U.D.C. 547.673.6 : 582.28

59. Shoji Shibata and Isao Kitagawa : Metabolic Products of Fungi. X.* The Structure of Rubroskyrin and Its Relation to the Structure of Luteoskyrin.

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Rubroskyrin

Rubroskyrin, a dark red coloring matter, was first isolated by Howard and Raistrick[1] along with other pigments[1,2] from the cultures of *Penicillium islandicum* Sopp N.R.R.L. 1036 and some other strains.

Howard and Raistrick gave a molecular formula $C_{30}H_{22}O_{12}$ for rubroskyrin, and elucidated its dehydration reaction by the action of conc. H_2SO_4 giving iridoskyrin[1,3] and its thermal decomposition yielding islandicin, catenarin, and iridoskyrin.

$$C_{30}H_{22}O_{12}\ (\text{Rubroskyrin}) \begin{cases} \nearrow\ 2\,H_2O + C_{30}H_{18}O_{10}\ (\text{iridoskyrin}) \\ \searrow\ H_2O + C_{15}H_{10}O_5\ (\text{islandicin}) + C_{15}H_{10}O_6\ (\text{catenarin}) \end{cases}$$

As pointed out by Raistrick and his co-workers,[4] and also by us,[2] there could be seen some similarity between rugulosin[3,4,5,6] and rubroskyrin in their behavior to degradation reactions. Nevertheless it should be noted that these pigments also exhibit

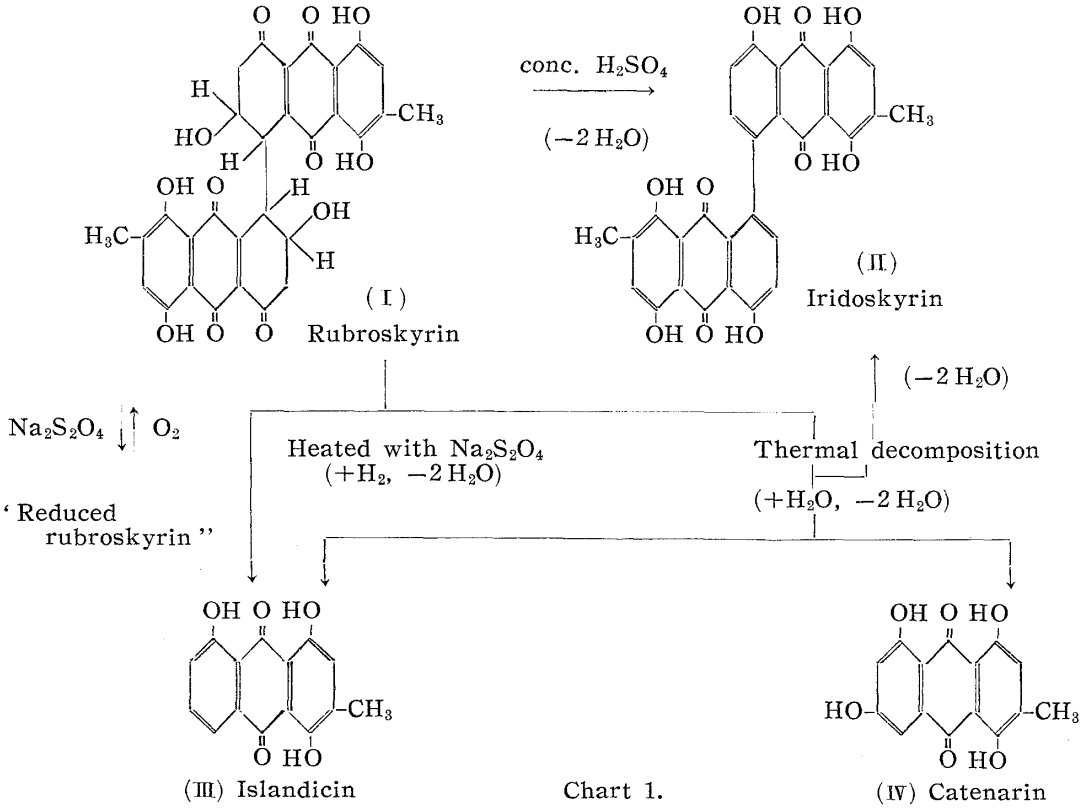


Chart 1.

* Part IX. This Bulletin, **4**, 303(1956)

** Hongo, Tokyo (柴田承二, 北川 勲).

1) B. H. Howard, H. Raistrick : Biochem. J. (London), **57**, 212(1954).
2) S. Shibata, M. Takido, T. Nakajima : This Bulletin, **3**, 286(1955).
3) S. Shibata, T. Murakami, I. Kitagawa, T. Kishi : *Ibid.*, **4**, 111(1956).
4) J. Breen, J. C. Dacre, H. Raistrick, G. Smith : Biochem. J. (London), **60**, 618(1955).
5) S. Shibata, T. Murakami, O. Tanaka, G. Chihara, M. Sumimoto : This Bulletin, **3**, 274(1955).
6) S. Shibata, T. Murakami, M. Takido : *Ibid.*, **4**, 303(1956).

some remarkable differences in their properties.

A quinonoid structure of rubroskyrin, which was suggested by Howard and Raistrick[1] on obtaining "reduced rubroskyrin" by the action of sodium dithionite has really been proved by the green coloration with magnesium acetate in alcoholic solution, whereas it is not the case in rugulosin. Regarding the structure of rugulosin which has fully been elucidated in the preceding paper,[6] we suggest that rubroskyrin should be represented by structure (I) and all its reactions and properties can be explained by the scheme shown in Chart 1.

The infrared spectrum* of rubroskyrin (Fig. 1) showed a chelated quinonoid C=O band at 1623 cm^{-1} and a band at 1703 cm^{-1} indicative of a carbonyl stretching absorption, which is shifted toward higher frequencies than predicted for a six-membered α,β-unsaturated ring ketone, as represented in the 4 and 4′ positions in the structure (I). This may be explained by the interaction of ketones as demonstrated by Jones *et al.*[7] in steroidal ketones.

The presence of a non-chelated hydroxyl in the rubroskyrin molecule was also indicated in its infrared spectrum (3559 (weak), 3341 cm^{-1}).

The hydroxyl must be alcoholic, present in the 2 and 2′ positions as suggested by the formation of catenarin on thermal decomposition of rubroskyrin. On dehydration reaction the hydroxyls were readily eliminated to form iridoskyrin (II).

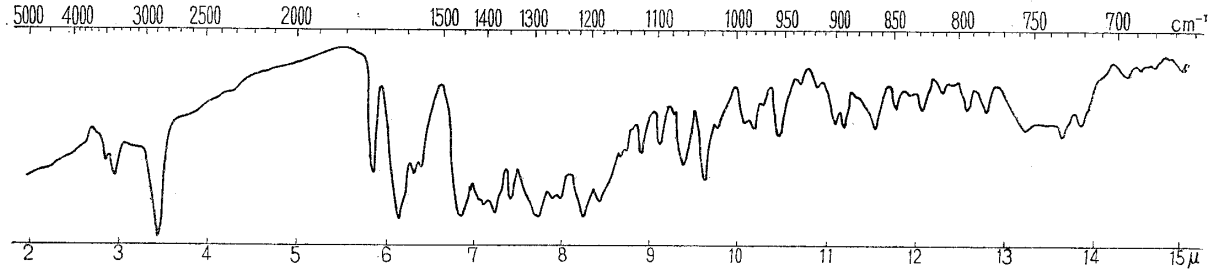


Fig. 1. Rubroskyrin

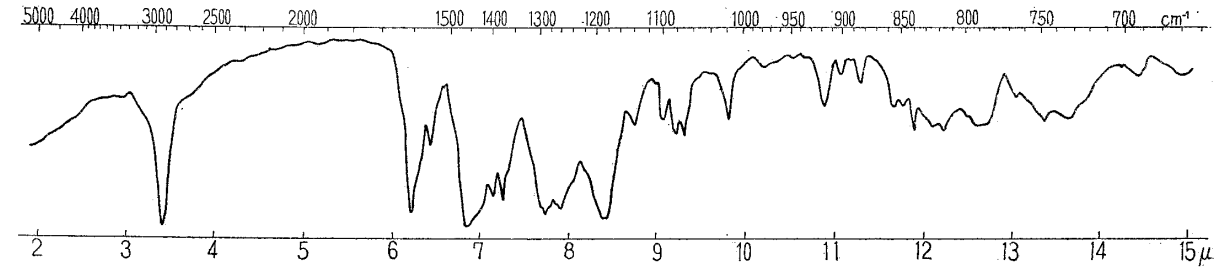


Fig. 2. Iridoskyrin

Luteoskyrin

Recently, the problem of the toxic effect caused by the moldy rice imported from Southeast Asian countries has seriously been discussed in this country and some toxic substances which give harmful effect in animal organs are reported to have been obtained from the cultures of some strains of *Penicillium islandicum* Sopp isolated from moldy rice grains.

A yellow coloring matter, m.p. 273°, $[\alpha]_D$ −880°, was proved by Yamamoto *et al.*[8] and almost simultaneously by Tatsuno *et al.*[9] as accounting for the toxic effect of

* All the infrared spectra cited in this paper were measured in Nujol mull.

7) R. N. Jones, K. Dobriner : J. Am. Chem. Soc., **72**, 956(1950).

8) Y. Yamamoto, T. Yamamoto, S. Kanamoto, Y. Tanimichi, K. Kikui : J. Pharm. Soc. Japan, **76**, 192(1956).

9) T. Tatsuno, M. Tsukioka, Y. Sakai, Y. Suzuki, Y. Asami : This Bulletin, **3**, 476(1955).

the mold. Comparing its properties and Rf value, it became almost certain that the toxic yellow pigment is identical with pigment-A, the occurrence of which in the culture of *P. islandicum* Sopp N.R.R.L. 1036 was indicated on paper chromatogram.[2] The formation of iridoskyrin by dehydration reaction and islandicin by reductive cleavage of pigment-A were shown microchemically.

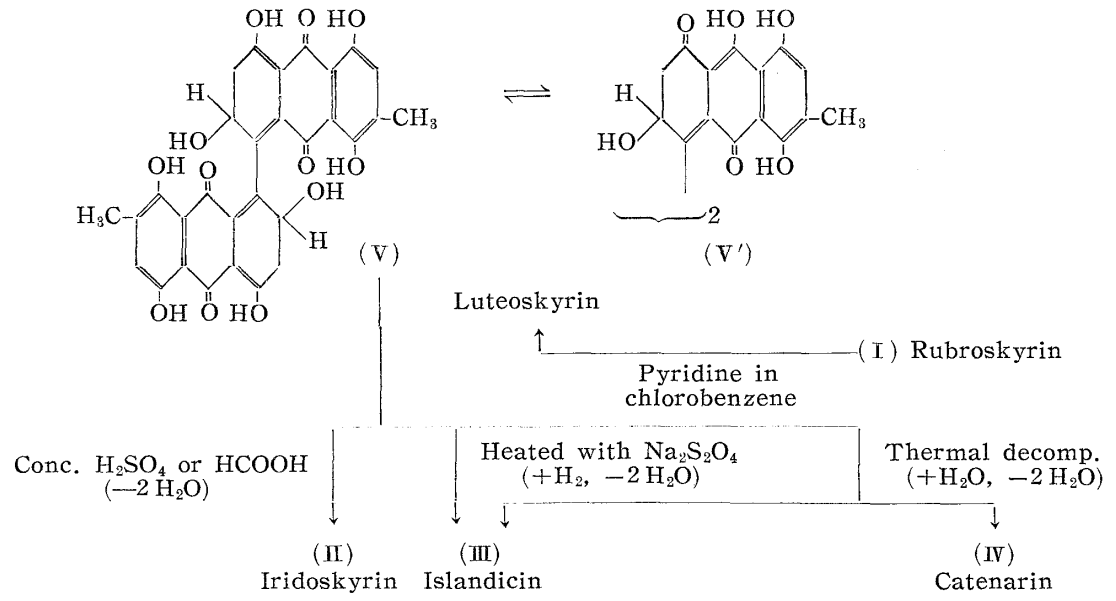
The chemical structure of the toxic yellow pigment, now being named luteoskyrin, has recently been studied by Yamamoto *et al.*,[10] and by Tastuno *et al.*[11] According to these workers, luteoskyrin, soluble in aqueous bicarbonate, yields islandicin and iridoskyrin by the action of conc. sulfuric acid or formic acid, and islandicin and catenarin by thermal decomposition. The infrared spectrum showed the presence of a chelated carbonyl (1623 cm^{-1}) and a hydroxyl (3378 cm^{-1}). Yamamoto *et al.*[10] proposed a structural formula, which, however, should be adopted for an enolized form of rubroskyrin. Similar to rugulosin and differing from rubroskyrin, luteoskyrin shows no marked color change in its alcoholic solution by the addition of magnesium acetate, suggesting the absence of the true quinone structure.

This and other behaviors against degradation and dehydration reactions showed that luteoskyrin should be a homolog of rugulosin with two more hydroxyls whose positions correspond to those in rubroskyrin, which should be a structural isomer of luteoskyrin.

A structural formula (V), therefore, is proposed to represent luteoskyrin, by which all its properties and reactions can reasonably be explained.

On the other hand, an additional proof for the structure (V) for luteoskyrin has been provided by isomerization of rubroskyrin into luteoskyrin, which can be effected by heating the former with pyridine in chlorobenzene by analogy of the conversion of 1-oxo- or 1-oxo-8-hydroxy-1,2,3,4-tetrahydroanthraquinone into 1-hydroxy- or 1,8-dihydroxy-2,3-dihydroanthraquinone (see formulae IXa, b and Xa, b in the preceding paper[6]).

In connection with the isomerization reaction of the model compounds, it was noted that 1-oxo-8-hydroxy-1,2,3,4-tetrahydroanthraquinone is very unstable and readily



10) Y. Yamamoto, T. Yamamoto, S. Kanamoto, Y. Tanimichi, K. Kikui : J. Pharm. Soc. Japan, **76**, 670(1956).

11) T. Tatsuno *et al.* : Paper presented before the 76th Annual Meeting of the Pharmaceutical Society of Japan at Fukuoka, April 7, 1956.

convertible into the nonquinonoid isomer, even by the mild recrystallization process. This may provide an explanation for the stability of rugulosin and may suggest that the quinonoid isomer of rugulosin, which would correspond to a two hydroxylless-homolog of rubroskyrin, would scarecely occur in nature. The presence of fully hydrogen-bonded ketones which constitute the quinonoid structure would make rubroskyrin stable enough to exist in nature, though it can be isomerized by prolonged heating with pyridine in chlorobenzene into nonquinonoid luteoskyrin.

Luteoskyrin is very sensitive to light, being converted into a deep brownish red colored quinonoid compound which gives a distinct sky-blue coloration with magnesium acetate in alcohol, and exhibits a nonchelated C=O band (1690 cm^{-1}) along with the initially existing chelated C=O(1620 cm^{-1}) absorption in its infrared spectrum. The mechanism of the photoxidation which would cause a highly extended conjugation has not yet fully been investigated.

We wish to thank Prof. H. Raistrick, Dr. B. H. Howard, and Mr. G. Smith for their kind help in supplying the mold strains and the samples of pigments, and agreeing to the continuance of the work on rubroskyrin, the earlier study of which was carried out by them. We are also grateful to Dr. T. Tatsuno, Pharmacological Department of this University, for giving us a sample of luteoskyrin. The infrared spectra were taken by Mr. H. Shindo of the Takamine Research Laboratory, Sankyo Co. Ltd., and microanalyses were carried out by the members of the microanalytical laboratories of this Institute, to all of whom our thanks are due.

Experimental

Rubroskyrin—The dried mycelium of *Penicillium islandicum* Sopp, N.R.R.L. 1036 strain incubated on Czapek-Dox solution for 3 weeks at 25° was extracted successively with ether and acetone. The extract was chromatographed on a column of $CaHPO_4$ (pretreated with H_3PO_4) developing with $CHCl_3$, when the mixture of pigments was separated into several following bands, from bottom to top : (i) Orange (islandicin), (ii) purple (iridoskyrin), (iii) yellow (erythroskyrin ?), (iv) light pink (catenarin), (v) yellow (luteoskyrin), (vi) brownish red (rubroskyrin), (vii) yellowish orange (skyrin).

From the brownish red band, crude rubroskyrin was obtained by elution in 10% yield of the pigment mixture and 1% of the weight of the dried mycelium. The crude rubroskyrin was chromatographed repeatedly (4 times) as above and the purified pigment was isolated in brownish red crystals, m.p. 273°(decomp.),* by repeated crystallization from EtOH. The measurement of optical rotation of rubroskyrin was difficult because of its deep red color in solution.

Rubroskyrin dissolves in EtOH, acetone, $CHCl_3$, and dioxane, sparingly in benzene and ether. It is readily soluble in 2*N* Na_2CO_3 and 2*N* NaOH forming a green solution, and slightly soluble in 5% $NaHCO_3$.

On addition of $Na_2S_2O_4$ to the alkaline solution, the green color turns into orange red which on bubbling air regenerates the original green coloration. It gives an emerald green coloration with $Mg(OAc)_2$ in EtOH. *Anal.* Calcd. for $C_{30}H_{22}O_{12}$: C, 62.72; H, 3.83. Found : C, 62.35; H, 4.03.

On standing in cold conc. H_2SO_4, rubroskyrin which dissolves at first to form a red solution is converted into iridoskyrin giving an intense blue color.

Formation of Islandicin from Rubroskyrin—A solution of a small amount of rubroskyrin in aq. 2*N* Na_2CO_3 was added with $Na_2S_2O_4$ heating on a boiling water bath, when the original green color of the solution changed to red. After 25 mins.' heating, aq. 2*N* NaOH was added to the mixture when the brownish precipitate dissolved to form a purple solution. After further 5 mins.' heating, the solution was cooled and neutralized with 2*N* HCl. The orange red precipitate thus formed was extracted with $CHCl_3$. The $CHCl_3$ extract was developed on paper chromatogram using upper layer of a mixture of acetone, benzine, and water (5 : 5 : 3.5), NH_4OH-saturated BuOH, or petroleum ether. Islandicin was confirmed by the comparison of its Rf value with that of the authentic sample.

Conversion of Rubroskyrin into Luteoskyrin—Rubroskyrin (100 mg.) was suspended in chlorobenzene (10 cc.) added with 3 drops of pyridine and the mixture was refluxed for 2 hrs. shielded from light. The color of the mixture changed from reddish brown to yellowish brown, a small amount of a precipitate of rubroskyrin remaining unchanged. The paper chromatogram of the reaction mixture developed with the upper layer of a mixture of acetone, benzine, and water (5 : 5 : 3.5) show-

* Howard and Raistrick recorded that rubroskyrin softened at 270° and melted at 289~290° under vigorous effervescence and blackening.

ed the spots of islandicin, iridoskyrin, catenarin, luteoskyrin (the main part), and rubroskyrin.

The reaction mixture was diluted with $CHCl_3$ and chromatographed on $CaHPO_4$, shielded from light, developing with $CHCl_3$, when it separated into the following six bands (from bottom to top): (i) Islandicin, (ii) iridoskyrin, (iii) catenarin, (iv) luteoskyrin (the main part), (v) rubroskyrin, and (vi) an unidentified purple band. The yellow band of luteoskyrin was chromatographed repeatedly and yellow crystalline substance obtained was recrystallized from EtOH to give yellow needles, m.p. 273°(decomp.). The identification of the product with luteoskyrin was made by the comparison of their infrared spectra.

Luteoskyrin is laevo-rotatory, $[\alpha]_D^{25}$ −880°(0.1% in acetone), soluble in 5% $NaHCO_3$, 2*N* Na_2CO_3, and 2*N* NaOH. It is readily soluble in acetone, less soluble in $CHCl_3$ and benzene. It gives no marked change of color in ethanolic solution by the addition of $Mg(OAc)_2$. Luteoskyrin is photosensitive and color of its solution in organic solvent changes into pink red on standing in light. Irradiated luteoskyrin, m.p. $>$ 370°, thus obtained showed a sky-blue coloration with $Mg(OAc)_2$ in EtOH, indicating the presence of a quinone structure in its molecule.

Summary

i) A possible structural formula of rubroskyrin (I) was forwarded from its relation to the structure of rugulosin, which was discussed in the preceding paper[6] and by the result of infrared spectral analyses.

ii) Rubroskyrin was converted into luteoskyrin by the action of pyridine in chlorobenzene.

iii) In relation to rubroskyrin and rugulosin, the structure of luteoskyrin was discussed, for which the formula (V) has been proposed.

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