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**Studies on the Metabolites of Phytotoxic Fungi. I. Isolation of
Macrosporin and 6-Methylxanthopurpurin 3-Methyl Ether
from *Alternaria bataticola* IKATA ex YAMAMOTO**

SHOICHI NAKAJIMA

Hoshi College of Pharmacy¹⁾

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In the course of the investigation on various phytotoxic fungi, the author has come across the isolation from *Alternaria bataticola* IKATA ex YAMAMOTO of two anthraquinone pigments, macrosporin (I) and 6-methylxanthopurpurin 3-methyl ether (II), along with mannitol. The mold is known as a causal fungus to black spot of sweet potato, but has not been examined chemically.

During cultivation of the mold on Czapek-Dox nutrients, the culture liquid turned dark reddish brown. When an aliquot of the liquid was made alkaline with sodium hydroxide, it showed red coloration, and when added with ferric chloride, it gave purple color. In order to obtain the substance which is responsible for these color reactions, the culture filtrate was mixed with carbon, and the carbon was extracted with methanol. The methanol extract gave a mixture of two yellow pigments as revealed by thin-layer chromatography (TLC). These two pigments, both obtained in small quantity, were macrosporin (I) and 6-methylxanthopurpurin 3-methyl ether (II), as mentioned later.

The mycelium was first defatted by extraction with petroleum ether, and then extracted successively with ether and acetone. The purification of the ethereal extract gave a yellow pigment which melted at 306° with decomposition. This pigment presented orange-brown color reaction by adding Shibata's magnesium acetate reagent²⁾ in methanol, indicative of being an anthraquinone having the hydroxyl at the position chelating to the carbonyl. The molecular formula was determined as C₁₆H₁₂O₅ from the results of the elementary analysis and the mass spectrum in which the molecular ion peak centered at *m/e* 284. The infrared (IR) spectrum was also suggestive of the anthraquinone structure, showing the absorption bands at 1665 cm⁻¹ of free carbonyl, at 1635 cm⁻¹ of chelated carbonyl, at 1504 cm⁻¹ and 1583 cm⁻¹ of aromatic moiety, at 3280 cm⁻¹ of associated phenolic hydroxyl, and in the wide range from 2400 cm⁻¹ to 3080 cm⁻¹ of chelated phenolic hydroxyl. On account of its less solubility in the ordinary organic solvents at room temperature, the nuclear magnetic resonance (NMR) spectrum was taken at 100° in deuterated dimethylsulfoxide, and the most significant signals could be detected: a singlet at 7.7 τ assigned to an aryl methyl, a singlet at 6.1 τ assigned to a methoxyl, and four benzenoid protons signals in the lower field. All these experimental data were indicating that the compound was possible to be 1,7-dihydroxy-3-methoxy-6-methylanthraquinone (macrosporin) (I), which had previously been isolated³⁾ from *Alternaria porri* ELLIOT and structurally determined⁴⁾ by Suemitsu, *et al.*, and later isolated also by Stoessl from *Alternaria solani* (ELLIS *et al.*) JOHNES *et al.*⁵⁾ Therefore, the compound was acetylated to a diacetate, which had sharp melting point at 213° and was compared with macrosporin diacetate, prepared similarly from the authentic sample of macrosporin offered by

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2) S. Shibata, *J. Pharm. Soc. Japan*, **61**, 103 (1941).

3) R. Suemitsu, Y. Matsui and M. Hiura, *Bull. Agr. Chem. Soc. Japan*, **21**, 1 (1957).

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5) A. Stoessl, *Can. J. Chem.*, **47**, 767 (1969).

I : R=OH

II : R=H

Chart 1

Dr. Suemitsu. The identity was established by mixed melting point measurement and comparison of their IR spectra.

The acetone extract gave a substance which melted at about 162° and showed a similar Molish color reaction as mannitol. The substance was therefore acetylated to a hexaacetate and identified with authentic mannitol hexaacetate.

The culture broth afforded two yellow pigments which were separated by TLC. One of them was identified with macrosporin by comparison of their *R_f*s and also by mixed fusion.

Another pigment melting at 182° gave a positive color reaction of α -hydroxyanthraquinone derivative with magnesium acetate reagent.²⁾ The IR spectrum showed the absorption bands at 1629 cm⁻¹ of chelated and at 1672 cm⁻¹ of non-chelated carbonyls of an anthraquinone. The mass spectrum of the compound showed the molecular ion peak at *m/e* 268, which was sixteen mass unit less than that of macrosporin. Consequently, the compound, although the amount was not enough for the elementary analysis, was presumed to be 6-methylxanthopurpurin 3-methyl ether (II), and the presumption was proved correct by the mixed fusion and comparison of IR spectra with the authentic sample sent by Dr. A. Stoessl who isolated the substance from *Alternaria solani* (ELLIS *et* MARTIN) JOHNES *et* GROUT.⁵⁾

Macrosporin (I) and 6-methylxanthopurpurin 3-methyl ether (II) are the anthraquinone derivatives that have been isolated merely from the molds of *Alternaria* genus in nature, that is, *A. porri*, *A. solani*, *A. cucumerina*⁶⁾ and now *A. bataticola* with respect to the former, and *A. solani* and now *A. bataticola* with respect to the latter compound. It is for this reason that Stoessl stated that these anthraquinones might have a chemotaxonomical significance. It is assumed that macrosporin (I) is biogenetically a hydroxylation product of 6-methylxanthopurpurin 3-methyl ether (II).

Experimental⁷⁾

Cultivation of *Alternaria bataticola*—*Alternaria bataticola* IKATA *ex* YAMAMOTO (strain, IFO No. 6187) obtained from Institute for Fermentation, Osaka, was grown in 170 Roux bottles each containing 200 ml of Czapek-Dox medium at 20° for 15 days. The mycelial mats were washed with water, dried and ground in mortar to give 190 g of the mycelial powder. The culture filtrate was kept frozen until examined.

Isolation of Macrosporin (I) from Mycelium—The pulverized mycelium was extracted with petroleum ether for a period of 2 days in an Asahina-type extractor. Evaporation of the petroleum ether gave a red-colored oil (12 g), which was not examined further. The defatted mycelium was then extracted with ether for succeeding 5 days until the extracting ether became colorless. Yellow crystal mass (1.3 g) was obtained after removal of the solvent. It was recrystallized from acetone to give yellow needles of macrosporin (I) of mp 306° (decomp.). *Anal.* Calcd. for C₁₆H₁₂O₅: C, 67.60; H, 4.26. Found: C, 66.95; H, 4.10. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1504, 1583 (aromatic ring), 1665 (non-chelated carbonyl), 1635 (chelated carbonyl), 2400—3080 (chelated phenol), 3280 (non-chelated phenol). NMR (60 Mc, in (CD₃)₂SO) τ : 7.7 (singlet, Ar-CH₃), 6.1 (singlet, OCH₃). Mass Spectrum *m/e*: 284 (M⁺). The diacetate was prepared by acetylation with acetic anhydride in presence of a drop of H₂SO₄, and crystallized from ethanol to give yellow needles, mp 213°. Authentic diacetate was prepared from macrosporin by the same procedure.

Isolation of Mannitol from Mycelium—The mycelium after removal of macrosporin as above was further extracted with acetone. After 5 days, the extracting solvent became colorless. The dark residue left after evaporation of the solvent was washed with *n*-hexane, small amount of acetone, and crystallized from methanol. The crude material (280 mg) thus obtained melting at about 162° was treated with acetic anhydride in presence of a drop of H₂SO₄ by heating for 40 min in a boiling water bath, and the acetylated product was recrystallized from ethanol to give cubic crystals of melting at 123°. The identity of the compound with the authentic hexaacetate was established by mixed fusion and comparison of the infrared spectra.

Isolation of 6-Methylxanthopurpurin 3-Methyl Ether (II) and Macrosporin (I) from Culture Liquid—The culture filtrate was treated with carbon (added 10 g per liter) to decolorize the reddish brown color. After

6) A.N. Starrat: Private communication to Dr. A. Stoessl (refer to 5).

7) Hitachi-Perkin-Elmer R-20, Hitachi model 215, and Hitachi RMS-4 apparatus were used for measurement of NMR, IR and mass spectra, respectively.

the treatment with carbon, the color reaction of the culture filtrate with NaOH and FeCl₃ became negative. The carbon which adsorbed the pigment was then extracted with MeOH, and MeOH extract was evaporated *in vacuo*. The residue thus obtained was suspended in water to remove soluble carbohydrate portion, and the water-insoluble fraction was collected by centrifugation as a red powder (180 mg). The powder was then treated with acetone. The acetone-insoluble orange powder obtained in a very small amount did not melt below 330°, and was not further examined. The acetone-soluble fraction was examined by TLC using silica gel as the adsorbent and chloroform as the solvent. Two yellow spots were detected on the plate. One spot, whose *R_f* was the same as that of macrosporin, was extracted with acetone and identified with macrosporin by mixed fusion. Another yellow spot was also extracted with acetone and recrystallized from EtOH to give yellow needles of mp 182°. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1588, 1601 (aromatic ring), 1629 (chelated carbonyl), 1672 (non-chelated carbonyl). Mass Spectrum *m/e*: 268 (M⁺). This compound was identified with the authentic sample of 6-methylxanthopurpurin 3-methyl ether (II) by mixed fusion and comparison of infrared spectra.

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Comparative Studies on Angiotensins. III.¹⁾ Structure of Fowl Angiotensin and Its Identification by DNS-Method²⁾

TAEKO NAKAYAMA, TERUMI NAKAJIMA,^{3a)} and HIROFUMI SOKABE^{3b)}

*Institutes of Pharmaceutical Sciences, Hiroshima University School of Medicine^{3a)} and
Department of Pharmacology, Toho University School of Medicine^{3b)}*

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The structure of native angiotensin I, produced by incubating homologous renin with homologous substrate, is Asp¹-Arg²-Val³-Tyr⁴-Ile⁵-His⁶-Pro⁷-Phe⁸-His⁹-Leu¹⁰ in the human⁴⁾ and rat.^{1b)} Val⁵-angiotensin I was obtained when ox plasma was incubated with rabbit renin.⁵⁾

Fowl angiotensin was different from Asp¹-Ile⁵-angiotensin I, II, or Asn¹-Val⁵-angiotensin II by its SE-Sephadex chromatographic behavior, ratio of oxytocic to pressor activity (O:P ratio), and susceptibility to carboxypeptidase (CPase) A.^{1a)} We have identified fowl angiotensin as Asp¹-Arg²-Val³-Tyr⁴-Val⁵-His⁶-Pro⁷-Phe⁸-Ser⁹-Leu¹⁰ by its amino acid composition and characteristics of its DNS- (1-dimethyl amino naphthalene 5-sulphonyl-) derivatives.

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- 5) L.T. Skeggs, Jr., K.E. Lentz, J.R. Kahn, N.P. Shumway, and K.R. Woods, *J. Exptl. Med.*, **104**, 193 (1956).