4,5,7-trimethoxy-2-methylanthraquinone (=8-bromoemodin trimethyl ether) (IV) (650 mg.), active Cu-bronze (800 mg.), and naphthalene (1.8 g.) was refluxed for 2.5 hrs. in an oil bath (bath temp. 225°). The cooled mixture was extracted with warm EtOH and acetone to remove naphthalene and the dried residue was extracted with $CHCl_3$. The concentrated $CHCl_3$ solution was chromatographed using acetone-$CHCl_3$ mixture (1:1) as a developing solvent. To the orange yellow eluate a small amount of EtOH was added and the mixture was concentrated to separate yellow crystals (300 mg.), which were collected, washed with warm EtOH, and crystallized from aq. HOAc to give orange yellow microneedles, m.p. about 350°(decomp.). The properties agree with those of hexamethylskyrin.[3] *Anal.* Calcd. for $C_{36}H_{30}O_{10}$: C, 69.45; H, 4.82. Found: C, 69.20, 69,19; H, 4.70, 4.56,

2,2′-Dimethoxy-4,5,4′,5′-tetrahydroxy-7,7′-dimethylbianthraquinone-(1,1′) (II) (β,β′-Dimethylskyrin (V: R=CH_3 in the preceding paper[1])—2,4,5,2′,4′,5′-Hexamethoxy-7,7′-dimethylbianthraquinone-(1,1′) (150 mg.) was boiled in a mixture of 48% HBr and glacial HOAc (1:1). During the reaction, orange red crystals separated out. After boiling for 1.5 hrs. the crystals were collected, washed successively with water, glacial HOAc, and warm acetone, then crystallized from nitrobenzene or nitrobenzene containing BuOH. The product, orange red microcrystals, does not melt below 360°(darkens from 321°) and gives a stable red solution with conc. H_2SO_4. On reduction with alkaline $Na_2S_2O_4$, it quantitatively affords physcion.

The properties and the infrared spectrum of this compound agree with that of β,β′-dimethylskyrin. *Anal.* Calcd. for $C_{32}H_{22}O_{10}$: C, 67.84; H, 3.89. Found: C, 68.09; H, 3.90.

On treatment with $ClCO_2Et$ in pyridine, 2,2′-dimethoxy-4,5,4′,5′-tetrahydroxy-7,7′-dimethylbianthraquinone-(1,1′) gives yellow needles, m.p. 247~249°. It gives no melting point depression by admixture with β,β′-dimethylskyrin tetraethoxycarbonyl ether (m.p. 247~249°). *Anal.* Calcd. for $C_{44}H_{38}O_{18}$: C, 61.83; H, 4.45. Found: C, 62.08; H, 4.62.

Summary

Skyrin β,β′-dimethyl ether was synthesized by the Ullmann condensation of 8-bromoemodin trimethyl ether followed by a partial demethylation. The structure of skyrin, therefore, was unequivocally established as 2,4,5,2′,4′,5′-hexahydroxy-7,7′-dimethylbianthraquinone-(1,1′).

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57. Shoji Shibata, Michio Takido, and Terumi Nakajima: Metabolic Products of Fungi. VII*. Paper Chromatography of the Coloring Matters of *Penicillium islandicum* Sopp.

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Howard and Raistrick[1~3] isolated several coloring matters from some strains of *Penicillium islandicum* Sopp. Of these pigments, chrysophanol[2] is well known as it is widely distributed in higher plants though it has been isolated for the first time from fungi.

A deep red compound which was named islandicin[1] was shown to be 1,4,5-trihydroxy-2-methylanthraquinone, and another orange red pigment named skyrin[3] has also been found in the mycelium of Endothia spp., whose structure has recently been established by us as shown in the preceding reports.[5~7] No decisive evidences have yet been obtained for the chemical structures of the remainder of the pigments of *Penicillium islandicum*, termed flavoskyrin[3], rubroskyrin, iridoskyrin, and erythroskyrin[4],

* Part VI: This Bulletin, **3**, 284 (1955).

** Hongo, Tokyo (柴田承二, 滝戸道夫, 中島暉躬).

1) B. H. Howard, H. Raistrick: Biochem. J., **44**, 227 (1949).
2) B. H. Howard, H. Raistrick: *Ibid.*, **46**, 49 (1950).
3) B. H. Howard, H. Raistrick: *Ibid.*, **56**, 56 (1954).
4) B. H. Howard, H. Raistrick: *Ibid.*, **57**, 213 (1954).

though some degradation reactions were elucidated.

As a preliminary to the study of the chemical structures of these pigments, a chromatographic examination has been carried out by us, with which the present paper is chiefly concerned.

It has been shown by paper chromatography that some undescribed coloring matter appears in the mycelium of *Penicillium islandicum* along with the pigments mentioned above.

Microchemical investigation suggests that a close relationship might be expected in their chemical structures. The occurrence of these pigments during the cultivation of the mold has also been followed by paper chromatography, testing the mycelium at several stages of growth. The paper chromatography of the extracts of the mycelium would be available for the identification of the chemical strains of the mold.

Experimental

Strains—*Penicillium islandicum* Sopp NRRL 1036 and NRRL 1175 were received from Professor H. Raistrick and Mr. G. Smith, London School of Hygiene & Tropical Medicine, in June, 1954. A strain of *P. islandicum* Sopp N. I. 6299 isolated from the yellowsis rice was received from Mr. K. Tsubaki, Nagao Institute, Tokyo, in October, 1954.

Cultivation—The strains were sown in Czapek-Dox solution and incubated for 3~4 weeks at 25°. The samples were picked up at a suitable interval of time during the cultivation as indicated below.

Procedure of Paper Chromatography—The dry mycelium was extracted with acetone at room temperature and a drop of the concentrated extract was placed on a filter paper (Toyo Roshi No. 3, 1.5×25 cm.) and developed at 18~20° by one-dimensional ascending method using the upper layer of a mixture of acetone-petroleum benzine (b.p. 60~70°)-water (5 : 5 : 3.5) (saturated at 18°) as a developing solvent. The developed paper was sprayed with the $Mg(OAc)_2$ reagent[8,9] and the Rf value and the color of spot were examined.

Results

The strain NRRL 1036 of *Penicillium islandicum* Sopp gave a paper chromatogram on which ten spots (a~j) of the pigments were observed (Fig. 1 A). In Table I are presented the color reactions and Rf values of the spots.

Table I.

Spot	Color	Coloration with $Mg(AcO)_2$	Rf[a]	Identification	Remarks
a	Orange	Purple	0.97	Islandicin & iridoskyrin	Note (1). Ref.[1,4]
b	〃	〃	0.80	Catenarin	
c	Yellowish brown	Yellow	0.63	Erythroskyrin (?)[b]	The color faded by exposure to light. Ref[4]
d	〃	〃	0.58	〃	〃
e	Yellow	Yellow-brown	0.52	Pigment-A	Note (2).
f	Red	Red	0.45		
g	Yellow-brown	Orange red	0.42	Skyrin	Ref.[3,6,7]
h	Brown-red	Green	0.25	Rubroskyrin	Ref.[4]
i	Yellow-brown	Orange	0.19		Soluble in aq. $NaHCO_3$ to give a red solution
j	Red	Greenish blue	0.05		

a) Rf value ± 0.01 *b*) Not identified with the authentic specimen.

The strain N. I. 2699 of *Penicillium islandicum* Sopp gave a paper chromatogram (Fig. 1 B) which is very similar to that of the strain NRRL 1036 but it is distinguished at spot b (Rf. 0.70, $Mg(AcO)_2$: orange red) and spot i (Rf. 0.10) which shows no change of color with $Mg(OAc)_2$ reagent.

5) S. Shibata, T. Murakami, O. Tanaka, G. Chihara, M. Sumimoto : This Bulletin, **3**, 274 (1955).
6) S. Shibata, O. Tanaka, I. Kitagawa : *Ibid.*, **3**, 278 (1955).
7) O. Tanaka, C. Kaneko : *Ibid.*, **3**, 284 (1955).
8) S. Shibata : J. Pharm. Soc. Japan, **61**, 103 (1941) (C. A., **44**, 9396 (1950)).
9) S. Shibata, M. Takido, O. Tanaka : J. Am. Chem. Soc., **72**, 2789 (1950).

The strain NRRL 1175 of *Penicillium islandicum* Sopp showed nine spots on the paper chromatogram (Fig. 1C, Table II).

Table II.

Spot	Color	Coloration with $Mg(OAc)_2$	Rf	Identification	Remarks
a	Yellow	Orange red	0.97	Chrysophanol	Ref.[2]
b	〃	〃	0.80		very slight
c	Yellowish brown	Yellow	0.63	Erythroskyrin(?)	Cf. Table I, spot c, d
d	〃	〃	0.58	〃	〃
e	Yellow	〃	0.49	Flavoskyrin	Ref.[3]
f	Yellow-brown	Orange red	0.42	Skyrin	Cf. Table I, spot g.
g	〃	〃	0.29		very slight
h	〃	〃	0.16	Pigment-B	Note (3)
i	〃	〃	0.01	Pigment-C	Note (4)

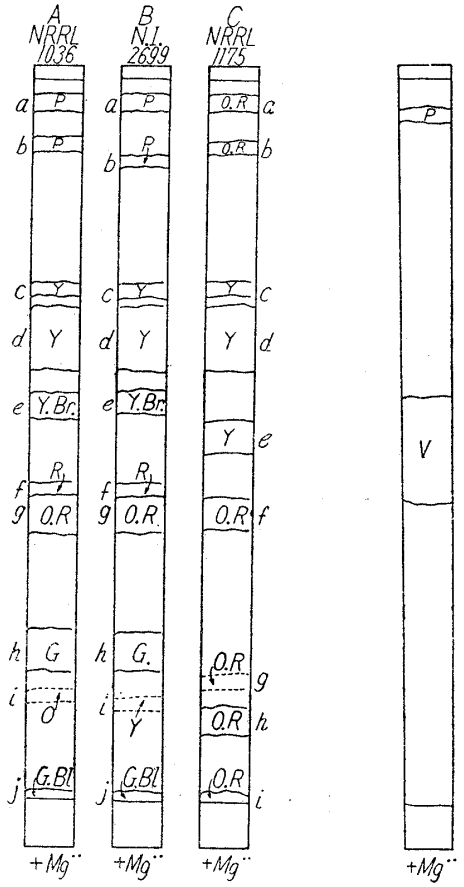

Fig. 1

Fig. 2

P = purple
O = orange
R = red
Y = yellow
G = green
V = violet
Br = brown
Bl = blue

Note (1) : **Islandicin and Iridoskyrin**—Were separated by paper chromatography developing with petroleum ether (b.p. 40~50°) (Fig. 2, Table III).

Table III.

Spot	Color	Coloration with $Mg(AcO)_2$	Rf	Identification
a	Orange	Purple	0.95	Islandicin
b	Red	Violet	0.55~0.40 (tailing)	Iridoskyrin

Note (2) : **Pigment A**—Yellow needles, blackening from 250°. It is soluble in aq. bicarbonate to give a yellow solution. On heating to above 300° in vacuum, it decomposed to give islandicin and catenarin which were confirmed by paper chromatography in comparison with the authentic samples. By heating with conc. H_2SO_4 for 10 mins. in a boiling water bath, it dissolves to form a violet solution. The reaction mixture was poured into water and shaken with ether. The ethereal extract gave islandicin and iridoskyrin. On heating in alkaline $Na_2S_2O_4$, pigment A afforded islandicin.

Note (3) : **Pigment B**—Orange crystals, do not melt below 300°. It partially dissolves in aq.

bicarbonate and completely in aq. carbonate to give a purple solution. It forms a purple solution with conc. H_2SO_4. Within a few seconds of dissolution the color changes to an emerald green, similarly as observed in skyrin.[3,6] By the action of alkaline $Na_2S_2O_4$, it was decomposed to give emodin and ω-hydroxyemodin which were confirmed by paper chromatography in comparison with the authentic samples.

Note (4) : **Pigment C**—The brownish orange extract obtained from the spot cut out and dissolved in 5% aq. bicarbonate gives a purple solution. It exhibits a purple color in conc. H_2SO_4 which changes within a few seconds to green. On treatment with alkaline $Na_2S_2O_4$, it gave ω-hydroxyemodin which was confirmed on the paper chromatogram.

Occurrence of Coloring Matter in the Mycelia of *Penicillium islandicum* NRRL 1036 and NRRL 1175 during Cultivation—Twenty 100-cc. flasks containing 50-cc. of Czapek-Dox solution (pH 5.2) were used for the cultivation of each strain. During the incubation at 25°, two each of the flasks were picked up at a suitable interval of time for testing the pigment formation. The mycelium was collected and extracted while wet with acetone by allowing to stand overnight in a room temperature.

The acetone extracts were examined by paper chromatography as described above.

Table IV.

Days	Observation	Pigment identified	pH
P. islandicum NRRL 1036			
4	Colonies developing; reverse yellowish	Eryth.	—
5	Spores forming; reverse yellowish orange	〃	2.8
6	Mycelium covers surface of the medium; reverse orange ; medium orange	Eryth., Sk., Is., Ir.	—
7	〃	Eryth., Sk., Is., Ir., Rb.	3.0
8	〃	Eryth., Sk., Is., Ir., Rb., Spot-j(Table I)	3.1
9	Reverse dark red; medium orange	Pigment formation completed	3.2
10	〃		—
15	〃		4.0
25	〃		4.0
P. islandicum NRRL 1175			
5	White colonies developing		—
6	Reverse orange; medium pale yellow	Eryth., Sk.	3.1
7	Spores forming; reverse reddish brown	Eryth., Sk., Pigment-B	3.1
8	Medium yellowish brown	Eryth., Sk., Pigment-B, Chrys., Pigment-C	3.2
9	〃	Pigment formation completed	3.2
10	〃		3.4
11	〃		3.6
12	〃		3.6
21	〃		4.0
25	〃		4.2

Eryth : Erythroskyrin; Sk. : Skyrin; Is. : Islandicin; Ir. : Iridoskyrin; Rb. : Rubroskyrin; Chrys. : Chrysophanol

Discussion and Conclusion

It has been shown by the present work on chromatography that catenarin, which has been assumed to be one of the constituents of iridoskyrin,[4] occurs as a single molecule in the mycelium of the NRRL 1036-strain and three undescribed pigments tentatively named pigments A, B, and C are added to the list of the coloring matter of *Penicillium islandicum*.

The pigment A isolated from the strain NRRL 1036 resembles rubroskyrin in its behavior giving the same degradation products but differs in the color reaction with magnesium acetate and the Rf value on the paper chromatogram. It would not be unreasonable to assume that both piments are in a very close correlation in their chemical structures.

On the other hand, it was strongly suggested by their color reactions with magne-

sium acetate* and conc. H_2SO_4** as well as by the behavior against reductive cleavage with $Na_2S_2O_4$ that the pigments B and C would belong to a group of the skyrin-type aromatic bianthraquinones. Based on the degradation products and in analogy of the structure of skyrin (2,4,5,2′,4′,5′-hexahydroxy-7,7′-dimethylbianthraquinone-(1,1′)) (formula (I) in the preceding report[7]), it is highly probable that the pigments B and C are to be represented by a bianthraquinone consisting of emodin and ω-hydroxyemodin or two molecules of ω-hydroxyemodin, respectively, which link together at the 8,8′-positions (methyl in 2).

We are greatly indebted to Professor H. Raistrick, Dr. B. H. Howard, and Mr. G. Smith, London School of Hygiene & Tropical Medicine, for supplying us the strains of *Penicillium islandicum* NRRL 1036 and NRRL 1175, and the authentic specimens of the mold pigments. Thanks are due to Mr. K. Tsubaki, Nagao Institute, Tokyo, for his help in supplying the strain N. I. 2699.

Summary

The pigments of *Penicillium islandicum* Sopp were investigated by paper chromatography. The strains NRRL 1036 and N.I. 2699 gave almost the same paper chromatogram. Catenarin and pigment-A were newly found in the strain NRRL 1036, and piment B and C were added to the group of the pigments of the strain NRRL 1175.

The similarity in the behaviors of pigment A and rubroskyrin, and the possible structures of pigment B and C were suggested.

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* Orange red color given with magnesium acetate indicates a *meta*-disposition of hydroxyls in anthraquinone ring[8,9] as shown by emodin and skyrin.

** The purple color given with conc. H_2SO_4 changes within a few seconds to an emerald green color. This suggests the presence of free β-hydroxyls in the *ortho*-position of the linkage in bianthraquinone-(1,1′).[6]