[Chem. Pharm. Bull. 13(3) 385~386 (1965)]

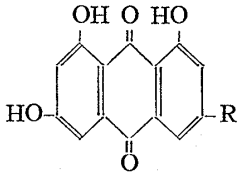
UDC 581.19[547.672.5] : 582.282

Shinsaku Natori, Fusako Sato, and Shun-ichi Udagawa : Anthraquinone Metabolites of *Talaromyces avellaneus* (Thom et Turreson) C. R. Benjamin and *Preussia multispora* (Saito et Minoura) Cain.

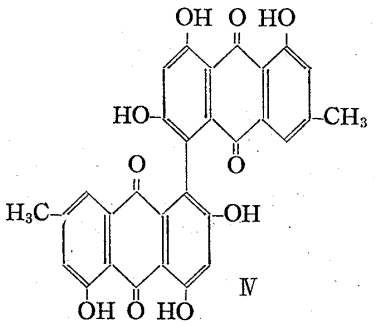
(*National Institute of Hygienic Sciences*[*1])

In the course of studies on mold metabolites in our laboratory, abundant production of red pigments by the two molds, *Talaromyces avellaneus* (Thom et Turreson) C. R. Benjamin (conidial stage : *Penicillium avellaneum* Thom et Turreson) (Ascomycetes, Eurotiaceae) and *Preussia multispora* (Saito et Minoura) Cain (Ascomycetes, Sporormiaceae), attracted our attention.

Now the pigments produced by *T. avellaneus* have been proved to be emodin (I), ω-hydroxyemodin (citréo-roseine) (II), and emodic acid (III). Although emodin has been isolated from many molds,[1] the other two anthraquinones have been reported only in



I : R=CH_3
II : R=CH_2OH
III : R=COOH



IV

a few cases as mold metabolites; namely ω-hydroxyemodin and emodic acid from *Penicillium cyclopium* Westl.[2] and the former from *P. cyaneo-fulvum* Biourge (*P. citreo-roseum* Dierckx).[3] The three pigments produced by the same mold differ in the oxydation stages of the side chain.

The pigment produced by *Preussia multispora* has been identified with skyrin (IV). The bianthraquinonyl is now becoming to be proved as a widely-distributed pigment in many fungi.[1]

Experimental[*2]

Strains——*Talaromyces avellaneus* was isolated from the soil from Chiba, Japan, and deposited as NHL 6081.

The strain of *Preussia multispora* was isolated on the soil from Walakamgiri, Qandakavania, India, and deposited as NHL 2313.

Cultural Conditions——Czapek-Dox medium and potato-dextrose medium were employed for *T. avellaneus*. After 3 weeks' incubation at 25°, the mycelia were separated from the culture filtrate; dry weight, 18~20 g./L. for Czapek-Dox medium, and 11 g./L. for potato-dextrose medium.

---

*1 Tamagawayoga, Setagaya-ku, Tokyo (名取信策, 佐藤惣子, 宇田川俊一).

*2 Melting points were determined in a Yanagimoto micro-melting point determination apparatus. IR spectra were taken in a Koken Model 301 Infrared spectrophotometer. Thin-layer chromatography was carried out, using Silica-gel G treated with oxalic acid solution as the absorbent and benzene-EtOAc mixture as the solvent.

1) S. Shibata, S. Natori, S. Udagawa : "List of Fungal Products," Tokyo University Press (1964).
2) W. K. Anslow, J. Breen, H. Raistrick : Biochem. J., **34**, 159 (1940).
3) T. Posternak : Compt. rend. sci. phys. hist. nat. Geneve, **56**, 29 (1939); T. Posternak, J. P. Jacob : Helv. Chim. Acta, **23**, 237 (1940).

In the case of *P. multispora*, potato-dextrose agar medium was used and the incubation was continued for 4 weeks at 25°. The mycelia were collected from the agar and dried; 9~10 g./L.

Metabolites in Cultural Medium of *T. avellaneus* ——The red-colored medium showed pH 7.6 after 3 weeks' incubation. Red precipitate formed by acidification was collected by ethereal extraction to afford a crude mixture of pigments; yield, 70~90 mg./L. for Czapek-Dox medium and 40 mg./L. for potato-dextrose medium. Thin-layer chromatography revealed that the mixture is chiefly composed of two pigments; both being positive to $Mg(OAc)_2$ reaction (emodin type anthraquinone), one soluble to $NaHCO_3$, and the other to Na_2CO_3. The benzene solution of the mixture was chromatographed through a column of $CaHPO_4$, eluted with benzene, benzene-EtOAc, and then EtOAc to give two main colored bands. The first band was collected and recrystallised from HOAc to orange-red needles of m.p. 260~265°, which was proved to be identical with emodin (I) by a mixed fusion, IR spectra, and thin-layer chromatography. Yield, 10~20 mg./L. The acetate, yellow needles of m.p. 201° from EtOH, was also identical with emodin triacetate.

The second band furnished orange-red needles of m.p. 345~350° (decomp.) from AcOH or MeOH, IR spectra and thin-layer chromatography of which showed the identity with emodic acid (Ⅲ). Yield, 30~50 mg./L. The acetate, m.p. 215~220° from AcOH, was prepared for further confirmation of the identity.*3

Metabolites in the Mycelium of *T. avellaneus* ——The dried mycelia were moistened with ethanolic HCl and then extracted succesively with ether and acetone. Ethereal extract, ca. 15% of the dried mycelia, was fractionated by chromatography through a column of $CaHPO_4$. First elute with benzene was composed of fatty oils, saponification of which showed the presence of palmitic and octadecenoic acids as the chief acidic components by gas chromatography.*4 Further elution with benzene-EtOAc mixture showed the presence of several coloring matters. Repeated chromatography of each fractions afforded three $Mg(OAc)_2$ positive fractions in pure state; emodin (I) (0.02% of the dry weight of mycelium), an emodin-type anthraquinone (Ⅱ) (0.004%), and emodic acid (Ⅲ) (0.02%), in the order of elution. The second anthraquinone, orange-red needles of m.p. >270° from AcOH, was proved to be identical with ω-hydroxyemodin (Ⅱ) by IR spectra and thin-layer chromatography.

Acetone extract of the mycelia afforded D-mannitol, m.p. 164~166°, in 0.6% yield.*5

The characteristic deep violet color of the mycelium could not be removed by extraction with ordinary organic solvents at acidic, neutral and basic conditions.

Metabolites in the Mycelium of *Preussia multispora* ——The dried orange-colored mycelia were extracted successively with hexane, ether, and acetone. Hexane extract was a fatty material and not investigated further. Ether extract, ca. 0.8% of mycelium, was further purified by $CaHPO_4$ chromatography. Elution with benzene, followed with benzene-acetone, showed the existence of six compounds by thin-layer chromatography of each fractions. The main colored band was collected and purified by repeated chromatography through the same column to give orange-red needles of m.p. >300°. The color reactions with $Mg(OAc)_2$ and H_2SO_4 and other properties suggested the similarity with skyrin (Ⅳ).[4] The identity was established by paper chromatography, thin-layer chromatography, and IR spectra. Yield, 0.3~0.4%. The identity was confirmed by the comparison of IR spectra of the acetate and the pyridine salt.[4]

Acetone extract of the mycelium gave D-mannitol, m.p. 165~167°, in 0.7% yield.*5

Ethyl acetate extract of the agar medium separated from the mycelium afforded a colorless phenolic substance of m.p. >270°.

The authors' thanks are due to Professor S. Shibata, Tokyo University, and Dr. M. Takido, Nihon University, for their generous gifts of the authentic specimens.

Summary

Emodin, ω-hydroxyemodin, and emodic acid were isolated from *Talaromyces avellaneus* C. R. BENJAMIN (conidial stage : *Penicillium avellaneum* THOM et TURRESON). Skyrin was proved to be the main pigment of *Preussia multispora* CAIN.

(Received November 13, 1964)

*3 IR spectrum of the crude acetate showed the absorption of an acid anhydride group, even after careful drying.

*4 Gas chromatography was carried out with methyl ester mixture on Shimadzu Gas-Chromatograph DC-1B type with a column of 3.5% SE-30 on Chromosorb W.

*5 D-Mannitol appears in two different crystal forms in IR spectra in Nujol mull according to the conditions of recrystallisation.

4) S. Shibata, *et al.* : This Bulletin, **4**, 274 (1955).