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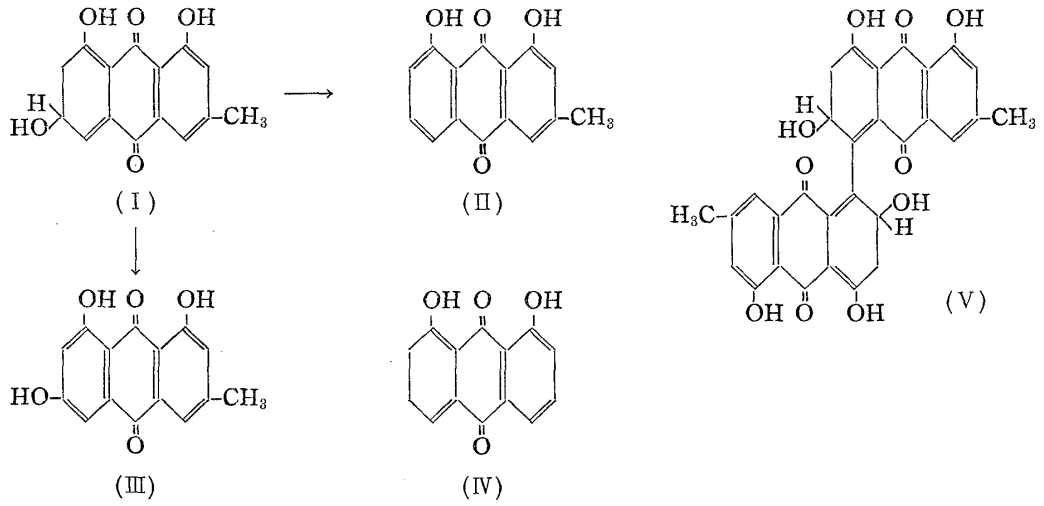
# 146. Shoji Shibata, Tetsuro Ikekawa,[*1] and Teruo Kishi[*2] : Metabolic Products of Fungi. XVII.[*3] The Structure of Flavoskyrin. (2).[*4]

(*Faculty of Pharmaceutical Sciences, University of Tokyo*[*1])

A yellow pigment, flavoskyrin, m.p. 208°(decomp.), was isolated from *Penicillium islandicum* Sopp NRRL-1175, accompanying erythroskyrin, chrysophanol, skyrin, oxyskyrin, and pigment C (skyrinol).[1,2]

Howard and Raistrick[1] who first isolated flavoskyrin proposed a molecular formula $C_{15}H_{12}O_5$ for it and they stated that it gave no crystalline derivative, while it yielded chrysophanol (II) on dehydration reaction.

In a previous report,[*4] the structural formula (I) was forwarded for flavoskyrin which was mainly deduced from the infrared spectral analysis and on analogy of the structure of rugulosin (V).




Since flavoskyrin, as in the case of rugulosin, showed no coloration with magnesium acetate, the presence of a true quinone structure in its molecule was excluded. However, it was found later that the spot of flavoskyrin on the paper chromatogram sprayed with methanolic solution of magnesium acetate changed its original yellow color gradually into intensive orange on standing for a week. It seemed that flavoskyrin was converted into a true quinone compound on exposure to air.

Subsequently, this reaction was confirmed on keeping a mixture of flavoskyrin and magnesium acetate in methanol at 25° for 5 days, when emodin was produced in 10% yield. This reaction indicated that flavoskyrin is converted into emodin by the catalytic air oxidation of alcoholic hydroxyl group into keto group to complete aromatization. Thus, the presence of alcoholic hydroxyl group in flavoskyrin was established.

The ultraviolet absorpiton curve of flavoskyrin measured in alkaline solution almost completely overlapped that of 1,8-dihydroxy-2,3-dihydroanthraquinone[*4] (IV). This experi-

*1 Hongo, Tokyo (柴田承二, 池川哲郎).
*2 Present address : Tokyo Research Laboratory, Kyowa Hakko Co. Ltd., Shibuya, Tokyo (貴志光雄).
*3 Part XVI. S. Shibata, I. Kitagawa : This Bulletin, 8, 884(1960).
*4 Part (1). S. Shibata, T. Murakami, M. Takido : *Ibid.*, **4**, 303(1956).
1) B. H. Howard, H. Raistrick : Biochem. J., **56**, 56(1954).
2) S. Shibata, M. Takido, T. Nakajima : This Bulletin, **3**, 286(1955).

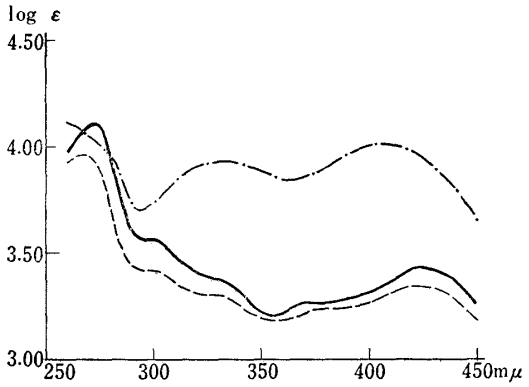



Fig. 1.
0.01*N* NaOH in dioxane
——— Flavoskyrin
– – – – Dihydrochrysazin
—·—·— Rugulosin

mental evidence also supported the correctness of the proposed structure of flavosyrin (I).

By the action of chromic acid or active manganese dioxide on flavoskyrin in attempting to oxidize the alcoholic hydroxyl into a ketone, the expected product, emodin, was not obtained, but a bimolecular coupled compound, dianhydrorugulosin (1,1′-bis(4,5-dihydroxy-7-methylanthraquinone)) was unexpectedly produced as a main product separated by chromatography on calcium hydrogenphosphate column.

On treatment with alkali, flavoskyrin was proved to be converted into a bianthraquinone derivative which was shown by paper chromatography to be similar to skyrin giving the same Rf value and coloration with magnesium acetate and conc. sulfuric acid.

It is noteworthy that flavoskyrin undergoes an oxidative coupling to form bianthraquinone derivative, in connection with the biogenetic relationship between flavoskyrin and other coexisting bianthraquinones and related partially hydrogenated bianthraquinones.

## Experimental

**Formatiom of Emodin from Flavoskyrin**—Flavoskyrin (50 mg.) was mixed with a saturated MeOH solution of $Mg(OAc)_2$ and the mixture was allowed to stand at 25° for 5 days. The solvent was removed and the residue was paper chromatographed, using the upper layer of the mixture of $Me_2CO$–benzine–$H_2O$ (5:5:3.5) as the developing solvent, and gave a spot at Rf 0.95. When benzine (b.p. 60～70°) saturated with 96% MeOH was used, the Rf value was 0.3.

The residue was treated with $Et_2O$ to separate $Mg(OAc)_2$, the $Et_2O$-soluble portion was dissolved in benzene, and chromatographed on a $CaHPO_4$ column to remove chrysophanol which was recovered from the first eluate. The developing solvent was altered to benzene–$Me_2CO$ (10:1) and from the second band emodin was obtained, which was proved by a mixed fusion with the authentic sample (m.p. and mixed m.p. 254°). Yield, 7 mg.

**Molecular Weight of Flavoskyrin**—The molecular weight of flavoskyrin was determined by Barger-Akiya's method using tetrahydrofuran as the solvent and azobenzene as the standard. Calcd. for $C_{15}H_{12}O_5$ : Mol. wt., 272. Found : Mol. wt., 309, 257, 296.

**Formation of Dianhydrorugulosin from Flavoskyrin**—a) To a solution of flavoskyrin (100 mg.) in pyridine (6 cc.), a mixture of $CrO_3$ (50 mg.) and pyridine (0.5 cc.) was added. After 3 hr., the mixture was poured into ice-water and the precipitate formed was collected. The product was chromatographed on a $CaHPO_4$-column using a mixture of benzene and $Me_2CO$ as a solvent. The lowest broadest band yielded yellow crystals which were identified by mixed fusion as dianhydrorugulosin (1,1′-bis(4,5-dihydroxy-7-methylanthraquinone)), m.p. and mixed m.p. 323°.

b) To a solution of flavoskyrin (200 mg.) in dioxane (15 cc.), active $MnO_2$ (the activity tested with benzyl alcohol) was added and the mixture was stirred for 30 min. at room temperature. The reaction mixture was filtered to remove $MnO_2$ and the solvent was distilled off *in vacuo.* On paper chromatogram, the residue gave the spots of unchanged flavoskyrin, chrysophanol, and two other unidentified spots.

The benzene-soluble portion of the product was chromatographed on a $CaHPO_4$-column using benzene as a solvent and yielded chrysophanol, dianhydrogulosin, and an unidentified yellow band.

The portion which was sparingly soluble in benzene was dissolved in $Me_2CO$ and chromatographed on $CaHPO_4$, washing with benzene to be separated into 9 bands. Chrysophanol was obtained from the lowest band and dianhydrorugulosin (m.p. and mixed m.p. 323°) from the broadest second band.

**Reaction of Alkali on Flavoskyrin**—Flavoskyrin (150 mg.) was dissolved in *N* methanolic NaOH (or KOH) and instantly formed a red solution, which was allowed to stand overnight at room temperature. The solution was diluted with $H_2O$ and MeOH was distilled off. The residual solution was acidified with HCl and shaken with $Et_2O$. The $Et_2O$ layer was washed with $Na_2CO_3$ solution and $H_2O$, and evaporated.

The residue dissolved in benzene was chromatographed on $CaHPO_4$ to separate chrysophanol which was recovered from the lowest band. The column was washed with a mixture of benzene and $Me_2CO$ (10:1) and orange red crystals were obtained from the second band. The crystals gave the same Rf value as skyrin on paper chromatogram and showed a red $Mg(OAc)_2$ reaction and a characteristic coloration (red to emerald green) with conc. $H_2SO_4$, as given by skyrin.

On treatment with alkaline $Na_2S_2O_4$, the red crystals yielded emodin as from skyrin by the same treatment. However, the final establishment of identity of the product with skyrin failed due to shortage of the material.

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## Summary

Emodin was obtained by the air-oxidation of flavoskyrin in the presence of magnesium acetate, which gave an evidence for the proposed structural formula (I). By the action of chromium trioxide or active manganese dioxide, flavoskyrin yielded dianhydrorugulosin which resulted by oxidative coupling.

In methanolic alkaline solution flavoskyrin was converted into a skyrin-like compound.

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