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ANTHRAQUINONES OF THE LICHENS OF *XANTHORIA* AND *CALOPLACA* AND THEIR CULTIVATED MYCOBIONTS*

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Key Word Index—*Xanthoria*; *Caloplaca*; Lichens; anthraquinone pigments; 2-chlorofallacinol.

Abstract—From the thalli of *Xanthoria mandschurica* erythroglaucin (I), parietin (II) and presumably erythroglaucin carboxylic acid were isolated, while from its cultivated mycobiont an unidentified anthraquinone was detected on TLC along with the above pigments. From the mycobiont of *X. fallax* fallacinal (III), emodin (IV) and fallacinol (V) were isolated along with I and II. From the thalli and the mycobiont of an unidentified *Caloplaca* species. fragilin (VI), 2-chloroemodin (VII), parietin (II) and emodin (IV) were isolated, and a new pigment, 2-chlorofallacinal, was also found in the thalli of this lichen.

ANTHRAQUINONE colouring matters are widely distributed in the lichens of *Xanthoria* and *Caloplaca* sp. (Teloschistaceae). The present paper is concerned with the pigments in *Xanthoria mandschurica*, *X. fallax* and an unidentified *Caloplaca* spp. and the production of colouring matters by the cultivated mycobionts isolated from these lichens.

Xanthoria mandschurica (Zahlbr.) Asahina was collected at two places in Nagano Pref. and near Tokyo. *Xanthoria fallax* (Hepp.) Arn. was collected at Ômiyama, Nagano Pref. The mycobionts were isolated from the fresh lichens and cultivated for 4–7 months in the laboratory. Yellow pigmentation was observed in the mycobionts from the second month of cultivation. The air dried thalli of the lichen and the colonies of mycobionts were extracted with ether and acetone, and the extracts were examined by TLC on silica gel impregnated with oxalic acid in benzene (Fig. 1).

The pigments were isolated by column chromatography on silicic acid, and pigment A of *Xanthoria mandschurica* was identified as erythroglaucin (I)[1] and pigment B as parietin (II). Spot A on TLC of the extract of *Xanthoria fallax* mycobiont seemed to be identical with I, but this could not be confirmed due to the poor yield. Pigment C, m.p, 244–246° (from acetone) gave a purple colour with magnesium acetate in ethanol, and the UV spectrum resembled that of erythroglaucin (λ_{max}^{dioxan} 307, 492 nm). The MS showed *m/e* 344 (M^+), 300 ($M\text{-}CO_2$) (base peak), 282, 270 and 260, and the IR absorptions appeared at 1720–1730 (weak) and 1605 cm^{-1}. These data suggest that pigment C is erythroglaucin carboxylic acid, with the carboxyl group being located adjacent to the methyl. Due to shortage of material, the structures of this compound and of pigment E (UV λ_{max}^{dioxan} 420 nm) from the mycobiont of *X. mandschurica* have not yet been confirmed.

* Part II in the series "Metabolism of Lichen Symbionts". For Part I see T. KOMIYA and S. SHIBATA, *Phytochem.* **10**, 695 (1971).

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Pigment D was identified as fallacinal (III)[2] and F and G as emodin (IV) and fallacinol V)[2] respectively. The occurrence of parietin, fallacinal and fallacinol in the lichen, *Xanthoria (allax*, has been reported by Murakami.[2]

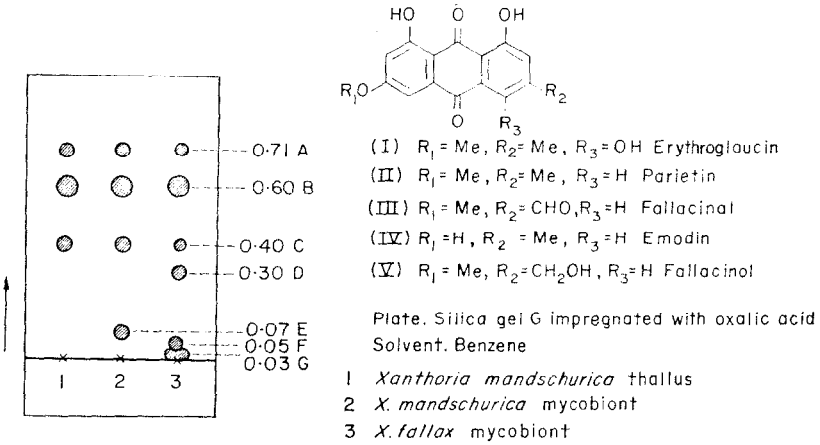

FIG. 1. SEPARATION OF ANTHRAQUINONES OF *Xanthoria* SPECIES.

A lichen belonging to the genus *Caloplaca*, collected at two places in Nagano and Gumma Pref., was extracted successively with ether and acetone. The mycobiont of this lichen was isolated from the fresh material and cultivated in laboratory. The culture medium and colonies exhibited brown and orange pigmentation after a few months cultivation. The pigments of the cultivated mycobiont were detected on TLC (Fig. 2) and isolated by column chromatography over silicic acid. The main pigments H and K were identified as fragilin (VI)[3] and 2-chloroemodin (VII),[4] respectively; Pigments I and L proved to be parietin (II) and emodin (IV).

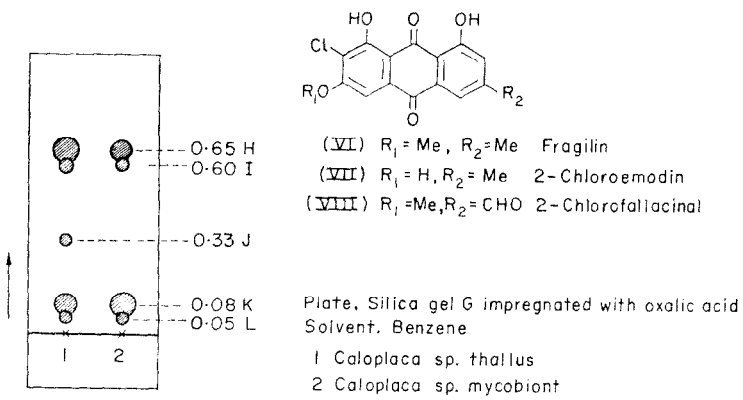

FIG. 2. SEPARATION OF ANTHRAQUINONES OF *Caloplaca* SPECIES.

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[3] T. BRUUN, D. P. HOLLIS and R. RYHAGE, *Acta Chem. Scand.* **19**, 839 (1965).
[4] G. BENZ, G. BOHMANN and J. SANTESSON, *Acta Chem. Scand.* **21**, 2889 (1967); I. YOSIOKA, H. YAMAUCHI, K. MORIMOTO and I. KITAGAWA, *Tetrahedron Letters* 1149 (1968).

In the lichen thalli of the *Caloplaca* species, a minute amount of pigment J was found, which was not in the extract of the mycobiont. This pigment gave a wine red magnesium acetate colour and showed IR absorptions at 1717 (CHO), 1678 (non-chelated C=O) and 1634 cm^{-1} (chelated C=O).

TABLE 1. THE LICHEN MS OF *Caloplaca* sp.

Culture No.	Place and date of collection	Lichen Thallis	Lichen Apothecium	Myocobiont	Remarks
154	Mt. Akagi 29 Sept. 1968	*m/e* 318 (320)	318 (320)	318 (320)	Fragilin
			304 (306)	304 (306)	2-Chloroemodin
		284	284	284	Parietin
			270		Emodin
190	Kurumayama	332 (334)			2-Chlorofallacinal
	1 Nov. 1969	318 (320)	318 (320)		Fragilin
		304 (306)	304 (306)		2-Chloroemodin
		284			Parietin
			270		Emodin

On TLC, this pigment J had almost the same R_f as fallacinal (III).[2] It is well known that chlorine containing anthraquinones have the same R_f on TLC as the corresponding non-chlorinated anthraquinones, but the former can be distinguished by their intense orange fluorescence from the latter which give yellow fluorescence in the UV. Pigment J showed an

TABLE 2. THE YIELDS OF METABOLITES OF LICHENS AND THEIR MYCOBIONTS

Compound	*Xanthoria mandschurica* Lichen	*Xanthoria mandschurica* Mycobiont	*X. fallax* Mycobiont	*Caloplaca* sp. Mycobiont*
Parietin	0·36%	0·10%	2·5%	trace %
Erythroglaucin	trace	trace	trace	—
Erythroglaucin carboxylic acid	trace	trace	trace	—
Fragilin	—	—	—	trace
2-Chloroemodin	—	—	—	0·44
Emodin	—	—	0·1	0·16
2-Chlorofallacinal	—	—	—	—
Fallacinal	—	—	0·1	—
Fallacinol	—	—	0·5	—

* The exact yield of pigments from the lichen could not be measured, due to contamination.

orange fluorescence and is a chlorofallacinal (VIII) in which the chlorine atom is probably at the 2-position, on biogenetic grounds. The MS giving M^+ 332 accompanied by the satellite ^{37}Cl isotope peak (334), 303 (305) (M–CHO), 289 (291) (M–CHO–Me) also support this structure (VIII).

Bohman[5] and Santesson[6] surveyed, about 230 species of *Caloplaca* for anthraquinones by means of lichen MS.[7] However, they failed to record 2-chloro-fallacinal as being present. The lichen MS of the present specimens of *Caloplaca* and its cultivated mycobiont are tabulated in Table 1.

EXPERIMENTAL

Materials. The lichen of *Xanthoria manschurica* (Zahlbr.) Asahina collected at Nunobikikannon, Nagano Pref. in November 1969 (dry wt: 320 g) was used for extraction. The mycobiont was isolated from the same lichen collected at Nippara, Nishitama-gun, Tokyo in October 1968 by means of the micromanipulator or test tube method.[8] *Xanthoria fallax* (Hepp.) Arn. collected at Ômiyama, Nagano Pref. was used for the isolation of mycobiont. The mycobionts were cultivated on malt-yeast extract agar or Hamada's 117 media[9] at 15–20°, pH 5·0–6·0 for 4–7 months. The colonies of the mycobionts were harvested and dried in air [dry wt: 2·5 g (*X. mandschurica*) and 0·8 g (*X. fallax*)].

The lichen of the unidentified *Caloplaca* species collected at Kurumayama, Nagano Pref. in October 1969 was used for extraction. From the same lichen collected at Mt. Akagi, Gumma Pref. in September 1968 the mycobiont was isolated by the same procedure as above, and cultivated under the same conditions. The weight of the lichen thalli could not be measured accurately due to contamination with basal wood bark. The dry wt. of the mycobiont was 3·2 g.

Isolation and purification of the metabolites. The lichen thalli were extracted successively with Et_2O and acetone, and the colonies of the mycobionts were extracted with acetone. The extracts were mixed with $CaHPO_4$ powder and placed on the top of a silicic acid column, and eluted successively with *n*-hexane, C_6H_6 to C_6H_6–acetone (10:1). Preparative TLC was also used for the separation of mycobiont metabolites.

Identification of the metabolites. The metabolites separated from the lichen thalli were identified by comparing with authentic samples, using m.m.p., IR, UV, MS and NMR spectrometry. The mycobiont metabolites were identified by TLC, UV and MS.

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