

Chemical Studies on Lichens

30.* Anthraquinonoid Pigments of *Trypetheliopsis boninensis*
and *Ocellularia domingensis*

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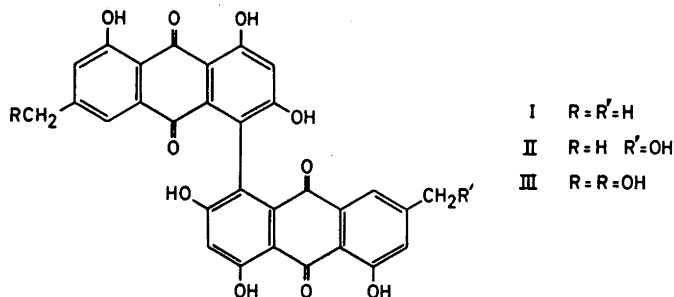
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Trypetheliopsis boninensis contains the bisanthraquinones (+)-skyrin, oxyskyrin, and skyrinol, the two latter pigments new for lichens. *Ocellularia domingensis* contains the anthraquinone endocrocin.

The crustaceous lichen *Trypetheliopsis boninensis* was originally described by Asahina, who reported¹ the presence of an unidentified yellow pigment.

The lichen has now been re-examined. Attempts to identify the pigment by lichen mass spectrometry² were unsuccessful, but by means of thin layer chromatography, the presence of three major and (at least) four minor pigments could be shown. The results are summarized in Table 1.

Because of the very small amount of lichen material available (100 mg), only (+)-skyrin (I), oxyskyrin (II), and skyrinol (III) could be isolated, each in about 1 % yield. (+)-Skyrin was identified by comparison with an authentic sample.



* Part 29: Santesson, J. *Phytochemistry*. In press.

Table 1. Pigments of *T. boninensis*.

| R_F in | | Colour | | | | Intensity | Identified as |
|-----------|------|-------------|----------------|------------------|--------------|-----------|---------------|
| BA | CA | in daylight | with H_2SO_4 | with $Mg(OAc)_2$ | with Et_3N | | |
| 0.58–0.62 | | pale yellow | <i>a</i> | <i>a</i> | <i>a</i> | very weak | |
| 0.45–0.55 | | yellow | | | | | |
| 0.38–0.41 | | orange | red → green | orange | wine red | strong | (+)-skyrin |
| | | pale yellow | reddish | faint | brownish | weak | |
| 0.20–0.23 | 0.75 | yellow | red | faint | | medium | |
| 0.12–0.14 | 0.45 | orange | red → green | orange | wine red | strong | oxyskyrin |
| 0.00–0.08 | 0.58 | yellow | red | faint | | weak | |
| | | | | orange | brownish | strong | skyrinol |
| 0.00–0.08 | 0.30 | orange | red → green | orange | wine red | | |

BA: silica gel H impregnated with 0.1 N oxalic acid; benzene-acetone 9:1 (v/v), two developments.⁵ CA: Eastman "Chromagram" 6060 (silica gel); chloroform-acetone 2:1 (v/v). ^a Too weak to be observed.

Oxyskyrin³ and skyrinol⁴ were tentatively identified by their chromatographic behaviour, colour reactions, *etc.* Verification could be obtained by reductive cleavage of the compounds with alkaline sodium dithionite solution.^{3,4} II afforded approximately equal amounts of emodin and ω -hydroxyemodin, while III gave only ω -hydroxyemodin.

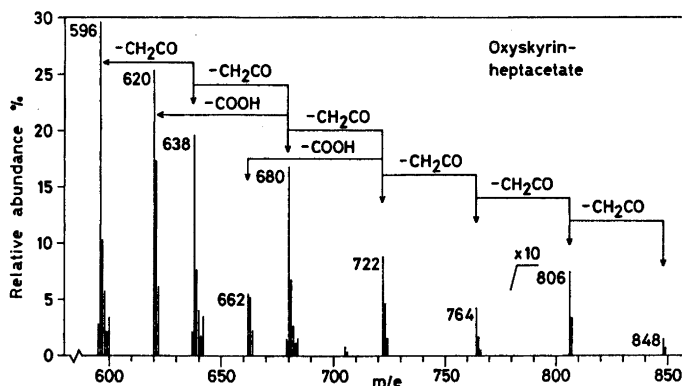


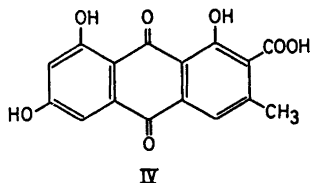
Fig. 1.

II was further identified by mass spectrometry of its heptacetate. The mass spectrum (Fig. 1) showed the expected successive loss of six ketene molecules (from the phenolic acetate units) together with loss of acetic acid or $CH_3COO\cdot$ (from the hydroxymethyl acetate unit).

The four minor pigments have not been identified. However, judging from their properties (including their involatility in the mass spectrometer) it seems likely that they are related to I–III.

Skyrin has recently been reported from the lichen *Acrosyphus sphaerophoroides* Lév.,⁵ where it occurs together with rugulosin. Oxyskyrin and skyrinol have not previously been found in lichens. They have, however, been reported from several non-lichenized fungi,^{6,7} e.g. *Penicillium islandicum*.

In *P. islandicum* Gatenbeck⁸ also found the anthraquinone endocrocin (IV), evidently very closely related to the assumed monomeric precursors⁷



of I–III. Endocrocin has been reported from only one lichen species: *Cetraria endocrocea* (Asah.) Sato (Parmeliaceae).

It has now also been found in *Ocellularia domingensis* (Nyl.) Müll. Arg. (Thelotremaceae). It was identified by lichen mass spectrometry² and thin layer co-chromatography. No traces of emodin or other anthraquinones could be detected in *O. domingensis*.

EXPERIMENTAL

Mass spectra were recorded with an LKB 9000 gas chromatograph-mass spectrometer using the direct inlet system: ion source temp. 290°, energy of the ion beam 70 eV, electron current 60 μ A.

The lichen material. Voucher specimens are to be found in the herbarium of Uppsala Botanical Museum (UPS). *Trypetheliopsis boninensis*, from Bonin Isl., collected in 1937, ref. designation Okabe (isotype). *Ocellularia domingensis*, Cuba, Wright Lich. Cubae 163.

Isolation of skyrin, oxyskyrin, and skyrinol. Dry *T. boninensis* (100 mg) was continuously extracted with acetone (10 ml) for 48 h. TLC of the crude extract in BA (see Table 1) gave three main and four minor coloured bands. The band with R_F 0.45–0.55 afforded (+)-skyrin (1.1 mg), m.p. above 350° (lit.⁶ 360°), identified by comparison TLC) with an authentic sample. The bands with R_F 0.12–0.14 and 0.00–0.08 were rechromatographed in CA (see Table 1) and afforded oxyskyrin (0.9 mg), m.p. above 350° (lit.⁶ 360°), and skyrinol (0.8 mg), m.p. above 350° (lit.⁶ 360°), respectively.

Alkaline cleavage. Oxyskyrin (0.4 mg) in 5% sodium carbonate (0.5 ml) was treated with an aqueous solution of sodium dithionite (0.1 g in 0.5 ml) according to the procedure of Shibata *et al.*¹⁰ TLC of the reaction product afforded approximately equal amounts of emodin and ω -hydroxyemodin, identified by comparison with authentic samples (TLC, MS).

In the same way skyrinol (0.4 mg) afforded only ω -hydroxyemodin, identified as above.

Oxyskyrinheptacetate was prepared directly in an MS direct inlet sample tube by treating oxyskyrin (0.1 mg) with acetic anhydride-pyridine (4:1, 5 μ l). MS see Fig. 1.

Endocrocin in O. domingensis. The lichen mass spectrum showed peaks at m/e 314 (5%), 296 (8%), and 270 (100%), and "metastable peaks" corresponding to the transi-

tions 314–296 (loss of H₂O) and 314–270 (loss of CO₂). TLC (in CA—see Table 1—and the solvent systems listed in Ref. 11) including co-chromatography with an authentic sample established the presence of endocrocin.

Acknowledgements. I wish to express my sincere thanks to Dr. Rolf Santesson for the gift of the lichens, and to Professor Shoji Shibata for the gift of a sample of (+)-skyrin. The costs of the investigation were defrayed by a grant from the *Swedish Natural Science Research Council* to Dr. Gerd Bendz at this Institute.

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Received April 1, 1970.