line solid was separated and filtered to obtain the methiodide of (IV) (0.3 g.) as yellow needles (AcOEt + MeOH), m.p. 235~236°. A mixed melting point of this compound with the authentic sample of methiodide of (IV) was not depressed.

A mixture of the methiodide of the methoxy compound (IV) (0.2 g) was warmed with moisted fresh AgCl on a water bath and converted to the methochloride. AgI was filtered off and the combined filtrate was concentrated to dryness. The residue was distilled *in vacuo* (2 mm. Hg) to yield colorless, very viscous oil at 240° (bath temp.). Hydrochloride : Colorless needles (AcOEt + EtOH), m.p. 249°. This compound was determined as the starting material (IV) by admixture.

Summary

Some considerations were made on the steric configuration of 3-hydroxy-9-aza-des-N-morphinan synthesized previously. It was thereby established that the octahydroquinoline formed during the course of its synthesis has a *trans*-configuration and that its cyclized product and demethylated, objective compound have the same steric configuration as that of morphine.

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Shoji Shibata, Junzo Shoji, Akihiro Ohta, and Mitsuo Watanabe : Metabolic Products of Fungi. XI.* Some Observation on the Occurrence of Skyrin and Rugulosin in Mold Metabolites, with a Reference to Structural Relationship between Penicilliopsin and Skyrin.

(*Pharmaceutical Institute, Medical Faculty, University of Tokyo***)

Rugulosin was reported to be found accompanying skyrin in the mycelium of *Penicillium rugulosum* THOM, *P. wortmanni* KLÖCKER,[1] *P. tardum* THOM,[2] and *Endothia* spp.[3] Furthermore, skyrin, not accompanying rugulosin, was produced by *Penicillium islandicum* SOPP[3,4] along with some of other anthraquinones and related coloring matters.

The studies of rugulosin and skyrin were elucidated by Raistrick and his co-workers,[1,4] and also by us.[3,5~8] According to our findings on the chemical structures of skyrin (diemodin(8,8′)) and rugulosin (6,7,6′,7′-tetrahydro-diemodin(8,8′)), it is not improbable to assume that both pigments are in a close biogenetical relationship.

In the present communication, the occurrence of skyrin, rugulosin, and chrysophanol in the mycelium of *Sepedonium ampullosporum* DAMON, a fungicolous fungus,[9] is described, which provides a further example of wide distribution of skyrin and rugulosin in molds.

On the other hand, the metabolic products of *Penicilliopsis clavariaeformis* SOLMS-LAUBACH have been investigated. Penicilliopsin, an orange coloring matter of the above fungus, was first isolated and studied by Oxford and Raistrick,[10] and then by Brockmann

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** Hongo, Tokyo (柴田承二, 庄司順三, 太田明広, 渡辺光夫).

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and his co-workers.[11] The structural formula of penicilliopsin, for which Brockmann adopted diemodinanthronyl-(9,9′), was recently amended by himself[12] to diemodinanthronyl-(8,8′).

Brockmann and Eggers suggested at that time that skyrin could not be diemodin-(8,8′) since the oxidation product of penicilliopsin hexaacetate gave a lower melting point (m.p. 249°) than that recorded by Raistrick for skyrin hexaacetate (m.p. 295~296°).

We stated a comment[13] for Brockmann's idea referring to the fact that the melting point of skyrin acetate is variable while the structure of skyrin was fully established as diemodin-(8,8′) by the synthesis of its 7,7′-dimethyl ether,[6] though Brockmann had not noted the existence of our papers.

By the extraction of the mycelium of *Penicilliopsis clavariaeformis* grown on the medium indicated by Raistrick, *et al.*, penicilliopsin was obtained in a fairly good yield. The chromatographical investigation of the remaining portion of the product showed the presence of skyrin, emodin, and an unidentified orange pigment.

By a cautious treatment, it was well established that skyrin and emodin occur initially in the mycelium, and the possibility of their formation by secondary conversion from penicilliopsin during the course of isolation was excluded.

For the purpose of clarifying the structural relationship between penicilliopsin and skyrin which has not clearly been demonstrated, we attempted the conversion of penicilliopsin directly into skyrin.

On oxidation with selenium dioxide, penicilliopsin was found to yield skyrin, and the latter was identified by a mixed fusion of its hexaethoxycarbonyl ether (m.p. and mixed m.p. 173°). Thus the slight confusion in dealing with the structures of penicilliopsin and skyrin has now completely been solved.

We are grateful to Professor H. Raistrick and Mr. G. Smith of London School of Hygiene and Tropical Medicine for sending us the strain of *Penicilliopsis clavariaeformis*. We wish to thank Mr. K. Tsubaki, Nagao Institute, for supplying us the culture of *Sepedonium ampullosporum*. The microanalyses were carried out by the members of Microanalytical Laboratories of this Institute, and the infrared spectra were measured by the staff of the Central Laboratories for Clinical Inspection, Tokyo University Hospital, to whom our thanks are due.

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Experimental

Cultural Condition for *Sepedonium ampullosporum* Damon—The culture used in the present investigation was received in April, 1956, from Mr. K. Tsubaki, Nagao Institute, Tokyo. Tsubaki[9] described that *Sepedonium ampullosporum* is parasitic on *Boletus* spp. The Roux flasks containing 5% glucose Czapek-Dox solution (pH 4.2) were sterilized and inoculated with the above strain. The flasks were incubated at 25~30° for 4 weeks. At the end of incubation the culture fluid showed pH 6.8.

Extraction and Isolation of the Pigments—The dried mycelium was defatted by extraction with petroleum ether. The defatted mycelium was reëxtracted with ether, acetone, and MeOH, successively, or extracted with MeOH only(It is noteworthy that MeOH is most effective in extracting rugulosin from the mycelium). The solvent was removed and the residue was dissolved in ether. The ethereal solution was shaken successively with 5% $NaHCO_3$, 2*N* Na_2CO_3, and 2*N* NaOH. The alkaline solutions were acidified with dil. HCl and each fraction was examined preliminarily by paper chromatography employing the solvent systems of (i) NH_4OH-saturated BuOH, (ii) MeOH-saturated benzine, (iii) acetone : benzine : H_2O (5 : 5 : 3.5)(upper layer), and (iv) petroleum ether. The presence of rugulosin, skyrin, and chrysophanol in the extracts was suggested by Rf values on the paper chromatograms.

The crude pigment (400 mg.) obtained from $NaHCO_3$-soluble portion of the methanolic extract of

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dry mycelium (42.2 g.) was recrystallized from EtOH to yellow prisms, m.p. 290°(decomp.), which was identified with rugulosin by comparison of its properties and the infrared spectrum. Methyl ether of the product was also prepared to identify with rugulosin hexamethyl ether (m.p. 279°).

Cultural Condition for *Penicilliopsis clavariaeformis* Solms-Laubach—The strain of *Penicilliopsis clavariaeformis* used for the present study was received from Prof. H. Raistrick and Mr. G. Smith, London School of Hygiene and Tropical Medicine, in March, 1956. The mold was grown on the following medium : Glucose, 50 g.; monoammonium citrate, 2.0 g.; KH_2PO_4, 1.0 g.; KCl, 0.5 g.; $MgSO_4 \cdot 7H_2O$, 0.5 g.; $FeSO_4 \cdot 7H_2O$, 0.01 g.; extract of orange or Chinese citron; distilled water to 1 L., pH was adjusted to 4.5 with 2*N* HCl. For the preparation of orange or Chinese citron extracts, 150 g. of orange or 190 g. of Chinese citron was crashed in a mixer and the pulp was boiled twice with 500-cc. and 200-cc. portions of water. The extract filtered through muslin was added to 1 L. of the medium. The medium (150 cc. each) was placed in Roux flask which was sterilized by steam 1 hr. on each of 3 consecutive days. Each flask was inoculated and incubated for 2 weeks at 25° in the dark. The final pH was 4.6~4.8 (orange extract medium) or 5.0~5.2 (Chinese citron extract medium).

Isolation of the Pigments—The dried mycelium was defatted by extracting with petroleum ether and then reëxtracted with ether. The ethereal extract was recrystallized from dioxane to orange needles (penicilliopsin), m.p. about 320°(decomp.). *Anal.* Calcd. for $C_{30}H_{24}O_8 \cdot \frac{1}{2}H_2O \cdot C_4H_8O_2$: C, 66.95; H, 5.43. Found : C, 66.47; H, 5.35.

Medium	Amt. of medium	Dry wt. of mycelium	Penicilliopsin obtained
Orange extract	150 cc. × 20	52 g.	2.1 g. (4.1%)
Chinese citron	150 cc. × 45	115 g.	1.5 g. (1.3%)

The ether extract separated from penicilliopsin was chromatographed on a $CaHPO_4$-column using petroleum ether : acetone : H_2O (4 : 2 : 0.2)(upper layer) as the developing solvent to give three separated bands. The lowest band was separated and chromatographed again through the same adsorbent using $CHCl_3$ as the solvent, and emodin was obtained and identified by a mixed fusion with authentic specimen. The remaining two fractions were chromatographed on the $CaHPO_4$-column with a mixture of petroleum ether : acetone : H_2O (4 : 1 : 0.2)(upper layer), and the lowest band was proved to be skyrin, by comparison of its properties with those of the authentic specimen. The orange crystals separated from the second portion resembled the pigment B of *P. islandicum* N.R.R.L. 1175,[14] though it could not be confirmed due to its poor yield.

Oxidation of Penicilliopsin into Skyrin—Penicilliopsin (500 mg.) and SeO_2 (1 g.) were mixed well and suspended in xylene. The mixture was refluxed for 2 hrs. and filtered after cooling. The precipitate was extracted with 2*N* NaOH and the xylene mother fluid was also shaken with the same reagent. The alkaline solution was acidified with dil. HCl and extracted with ether. The red-colored ethereal layer was shaken with 5% $NaHCO_3$, washed with water, and dried. The concentrated ethereal solution was repeatedly chromatographed on a $CaHPO_4$-column, developed with petroleum ether : acetone : H_2O (4 : 2 : 0.2)(upper layer) or petroleum ether : acetone : H_2O (4 : 1 : 0.2) (upper layer). The lowest band was separated to give skyrin which was identified by paper chromatography and by a mixed fusion with its hexaethoxycarbonyl ether (m.p. 171~172°). The SeO_2-oxidation of penicilliopsin without any solvent was carried out, for which 5-fold amount of SeO_2 was used, heating at 150~160° for 2 hrs. The reaction mixture was extracted with ether, and examined by paper chromatography using benzine : acetone : H_2O (5 : 5 : 3.5)(upper layer) to prove the formation of skyrin.

Summary

1) It was shown that *Sepedonium ampullosporum* Damon produces rugulosin, skyrin, and chrysophanol.

2) Skyrin and emodin were found to be produced by *Penicilliopsis clavariaeformis* Solms-Laubach along with penicilliopsin.

3) On oxidation with selenium dioxide, penicilliopsin was directly converted into skyrin.

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