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Skyrin from Two Lichen Species

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Skyrin (I) is an orange coloring substance and has been known to occur fairly often as the fungal metabolite.[2] In 1968, Ogihara, Kobayashi, and Shibata demonstrated[3] for the first time the optical activity due to the restricted rotation of skyrin isolated from a strain of *Penicillium islandicum* Sopp, and they proposed a prefix (+) to skyrin exhibiting (+) Cotton effect at the highest wave length region of the optical rotatory dispersion (ORD) curve, so that possessing the R configuration at the C-C linkage. On the other hand, the occurrence of (+)-skyrin along with some other phenolic metabolites in a lichen, *Acroscyphus sphaerophoroides* Lév, has been reported by Shibata and his co-workers,[4] and this corresponds to the first example of the pigment elucidated in the lichen family. Recently, Santesson[5] has also reported the isolation of (+)-skyrin from a crustaceous lichen *Trypetheliopsis boninensis* Asah. in addition to two bisanthraquinone pigments, oxyskyrin[6] and skyrinol.[7]

During the course of the investigation on the lichen phenolics,[8] we have examined the coloring substances of two lichens, *Physcia* cf. *obscura* Nyl. var. *endococcina* Th. Fr. and *Pyxine endochrysina* Nyl. and elucidated the component as described in the present paper.

The orange needles mp >360°, obtained from the lichen thalli of *Physcia* cf. *obscura* Nyl. var. *endococcina* Th. Fr. by the successive extraction with ether and acetone followed by column chromatography and recrystallization, exhibits the hydroxyl and quinonoid carbonyl absorption bands (3450, 1674, and 1621 cm^{-1}) in the infrared (IR) spectrum (KBr). The crystals showed the pink coloration with $Mg(OAc)_2$ suggesting the existence of the chelated quinonoid structure.[9] On treatment with conc. H_2SO_4, they colored red at the beginning and then immediately the color turned to green, being reminiscent of the characteristic coloration of skyrin.[10] On the basis of the above evidences, the direct comparison of the substance with authentic skyrin was performed and the identity of both has been established through thin-layer chromatography (TLC) and IR.

Skyrin thus identified here showed no optical activity. In spite of the careful re-examination, for instance, under the low temperature condition, it has been unsuccessful to isolate optically active skyrin from the above lichen.

Furthermore, as demonstrated by Shibata and his co-workers[3,4,6,7], skyrin has quite often been accompanied by the related anthraquinone or protoanthraquinone derivatives

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[1] Location: *Toneyama, Toyonaka, Osaka.*

[2] S. Shibata, S. Natori, and S. Udagawa, "List of Fungal Products," University of Tokyo Press, Tokyo, Japan, 1964, p.82.

[3] Y. Ogihara, N. Kobayashi, and S. Shibata, *Tetrahedron Letters*, **1968**, 1881.

[4] S. Shibata, O. Tanaka, U. Sankawa, Y. Ogihara, T. Takahashi, S. Seo, D. Yang, and Y. Iida, *Journ. Jap. Bot.*, **43**, 335 (1968).

[5] J. Santesson, *Acta Chem. Scand.*, **24**, 3331 (1970).

[6] S. Shibata, M. Takido, A. Ohta, and T. Kurosu, *Pharm. Bull.* (Tokyo), **5**, 573(1957).

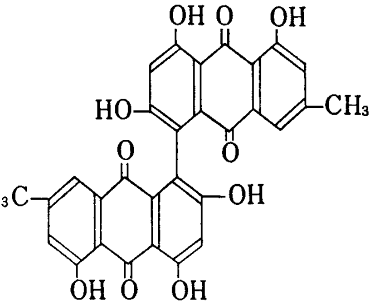
[7] S. Shibata, M. Takido, and T. Nakajima, *Pharm. Bull.* (Japan), **3**, 286(1955).

[8] The preceding paper on the subjects: I. Yosioka, K. Hino, M. Fujio, and I. Kitagawa, *Chem. Pharm. Bull.*(Tokyo), 19, 1070(1971), and the literatures cited therein.

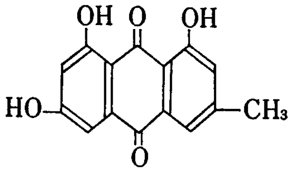
[9] S. Shibata, M. Takido, and O. Tanaka, *J. Am. Chem. Soc.*, **72**, 2789 (1950).

[10] S. Shibata, O. Tanaka, and I. Kitagawa, *Pharm. Bull.*(Japan), **3**, 278(1955).

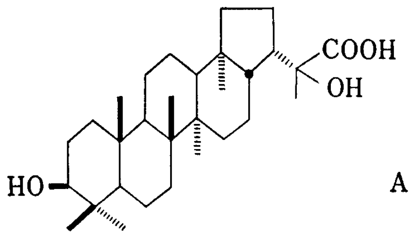
as the fungal or lichen metabolites. Therefore, the further survey in pursuit of these substances was performed. However, only a trace amount of emodin (II) was detected by TLC using three different kinds of the solvent systems.



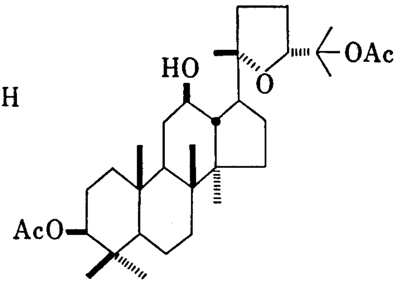
I : skyrin



II : emodin



III: pyxinic acid



IV : diacetylpyxinol

Next, the coloring substance of the lichen thalli of *Pyxine endochrysina* Nyl. has been examined, since only the triterpenoid constituents, such as pyxinic acid (III)[11] and diacetylpyxinol (IV),[12] have hitherto been elucidated from the lichen. In this case, skyrin was isolated as the sole coloring matter by virtue of polyamide column and preparative TLC separation, and it is interestingly pointed out that skyrin thus obtained possesses (+) Cotton effect ($[\theta]_{481}+2080$ (dioxane)). Judging from the molecular ellipticity,[3] the partial racemization during the isolation procedure is suspected.

The present results offer the third and fourth examples of the occurrence of skyrin in the lichen family.

Experimental[13]

Isolaion of Skyrin from *Physcia cf. obscura* Nyl. var, *endococcina* Thfr.——During the successive extraction of the lichen thalli(powdered, 10 g, collected at Nose in Osaka prefecture) with ether, was precipitated the orange needles (almost pure skyrin, 103 mg), which was collected by filtration. Further extraction with ether followed by acetone extraction, the additional amount (250 mg) of the extract was obtained. The latter extract was then purified by column chromatography with silica gel(Merck, 32 g) eluting with (i) $CHCl_3$ and (ii) $CHCl_3$–MeOH(30: 1). The eluate of (ii) furnished skyrin(66 mg) by recrystallization with acetone. Combined skyrin(169 mg) was further recrystallized with pyridine to afford a dark red complex, from which pure I was liberated by refluxing with acetone. mp>360°, IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3450, 1674, 1621. Identification with authentic skyrin by provided Prof. Shibata was accomplished by IR(KBr) and TLC using three solvent systems(*Rf* values in the parentheses): $CHCl_3$–MeOH(1:1) (0.58); acetone–benzene(1:4)(0.48); petr. benzine–ether(1:4) (0.51). The attempt to isolate optically active skyrin was also made, for instance, extracting at

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11) I. Yosioka, A. Matsuda, and I. Kitagawa, *Tetrahedron Letters*, **1966**, 613.

12) *a*) I. Yosioka, H. Yamauchi, and I. Kitagawa, *Tetrahedron Letters*. **1969**, 4241; *b*) H. Yamauchi, T. Fujiwara, and K. Tomita, *ibid*., **1969**, 4245.

13) The IR spectra were obtained with the Hitachi Infrared Spectrophotometer EPI-2 and the CD curve was taken on the JASCO UV/ORD-5 Automatic Recording Spectropolarimeter.

low temperature followed by TLC separation and recrystallization (without using pyridine), and it was however without success. From the eluate of (i), there was obtained a trace amount of an orange pigment, which was identified with II by TLC using the above described solvent systems with the *Rf* values of 0.82, 0.64, and 0.76 respectively.

Isolation of (+)-Skyrin from *Pyxine endochrysina* Nyl.——Ether extract of the lichen thalli (air dried, 800 g, colllected at Kirii in Gifu prefecture) was treated with 10% aq. Na_2CO_3 and the aq. layer, after acidification with dil.HCl, was extracted again with ether to give an acidic portion(5.4 g). TLC disclosed the acidic portion to be a complex mixture of the rich component of triterpenoids containing III in addition to an orange spot(skyrin) and another uncharacterized minor yellow-orange elongated spot. Polyamide column (Wako Pure Chem. Ind., C-300) chromatography eluting with $CHCl_3$-MeOH(25: 1) followed by preparative TLC(Camag SiO_2, $CHCl_3$-MeOH(20: 1)) and recrystallization with $CHCl_3$ afforded crystals of mp>300° (19 mg, 0.0024% from the lichen), $Mg(OAc)_2$(red), conc. H_2SO_4(red to green), CD(c=0.81, dioxane)[θ](mμ): +2080(481)(positive maximum). The substance was identified with skyrin by TLC and IR(KBr) as above.

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