od was found to be appropriate for the present method, and 20 μl of serum was shown to be sufficient for the glucose estimation.

Details of the experiment will be reported in the near future.

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Formation of Lichen Substances by Mycobionts of Lichens. Isolation of (+)Usnic Acid and Salazinic Acid from Mycobionts of *Ramalina* spp.

The formations of usnic acid, didymic acid, and rhodocladonic acid by mycobiont of lichen, *Cladonia cristatella*, were reported once by Castle and Kubsch.[1] However Ahmadjan[2] and Fox[3] denied the results of the earlier investigation by reexamination using the isolated fungi from the same lichen.

Recently Mosbach[4] revealed the formation of pulvic acid derivatives by the lichen fungus of *Candelariella vitellina*. Hess[5] suggested the contribution of algal symbiont of lichens to the formation of depsides and depsidones, while Mosbach[6] proved the presence of esterase in the algal symbiont of *Umbilicaria pustulata* suggesting its role in the depside biosynthesis in lichens.

We have found recently that (+)usnic acid and a depsidone, salazinic acid, are formed by the isolated mycobiont of lichen,[7] *Ramalina crassa* (DEL.) MOT. which was cultivated for 5 months at 8—20°C on Hamada's No.117 medium (glucose 20 g, dried yeast 5 g, agar 20 g, water 1 liter, pH 5.1—5.6). The former compound has also been obtained from the mycobiont of *Ramalina yasudae* RÄS. which was cultivated on the malt-yeast extract medium (malt extract 20 g, yeast extract 2 g, agar 20 g, dist. water 1 liter, pH 5.5). (+)Usnic acid, mp 201—202°, $[\alpha]_D$+480—519° (in $CHCl_3$), was isolated in a crystalline form from the above lichen mycobiont colonies in a yield of 0.022% and 1.8%, respectively, and identified by a mixed fusion as well as by IR and mass spectral comparisons with the authentic sample.

Salazinic acid was proved in the extract of mycobiont of *R. crassa* by thin-layer chromatography on silica gel G impregnated with 0.5N oxalic acid developed with benzene-acetone

1) H. Castle and F. Kubsch, *Arch. Biochem.*, **23**, 158 (1949).
2) V. Ahmadjian, *Bryologist*, **67**, 87 (1964).
3) C.H. Fox, personal communication (July 3, 1967).
4) K. Mosbach, *Acta Chem. Scand.*, **21**, 2331 (1967).
5) D. Hess, *Z. Naturforsch.*, **14b**, 345 (1959).
6) K. Mosbach, *Biochem. Biophys. Res. Comm.*, **22**, 145 (1966).
7) The mycobionts were isolated either by the micromanipulator or the test tube methods. (R.W. Davidson and T.E. Hinds, *Phytopathology*, **48**, 216 (1958)). Polyspore cultures were used for chemical analysis. All the lichen specimens used for the isolation of mycobionts are preserved in the Herbarium of the National Science Museum, Tokyo (TNS).

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(4:1), as well as by its characteristic yellow colouration with *p*-phenylenediamine. By the present investigation, we established the formation of usnic acid and salazinic acid by the isolated lichen mycobiont. It is also noted that the lichen substances are formed preferably in lichen mycobionts cultivated at lower temperature (10—20°).

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