Phytochemistry, 1975, Vol. 14, p. 2505. Pergamon Press. Printed in England.

PHYTOCHEMICAL REPORTS

SQUAMATIC ACID FROM THE MYCOBIONT OF *CLADONIA CRISPATA*

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(*Received* 16 *May* 1975)

Key Word Index—*Cladonia crispata*; fungus; lichen mycobiont; squamatic acid; depside.

In the course of our studies on the metabolites of isolated and cultivated lichen mycobionts, several anthraquinone pigments[1], usnic acid[2] and zeorin[3], which are peculiar in lichens were isolated in crystalline form. However none of the characteristic lichen depsides and depsidones have been found to occur in cultured mycobionts, though several attempts have been made by earlier workers[4, 5]. Schultz and Mosbach[6] isolated orsellinate depside hydrolase from a lichen, *Lasallia pustulata* and its phycobiont, *Trebouxia* spp. suggesting the algal partner played an important role in the biosynthesis of lichen depsides. On the other hand, evidence for the formation of depsides by the fungal partner was suggested by Komiya and Shibata[2] who demonstrated the presence of salazinic acid, a lichen depsidone, in the cultured mycobiont of *Ramalina crassa* by TLC.

We have now found, by changing the cultural conditions, such as temperature, light intensity, oxygen tension, or carbohydrate supply in media, that squamatic acid[7], a widely distributed depside in lichens, is formed by the cultivated mycobiont of *Cladonia crispata* (Ach.) Flot., after it was irradiated with an UV lamp for a short period. The identity of squamatic acid was confirmed by TLC, and TLC and MS of its permethylate.

It should be noted that this is first unequivocal evidence for the formation of a lichen depside from a cultured mycobiont of a lichen without participation of the phycobiont.

EXPERIMENTAL

The mycobiont of *Cladonia crispata*, precultured for 7 months in test tubes of Hamada's 117 medium (glucose 20 g, dried yeast 5 g, agar 20 g, H_2O 1 l; pH 5·1–5·6), was irradiated with a UV lamp (10 W) from 2 cm for 5 min, and then cultured for a further month under natural light at room temperature. The mycelia were harvested, dried in air, and extracted with hot Me_2CO. The extract was examined by TLC on Si gel GF_{254} (C_6H_6–Me_2CO–HOAc 50:5:1), and the occurrence of squamatic acid was confirmed by its fluorescence under UV light (254 nm), as well as by its colour with $FeCl_3$ in comparison with an authentic sample. Since the MS of squamatic acid gave no molecular ion peak, the extract was methylated with CH_2N_2 to obtain the dimethyl ether dimethyl ester which was isolated by column chromatography on Si gel treated with 0·5 N oxalic acid and identified by TLC and MS (M^+ 446) in comparison with authentic sample.

Acknowledgements—The authors wish to thank Dr. S. Kurokawa for his help in identification of the lichen material and Yakurikenkyukai for grant.

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