Note

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Shoji Shibata*1 and Shun-ichi Udagawa*2 : Metabolic Products of Fungi. XIX.*3 Isolation of Rugulosin from *Penicillium brunneum* UDAGAWA.

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Rugulosin is noted as one of the widely distributed fungal dianthraquinone series compounds.[1,2] It has been isolated frequently along with skyrin from some species of the Biverticillata-Symmetrica section in the genus Penicillium.

The present paper deals with the rugulosin formation in *P. brunneum* UDAGAWA, which has been described recently by one of the authors[3] as a new species of *P. funiculosum* series having brownish conidial heads.

Experimental

Production and Isolation of the Pigments of *Penicillium brunneum* UDAGAWA—The fungus *P. brunneum* (NHL 6054) was incubated at 25° for 21 days on a modified Czapek-Dox medium (glucose 50 g., $NaNO_3$ 2 g., KH_2PO_4 1 g., KCl 0.5 g., $MgSO_4 \cdot 7H_2O$ 0.5 g., $FeSO_4 \cdot 7H_2O$ 0.01 g., $ZnSO_4 \cdot 7H_2O$ 0.01 g., $CuSO_4 \cdot 5H_2O$ 0.005 g., distilled H_2O to 1 L.). The mycelial felts were collected, dried, and extracted in a Soxhlet extractor with petr. ether (b.p. 40~60°) to remove fatty substance. Subsequently, the defatted mycelium was extracted repeatedly and exhaustively with Et_2O. Concentration of the extracts yielded a mixture of crude pigments (12.5 g. from 100 g. of the dried mycelium) indicating the presence of several anthraquinones by paper chromatography which was developed in the usual way.[4] The Rf values showed the presence of rugulosin, skyrin and emodin. The ethereal solution of the mixture was shaken with 5% $NaHCO_3$ and 2*N* Na_2CO_3, successively.

Rugulosin——On acidification of the bicarbonate-soluble fraction, a considerable amount of rugulosin precipitated. Repeated recrystallization from EtOH resulted large yellow prisms, m.p. 290°(decomp.), yield 3 g. from 12.5 g. of crude ethereal extract. The product was identified as rugulosin by its chemical properties, chromatograms and IR spectrum (in Nujol). Furthermore, on treatment with benzoyl chloride, hexabenzoate was obtained as pale yellow rhombic crystals, m.p. 224~226°, giving no depression of melting point on admixture with an authentic sample.

Skyrin and Emodin——The carbonate-soluble portion contained skyrin and a small quantity of emodin, which were separated by chromatography on $CaHPO_4$-columns using hexane-Me_2CO-H_2O (4 : 1 : 0.1; upper layer) as the solvent.

Skyrin was obtained from the upper band, and purified by recrystallization first from pyridine and then from Me_2CO to form orange-red rectangular plates, m.p. >360°. Yield : 175 mg. from 12.5 g. of crude ethereal extract. It showed agreement with an authentic specimen of skyrin in its color reactions and IR spectrum (in Nujol).

The lowest yellow band was eluted and re-chromatographed using benzene and hexane-Me_2CO (9:1) by the same procedure. Thus the almost pure emodin was obtained, which was crystallized from MeOH to form orange needles, m.p. and mixed m.p. 255~256°, yield, 20 mg.

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Summary

Penicillium brunneum UDAGAWA was shown to produce rugulosin in good yield accompanying skyrin and emodin.

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