Macrosporium porri* Elliott, which is known as a pathogenic organism causing the black spot disease of stone-leek, cultured in liquid media for 25 days. For one of them, hitherto undescribed, the name "macrosporin" is proposed in connection of the species name, while the other pigment will be described later. Macrosporin, $C_{16}H_{12}O_5$, m.p. 300–302° (decom.) is an orange-yellow pigment having anthraquinone-like properties. It is easily soluble in $N-NaOH$ and $N-Na_2CO_3$ to give purple-red solution and, on acidifying with HCl the pigment is regenerated.

It dissolves in conc. H_2SO_4 to give an orange-red solution. It dissolves very sparingly in almost all of the organic solvents. Its glacial acetic acid solution is yellow in color, and does not show fluorescence in daylight. The color disappears on reducing

with zinc dust and the color is restored by standing for a while. This color-reaction is characteristic of the *p*-quinone type compounds. Its alcoholic solution gives dark-brown color with ethanolic $FeCl_3$ and orange-yellow color with ethanolic magnesium acetate¹⁾.

The ultra-violet absorption spectrum shows absorption bands, λ_{max} . 225 $m\mu$ (log. 4.32) indicating dihydroxyanthraquinone²⁾ structure, and λ_{max} . 285 $m\mu$ (log. 4.30)³⁾ also indicating the characteristic of quinoid nucleus of anthraquinone besides a broad band λ_{max} . 381 $m\mu$ (log. 3.90).

The infra-red spectrum of macrosporin gives a non-chelated carbonyl band at 6.02 μ , chelated carbonyl band at 6.11 μ , and a band by the hydroxyl group located the β -position to carbonyl group at 3.04 μ ⁴⁾. As it is apparent that in every case, when a hydroxyl group not adjacent to a carbonyl group is present, a band is to be observed at about 2.99 μ , and macrosporin is believed to contain a hydroxyl

1) S. Shibata, et al., *J. Am. Chem. Soc.*, **72**, 2789 (1950).

2) T. Ikeda, et al., *J. Pharm. Soc. Japan*, **76**, 217 (1956).

3) L.H. Briggs, et al., *J. Chem. Soc.*, **1952**, 1718.

4) M. St. C. Flett *J. Chem. Soc.*, **1948**, 1441.

FIG. 1. UV-absorption Spectrum of Macrosporin.
(5×10^{-5} mol ethanol solution)

FIG. 2. Infra-red Absorption Spectrum of Macrosporin.

group β -positioned to carbonyl group in it.

On treatment with acetic anhydride in the presence of a trace amount of conc. H_2SO_4 , macrosporin gives diacetate, $\text{C}_{16}\text{H}_{10}\text{O}_5(\text{COCH}_3)_2$, light yellow needles, m.p. $209-210^\circ$ and on boiling with dimethyl sulfate and K_2CO_3 in acetone, it gives a trimethylether $\text{C}_{16}\text{H}_7\text{O}_2(\text{OCH}_3)_3$, yellow needles, m.p. $260-261^\circ$. On determination of the methoxy group by Zeisel's method both macrosporin and diacetate give 1 methoxy group respectively, while trimethylether gives 3 methoxy groups.

Some reports concerning the paper chromatography of hydroxyanthraquinone^{1,5)} have been given by Shibata et al. By using Shibata's solvent system it was found that an orange spot of R_F 0.37 is given only with butanol saturated with 28% NH_4OH .

Judging from its general properties and both ultra-violet and infra-red absorption data of macrosporin, it is very probable that macrosporin may be a hydroxyanthraquinone derivative represented by formula, $\text{C}_{16}\text{H}_{12}\text{O}_5$, involving 1 methoxy group and 2 hydroxyl groups, one of which may be located at least in the β -position to the carbonyl group.

Six of the pigments produced by microorganisms have been previously reported to involve the methoxy group. They are: roseopurpurin (4-methoxy-emodin), phycion (7-methoxy-emodin), erythroglaucon (7-methoxy-catenarin), nalgiovinsin (4,5-dihydroxy-7-methoxy-2-hydroxypropylantraqui-

none), nalgliolaxin (1 or 8-chloronalgiovinsin), and carviolin (4-methoxy- ω -hydroxyemodin). Though a few hydroxyanthraquinone^{6,7,8)} derivatives having the methoxy group have been obtained from other sources they are not identical with macrosporin.

EXPERIMENTAL

Culture. The strain of *Macrosporium porri* Elliott used in this work, was obtained from the experimental field attached to our college.

Cultural Condition. Two % glucose solution of stone-leek decoction was used as the cultural medium. A number of 500-ml Erlenmeyer flasks, containing 150 ml of the solution, pH 6.5-7.0, were sterilized and inoculated with the strain which had been freshly cultured. The inoculated flasks were incubated at 25° in the dark. On the 5th day the mycelia were light-cream in color and covered the surface of the

6) R. Hill, *Nature*, **134**, 628 (1934).

7) A.G. Perkin, *J. Chem. Soc.*, **91**, 2066 (1907).

8) S. Nonomura, *J. Pharm. Soc. Japan*, **75**, 219 (1955).

5) M. Takido, *Pharm. Bull.*, **4**, 45 (1956).

FIG. 3. Isolation and Purification of the Pigment.

medium, and became yellow-orange on the 10th day. When harvested, at the end of 25 days, the mycelia became dark-red in color and the solution also turned dark-red.

Isolation and Purification of the Pigment. The mycelia were filtered by cloth, dried at 45° and powdered. The method of purification of the pigment is given in Fig. 3.

As it was doubtful that the pigment obtained by the method described in Fig. 3 might undergo change by NaOH treatment, the dried mycelia were directly extracted with ether and in this way the yellow-orange pigment was obtained. The pigment was identical with the one which was mentioned before. About 0.6 g of crystalline pigment was obtained from 10.4 l of substrate and its weight was about 2.5% of the dried mycelium.

General Properties of Macrosporin. Macrosporin crystallizes from glacial acetic acid as beautiful orange-yellow rhombic, m.p. 300–302° (decomp.) *Anal.* Found: C, 67.40; H, 4.39; OCH₃, 10.17; *Calcd.* for C₁₅H₉O₄-OCH₃: C, 67.60; H, 4.26; OCH₃, 10.91.

It readily sublimates at about 280°, does not dissolve

FIG. 4. Crystals of Macrosporin. ($\times 640$)

in sat. NaHCO_3 , but dissolves in $\text{N-Na}_2\text{CO}_3$ to a purple-red solution and readily dissolves in N-NaOH to a deep purple-red solution. Its ethanolic solution gives a darkbrown color with ethanolic FeCl_3 and orange-yellow color with ethanolic magnesium acetate. It dissolves in conc. H_2SO_4 to an orange-red solution. Its glacial acetic acid solution is yellow in color, and does not give rise to fluorescence in daylight. Macrosporin hardly dissolves in ether, benzene, chloroform and toluene, but dissolves in hot acetone, hot ethanol and hot glacial acetic acid.

Macrosporin Diacetate. Macrosporin (0.1 g) was dissolved in acetic anhydride (10 ml) and a drop of conc. H_2SO_4 was added. It was then cooled and poured into water. The dark-yellow solid separated was filtered, dried and crystallized from methanol to give fine light needles (0.07 g), m.p. 209–210°. *Anal.* Found: C, 65.26; H, 4.52; OCH_3 , 8.23; M.W. (Rast) 371; Cald. for $\text{C}_{15}\text{H}_7\text{O}_5(\text{COCH}_3)_2\text{OCH}_3$: C, 65.21; H, 4.38; OCH_3 8.42; M.W. 368.

Macrosporin Trimethylether. A mixture of macrosporin (0.55 g), anhydrous K_2CO_3 (30 g), redis-

tilled dimethyl sulfate (4 ml) and dry acetone (200 ml) was boiled under reflux. After boiling for 3 hrs., anhydrous K_2CO_3 (15 g) and dimethyl sulfate (4 ml) were added and the reaction was continued further for a period of 7 hours. The hot solution was filtered and evaporated to a yellow solid, which was recrystallized from acetone and finally from methanol to give yellow needles (0.4 g), m.p. 260–261°. *Anal.* Found: C, 69.09; H, 5.17; OCH_3 , 28.30; Cald. for $\text{C}_{15}\text{H}_7\text{O}_2(\text{OCH}_3)_3$: C, 69.22; H, 5.16; OCH_3 , 29.81.

Paper Chromatography of Macrosporin. Ethanol solution of the pigment was placed on a filter paper (Toyo Roshi No. 3, 1.5×25) and developed at 18° by one-dimensional descending method using butanol saturated with 28% NH_4OH as the developing solvent. The developed paper was dried and the spot was examined. color; orange, R_F ; 0.37.

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