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129. Tetsuro Ikekawa[*1] : Isoskyrin.

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Previously Shibata, Ikekawa and Kishi[1] reported that flavoskyrin (I), a pigment of *Penicillium islandicum* Sopp afforded dianhydrorugulosin (II) by the oxidative coupling accompanying dehydration, and yielded skyrin-like compound on treatment with methanolic potash. These results suggested that the bimolecular anthraquinones and their related mold pigments, such as skyrin[2] (III), luteoskyrin,[3] rubroskyrin[3] and rugulosin[4] would be biosynthesized from the preformed monomolecular precursors in the mold cells by the similar scheme of biological oxidation.

The present paper concerns with an experiment of oxidative coupling of emodin, which would provide an additional evidence for the formation of dimeric mold pigments.

After several unsuccessful attempts, it has been found that a small amount of the dimeric product was formed when oxygen was introduced into the alkaline solution of emodin. The same product was also yielded by the treatment of emodin with silver oxide. The dimeric product of emodin, $C_{30}H_{18}O_{10}$, orange red crystals, m.p. >360° from pyridine, shows almost the same properties as those of skyrin (diemodin (8,8′)) forming a red solution with caustic alkali and giving a red coloration with magnesium acetate in alcohol.

It should be noted particularly that the dimeric product of emodin gave a same change of color, red to emerald green in concentrated sulfuric acid, as shown by skyrin. Thus the product has now been named isoskyrin.

The ultraviolet spectra of isoskyrin and skyrin showed almost superimposable but slightly different curves (Fig. 1). Isoskyrin $\lambda_{\max}^{\mathrm{EtOH}}$ mμ (log ε) : 218.5 (4.83), 254 (4.59), 287 (4.63), 450 (4.36); Skyrin $\lambda_{\max}^{\mathrm{EtOH}}$ mμ (log ε) : 220 (4.94), 257.5 (4.84), 299 (4.63), 462.5 (4.45).

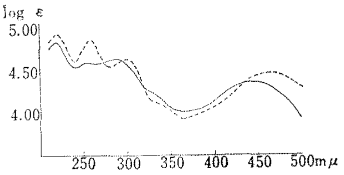

Fig. 1.

—— Isoskyrin
- - - Skyrin

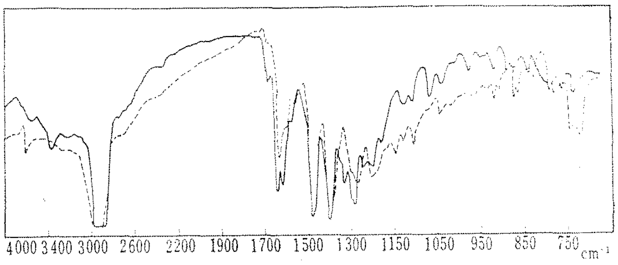

Fig. 2.

—— Isoskyrin
- - - Skyrin (in Nujol)

The infrared spectral curves of the both compounds also gave almost the same absorption bands at the functional group region, but a slight difference was observed at the finger print region (Fig. 2). Isoskyrin : IR $\nu_{\max}$ cm^{-1} : 1670 (non-chelated C=O); 1625 (chelated C=O); 3550, 3360 (OH).

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1) S. Shibata, T. Ikekawa, T. Kishi : This Bulletin, 8, 889 (1960).

2) S. Shibata, O. Tanaka, I. Kitagawa : *Ibid.*, 3, 278 (1955); O. Tanaka, C. Kaneko : *Ibid.*, 3, 284 (1955).

3) S. Shibata, I. Kitagawa : *Ibid.*, 4, 309 (1956); *Ibid.*, 8, 884 (1960).

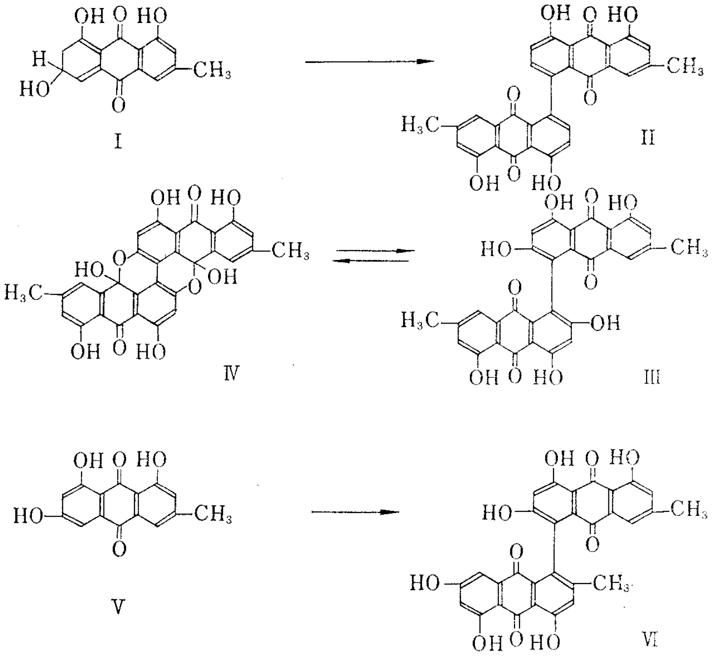
4) S. Shibata, T. Murakami, M. Takido : *Ibid.*, 4, 303 (1956).

Isoskyrin afforded hexaacetate $C_{42}H_{30}O_{16}\cdot H_2O$, m.p. 152° (decomp.) whose molecular weight determined by the Berger-Akiya method showed that it is a dimer of emodin triacetate.

On reductive cleavage with alkaline sodium dithionite, isoskyrin yielded emodin quantitatively.

The characteristic red to green color change in concentrated sulfuric acid suggested that isoskyrin is converted into a ketal type compound as being established by the formation of pseudoskyrin[5] (IV) from skyrin (III). This reaction showed the presence of at least one hydroxyl at the β-position which situates at the *ortho*-position of α,α'-C-C- linkage of bianthraquinone, and another free hydroxyl at the *para*-position of the linkage.

These results have reached a conclusion that isoskyrin is an assymmetric dimer of emodin as being represented by the formula, 2,4,4′,5,5′,7′-hexahydroxy-2′,7-dimethyl-1,1′-bianthraquinone (VI).



Experimental

Isoskyrin——i) A solution of emodin (3 g.) dissolved in 0.1*N* NaOH (600 cc.) was refluxed for 50 hr. under O_2 stream. The reaction mixture was neutralized with dil. HCl to obtain the precipitates which were washed with water and chromatographed on $CaHPO_4$ column using benzene-Me_2CO (10:1) as the developing solvent. From the first eluate emodin (330 mg.) was recovered, and from the third band isoskyrin (80 mg.) was obtained. The crude isoskyrin was purified repeatedly by the $CaHPO_4$ column chromatography and finally recrystallized from pyridine to obtain red crystals. The red crystals liberated pyridine on warming with EtOH or Me_2CO to give orange red crystals.

ii) A mixture of emodin (1.5 g.) and Ag_2O (5 g.) in EtOH (200 cc.) was refluxed for 20 hr. Ag_2O was removed by filtration and the solvent was distilled off. The residue was chromatographed on a $CaHPO_4$ column using benzene-Me_2CO (10:1) to separate first eluate from which emodin (320 mg.) was recovered. The remaining part was eluted out with AcOEt, and chromatographed again on the $CaHPO_4$ column using benzene-Me_2CO (30:1) as the solvent. Isoskyrin (75 mg.) was obtained from the eluate by the recrystallization as described above. The paper chromatographical examination of the products gave no evidence of formation of skyrin. Isoskyrin, darkens from 220°, but does not melt under 360°. It dissolves in aq. NaOH and NH_4OH to give deep violet. In Na_2CO_3, it is soluble to give red color. On

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[5] O. Tanaka : This Bulletin, **6**, 203 (1958).

addition of conc. H_2SO_4, isoskyrin gives a red color which however turns into emerald green within a few seconds. $(AcO)_2Mg$ in alcohol shows a red coloration and $FeCl_3$ reaction gives a brown color. On heating isoskyrin in 40% NaOH, or on treatment with conc. HCl, no remarkable change was observed, whereas skyrin was affected by these treatment. The paper chromatography using upper layer of Me_2CO-petr. benzin-H_2O (5:5:3.5) gave Rf 0.38 (skyrin gave Rf 0.42). Isoskyrin is soluble in dioxane, tetrahydrofuran and Me_2CO, but sparingly soluble in AcOEt, EtOH, MeOH, Et_2O, $CHCl_3$, benzene, glac. AcOH and hexane. *Anal.* Calcd. for $C_{30}H_{18}O_{10}\cdot 2H_2O$: C, 62.38; H, 3.84. Found : C, 62.14, 62.69; H, 4.05, 4.11.

Emodin was recovered unchanged in the following attempts :

i) Refluxing the solution of emodin in abs. xylene with anhydrous $FeCl_3$ for a few hours.
ii) Refluxing the solution of emodin in glac. AcOH with $FeCl_3\cdot 6H_2O$ for 2 hr.
iii) Refluxing the solution of emodin in xylene with chloranil for 16 hr.
iv) Introducing air into a mixture of emodin, arabic gum, ascorbic acid and $FeSO_4\cdot 7H_2O$ in phosphate buffer at 50~80° for 2 hr.
v) Refluxing the solution of emodin in aq. tetrahydrofuran with $K_3Fe(CN)_6$ for 10 hr.
vi) Standing the Me_2CO solution of emodin with the addition of periodate at acidic, neutral or alkaline state.

Emodin was reacted by refluxing in pyridine-piperidine (10:1) solution with the addition of aq. KOH to give a few spots on paper chromatogram, but the products were not determined.

O-Hexaacetylisoskyrin was obtained by the acetylation of isoskyrin with Ac_2O and pyridine. The product was purified by the $CaHPO_4$-column chromatography using benzene as the solvent, and recrystallized from EtOH-Me_2CO to give pale yellow needles, m.p. 152°(decomp.). *Anal.* Calcd. for $C_{42}H_{30}O_{16}\cdot H_2O$: C, 62.38; H, 3.99. Found : C, 62.45; H, 4.01. Mol. wt. Determination by the Berger-Akiya method : using tetrahydrofuran as the solvent; Calcd. Mol. wt. : 809. Found : 636~848. IR $\nu_{\max}^{\text{Nujol}}$ cm^{-1} : 1673 (non-chelated C=O), 1782 (acetate C=O).

Reductive Cleavage of Isoskyrin into Emodin——To a solution of isoskyrin (15 mg.) dissolved in 0.1*N* NaOH, $Na_2S_2O_4$ (ca. 15 mg.) was added, and the mixture was heated on a boiling bath for 30 min. The mixture was neutralized with dil. HCl cautiously after cooling, and exhaustively shaken with Et_2O. The product was purified by the $CaHPO_4$ column chromatography using benzene-Me_2CO (10:1) followed by sublimation in vacuum and recrystallization from MeOH to give crystals of emodin (11 mg.), which was identified by a mixed fusion with authentic sample. (m.p. and mixed m.p. 253~254°).

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Summary

Emodin afforded a dimeric product, 2,4,4′,5,5′,7′-hexahydroxy-2′,7-dimethyl-1,1′-bianthraquinone, which was named isoskyrin, on oxidative coupling by introducing oxygen in the alkaline solution or by the action of silver oxide.

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