UDC 547.673.6:582.282.12

99. Shoji Shibata,* Michio Takido,** Akihiro Ohta,* and Tama Kurosu**: Metabolic Products of Fungi. XIII.*** The Structure of Oxyskyrin.

(*Pharmaceutical Institute, Medical Faculty, University of Tokyo,* and Pharmaceutical Institute, Technological Faculty, Nihon University***)

In the previous paper,[1] we demonstrated by the chromatographical study on the products of *Penicillium islandicum* SOPP the existence of some new coloring matters in addition to the pigments which had been recorded by Howard and Raistrick.[2]

The strains of N. R. R. L. 1036 and N. R. R. L. 1175 are clearly distinguished by the difference in their pigment metabolisms.

The tentatively named pigment B, whose existence in the mycelium of the strain N. R. R. L. 1175 was shown by paper chromatography, has now been studied to determine the chemical structure. The pigment B, for which we propose the name "oxyskyrin," has also been found in the mycelium of *Endothia parasitica* ANDERSON ET ANDERSON accompanying rugulosin and skyrin.

Oxyskyrin was isolated by chromatography using a $CaHPO_4$-column as orange-red crystals, m.p. above 360°, gave a molecular formula $C_{30}H_{18}O_{11}$, and showed color reactions with sulfuric acid(purple turning immediately into emerald green) and magnesium acetate (orange red) similar to that given by skyrin.[2,3] These color reactions and the ultraviolet absorption curve suggested that oxyskyrin is likely to be a homolog of skyrin.

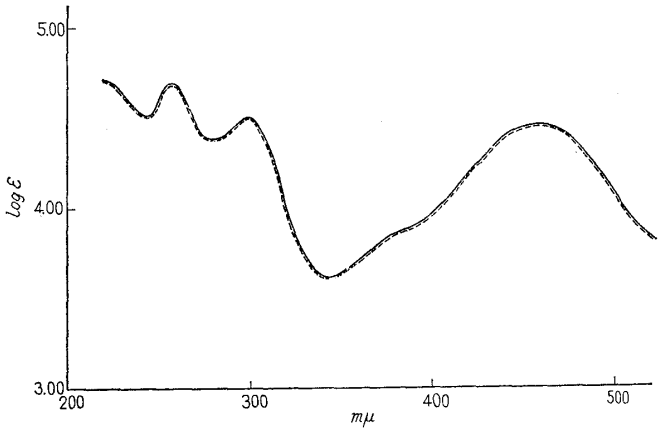


Fig. 1.

——— Skyrin (EtOH)
λmax 257 mμ log ε 4.69
300 mμ 〃 4.50
462 mμ 〃 4.37
------ Oxyskyrin (EtOH)

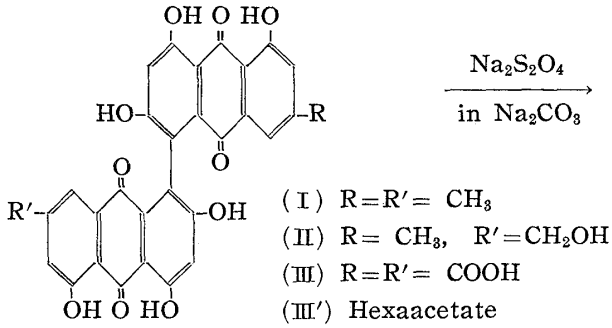
As tentatively suggested in the previous paper[1] and now chemically confirmed, oxyskyrin yielded ω-hydroxyemodin (V) and emodin (IV) on reductive cleavage with alkaline sodium dithionite.

On acetylation, oxyskyrin gave a heptaacetate, $C_{44}H_{32}O_{18}$, m.p. 271°, which showed split infrared absorption peaks of C=O of acetyl grouping at 1745 cm^{-1} (alcoholic acetate) and 1774 cm^{-1} (phenolic acetate) in chloroform solution.

* Hongo, Tokyo (柴田承二, 太田明広).
** Surugadai, Tokyo (滝戸道夫, 黒須 玉).
*** Part XII. This Bulletin, **5**, 383(1957).

1) S. Shibata, M. Takido, T. Nakajima: This Bulletin, **3**, 286(1955).
2) B. H. Howard, H. Raistrick: Biochem. J. (London), **56**, 56(1954); *Ibid.*, **57**, 212(1954).
3) S. Shibata, T. Murakami, O. Tanaka, G. Chihara, M. Sumimoto: This Bulletin, **3**, 274(1955); S. Shibata, O. Tanaka, I. Kitagawa: *Ibid.*, **3**, 278(1955); O. Tanaka, C. Kaneko: *Ibid.*, **3**, 286(1955).

The conversion of heptaacetyl-oxyskyrin to hexaacetylskyric acid (III′), which was identified by a mixed fusion and by comparison of infrared spectra, gave an evidence that oxyskyrin possesses the same disposition of substituents in the bianthraquinone structure as in skyrin (I). Oxyskyrin, therefore, is formulated as (II), which provides, together with other coloring constituents of *P. islandicum* and *Endothia* spp., an interesting scheme of biogenetical correlation.



(I) R=R′= CH_3
(II) R= CH_3, R′=CH_2OH
(III) R=R′= COOH
(III′) Hexaacetate

(IV) R= CH_3
(V) R= CH_2OH

The strain of *Penicillium islandicum* Sopp, N.R.R.L. 1175 was supplied by Prof. H. Raistrick and Mr. G. Smith, London School of Hygiene and Tropical Medicine, to whom we are very grateful.

The microanalyses were carried out by the members of the microanalytical laboratories of this Institute and the Research Institute of Applied Microbiology of this University. The infrared spectra were measured by the member of the Biochemical Laboratory of Clinical Inspection of the University Hospital, to all of whom our thanks are due. This work was supported partly by a Grant in Aid for Scientific Research from the Ministry of Education, to which we are also grateful.

Experimental

Isolation of Oxyskyrin (Pigment B)—(a) The mycelium (dry weight, 150 g.) of *Penicillium islandicum* Sopp N.R.R.L. 1175 grown on Czapek-Dox solution after 7~10 days' incubation was extracted with Et_2O. The ethereal extract was dissolved in acetone and mixed with $CaHPO_4$ powder, which was dried under infrared lamp. The dried mixture of the extract and $CaHPO_4$ powder was placed on the top of a $CaHPO_4$-column and chromatographed, using upper layer of a mixture of petr. ether-acetone-H_2O (2 : 1 : 0.1) as a developing solvent.

The third band from the bottom which showed orange-red color was eluted to separate the pigment B (yield, 100 mg.). The crude pigment thus obtained was acetylated with Ac_2O and pyridine by allowing to stand overnight. The acetate was purified by recrystallization from glacial AcOH to form yellow needles, m.p. 270~271°(decomp.).† *Anal.* Calcd. for $C_{30}H_{11}O_4(OCOCH_3)_7$: C, 62.26; H, 3.77; CH_3CO, 35.49. Found : C, 62.23; H, 4.07; CH_3CO, 35.81.

(b) The mycelium of *Endothia parasitica* Anderson et Anderson, incubated for 3~4 weeks at 25° in potato extract-glucose medium,[2] was extracted with Et_2O. Skyrin was separated during extraction and the filtrate was concentrated to give a mixture of coloring matters. It was proved by paper chromatography that the pigments were rugulosin, skyrin, and pigment-B (oxyskyrin). The solvent system used was an upper layer of benzine-acetone-H_2O (5 : 5 : 3.5).

The ethereal extract was shaken with 5% $NaHCO_3$ solution to remove rugulosin, the remaining portion was dissolved in acetone, and mixed with $CaHPO_4$ powder. The dried $CaHPO_4$-pigment mixture was placed on the top of $CaHPO_4$-column and developed with a solvent mixture consisting of petr. ether-acetone-H_2O (2 : 1 : 0.1) (upper layer). The pigments were separated on the column in the order, from top to bottom, into the bands of pigment B, skyrin, and rugulosin. The pigment B portion was chromatographed repeatedly through $CaHPO_4$ column with petr. ether-acetone-H_2O (4 : 1 : 0.2) (upper layer) to separate skyrin completely. Petr. ether was distilled off from the eluate to obtain the pigment B which was sparingly soluble in acetone.

The Properties of Oxyskyrin (Pigment B)—The acetate of oxyskyrin obtained as above was hydrolyzed by heating with 5% NaOH to give red crystals which were recrystallized from a mixture of acetone and benzine. Oxyskyrin forms orange red needles, m.p.>360°(darkens from 270°), gives with conc. H_2SO_4 a purple color which instantly changes into emerald green, similarly as

† As shown in the case of skyrin hexaacetate,[3] oxyskyrin heptaacetate is also very sensitive to alkali contamination in the capillary glass, which causes melting at a lower temperature by careless treatment.

shown in the case of skyrin.[2] The ultraviolet absorption curves of oxyskyrin and skyrin were completely superimposed (Fig. 1.). *Anal.* Calcd. for $C_{30}H_{18}O_{11}$: C, 64.98; H, 3.25. Found : C, 65.40; H, 3.70.

On treatment with alkaline $Na_2S_2O_4$ solution oxyskyrin was decomposed readily to yield emodin (IV), m.p. 256°, and ω-hydroxyemodin (V) (triacetate, m.p. 190.5°), which were identified by mixed fusion with the authentic specimens.

Heptabenzoyl-oxyskyrin—Oxyskyrin was dissolved in pyridine added with BzCl and allowed to stand overnight in a cool place. The product was purified by chromatography through Al_2O_3 column, using benene as a solvent. By recrystallization from EtOH-acetone mixture yellow needles, m.p. 259.5~261.5°(decomp.), were obtained. *Anal.* Calcd. for $C_{30}H_{11}O_4(OCOC_6H_5)_7$: C, 73.92; H, 3.61. Found : C, 73.84; H, 3.47.

Oxidation of Heptaacetyl-oxyskyrin—To the solution of heptaacetyl-oxyskyrin (130 mg.) in a mixture of glacial AcOH (5.5 cc.) and Ac_2O (5.5 cc.), a solution of CrO_3 (0.25 g.) in AcOH (1 cc.) and 1 drop of H_2O was gradually added (during 1 hr.) under vigorous stirring. The temperature was kept at 55° for 3 hrs. and the reaction was completed by heating on a boiling water bath for 10 mins. The reaction mixture was poured into ice water and allowed to stand overnight. The precipitate formed was recrystallized from MeOH to yellow plates, m.p. 250°(decomp.). Yield, 50 mg. The crystals were identified as hexaacetylskyric acid (III′) by comparing its infrared spectrum with that obtained by the oxidation of hexaacetylskyrin.[2]

Paper Chromatography of Oxyskyrin and Related Compound

Compound \ Solvent system	28% NH_4OH-saturated BuOH (upper layer) Rf*	Benzene-acetone-H_2O (1 : 1 : 1) (lower layer) Rf*
Skyrin	0.80	0.17
Oxyskyrin	0.67	0.36
Skyric Acid	0.41	0.83

Compound \ Solvent system	Benzene-acetone-H_2O (5 : 2 : 1) (upper layer) Rf*
Emodin	0.90
ω-Hydroxyemodin	0.20

* at 25~26°. Filter paper : "Toyo-Roshi" No. 3.

Summary

The pigment B, isolated from *Penicillium islandicum* SOPP, N.R.R.L. 1175, and also found in the mycelium of *Endothia parasitica* ANDERSON ET ANDERSON, was studied. The pigment B which was named oxyskyrin gave a molecular formula, $C_{30}H_{18}O_{11}$, and yielded heptaacetate and heptabenzoate. On reductive cleavage with alkaline sodium dithionite, oxyskyrin was decomposed to give emodin and ω-hydroxyemodin.

On oxidation with chromium trioxide, oxyskyrin heptaacetate was converted into skyric acid hexaacetate. Consequently, oxyskyrin was formulated as 2,4,5,2′,4′,5′-hexahydroxy-7-methyl-7′-hydroxymethyl-bianthraquinone-(1,1′).

(Received July 11, 1957)