

## Note

UDC 576.882.821.2,095.3

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

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Rugulosin is noted as one of the widely distributed fungal dianthraquinone series compounds.<sup>1,2)</sup> 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<sup>3)</sup> 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<sub>3</sub> 2 g., KH<sub>2</sub>PO<sub>4</sub> 1 g., KCl 0.5 g., MgSO<sub>4</sub>·7H<sub>2</sub>O 0.5 g., FeSO<sub>4</sub>·7H<sub>2</sub>O 0.01 g., ZnSO<sub>4</sub>·7H<sub>2</sub>O 0.01 g., CuSO<sub>4</sub>·5H<sub>2</sub>O 0.005 g., distilled H<sub>2</sub>O 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<sub>2</sub>O. 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.<sup>4)</sup> The R<sub>f</sub> values showed the presence of rugulosin, skyrin and emodin. The ethereal solution of the mixture was shaken with 5% NaHCO<sub>3</sub> and 2N Na<sub>2</sub>CO<sub>3</sub>, 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, hexabenzoylate 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<sub>4</sub>-columns using hexane-Me<sub>2</sub>CO-H<sub>2</sub>O (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<sub>2</sub>CO 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<sub>2</sub>CO (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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\*<sup>3</sup> Part XVIII. This Bulletin, 9, 352 (1961).

1) S. Shibata, T. Murakami, O. Tanaka, G. Chihara, M. Sumimoto: *Ibid.*, 3, 274 (1955).

2) S. Shibata, J. Shoji, A. Ohta, M. Watanabe: *Ibid.*, 5, 380 (1957).

3) S. Udagawa: *Jour. Agr. Sci. Tokyo Nogyo Daigaku*, 5, 5 (1959).

4) S. Shibata, M. Takido, T. Nakajima: This Bulletin, 3, 286 (1955).


### Summary

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

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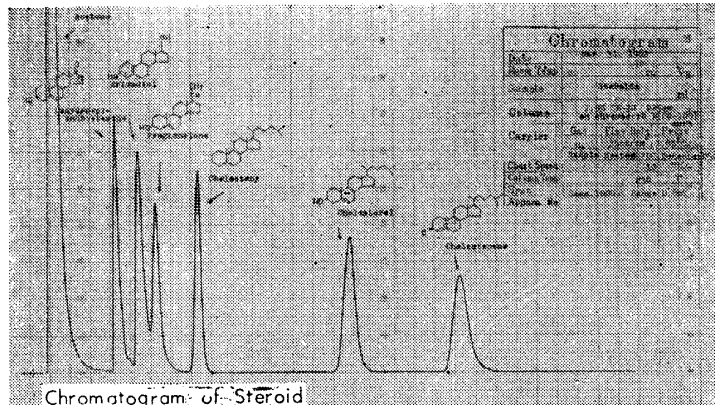
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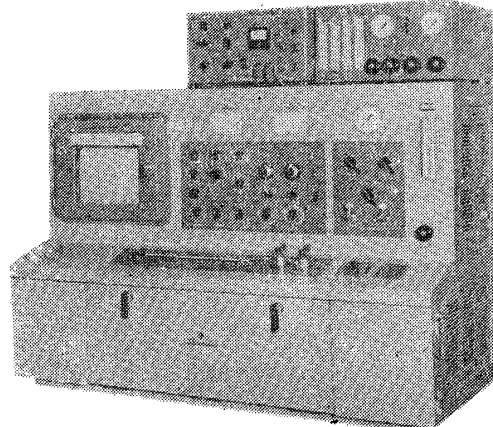
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