Phytochemistry, 1971, Vol. 10, pp. 695 to 699. Pergamon Press. Printed in England.

POLYOLS PRODUCED BY THE CULTURED PHYCO- AND MYCOBIONTS OF SOME *RAMALINA* SPECIES*

TAKEYA KOMIYA† and SHOJI SHIBATA
Faculty of Pharmaceutical Sciences, University of Tokyo, Japan

(*Received* 18 *June* 1970)

Abstract—The polyol metabolism of *Ramalina crassa* and *R. subbreviuscula* and their cultured phyco- and mycobionts was studied. Ribitol is produced by the phycobiont and is converted into arabitol and mannitol in the mycobiont.

INTRODUCTION

IT HAS generally been recognized that polyols occur regularly in the lichen composites and their symbionts.[1] Using ^{14}C-tracer techniques, Smith and his coworkers[2–4] studied the carbohydrate metabolism of the isolated symbionts of lichens to find that D-glucose initially formed in the blue-green algal partner, *Nostoc*, is released to the fungal partner where it is converted into D-mannitol, while ribitol formed in the green algal partner, *Trebouxia*, is transformed into D-mannitol and D-arabitol in the fungal partner. On the other hand, Richardson and Smith[3] studied the carbohydrate metabolism of the cultured symbionts of the lichen, *Xanthoria aureola*, and tried to identify the pentitols in the metabolites by paper chromatography, but failed to distinguish whether the product was ribitol or arabitol.

By surveying the isolated phyco and mycobionts of more than 60 species of lichens in 15 families (T. Komiya and S. Shibata: Unpublished data), we found that the symbionts of *Ramalina crassa* (Del.) Mot. and *R. subbreviuscula* Asahina are most suitable for studying the metabolism of the cultivated symbionts, as they grow on the culture media relatively faster than others.

The present study deals mainly with the carbohydrate metabolism of the symbionts of these lichens which are morphologically very similar but can be distinguished by the secondary metabolites.‡

RESULTS AND DISCUSSION

Although electrophoresis and paper partition chromatography have generally been used for the analysis of polyols or sugar alcohols of lichens, we have obtained a well defined pattern of the polyols by GLC after trifluoroacetylation of the metabolites.[5]

* Part I in a proposed series 'The Metabolism of Lichen Symbionts'.

† Present address: Experimental Station of Medicinal Plants, Takeda Chemical Industry Co. Ltd., Ichijoji, Sakyo-ku, Kyoto, Japan.

‡ *Ramalina crassa* contains (+)usnic acid and salzinic acid, while *R. subbreviuscula* (+)usnic acid and divaricatic acid (Y. ASAHINA, *J. Japan Bot.* **15**, 205 (1940)).

[1] D. H. LEWIS and D. C. SMITH, *New Phytologist* **66**, 143 (1967).
[2] D. H. S. RICHARDSON and D. C. SMITH, *New Phytologist* **67**, 469 (1968).
[3] D. H. S. RICHARDSON and D. C. SMITH, *New Phytologist* **69**, 69 (1968).
[4] D. H. S. RICHARDSON, D. C. SMITH and D. H. LEWIS, *Nature* **214**, 879 (1967).
[5] M. MATSUI, M. OKADA, T. IMANARI and Z. TAMURA, *Chem. Pharm. Bull.* **16**, 1383 (1968).

GLC of Carbohydrates of Phycobionts

The carbohydrate fractions of the phycobionts of *Ramalina crassa* and *R. subbreviuscula* cultured in the organic medium (Trebouxia organic nutrient medium I)[6] were trifluoroacetylated and analyzed by GLC. The results show that ribitol is the sole product of both phycobionts, and is also present in the extracts of the algal cells and in the culture filtrate (Fig. 1). The phycobiont of *R. crassa* also produced ribitol when it was cultivated in the inorganic medium (Bold's mineral solution).[6]

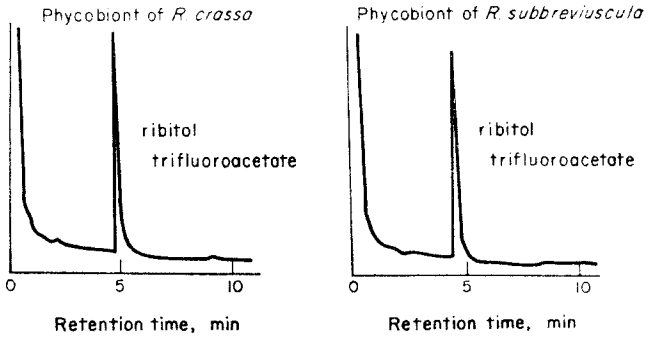

FIG. 1.

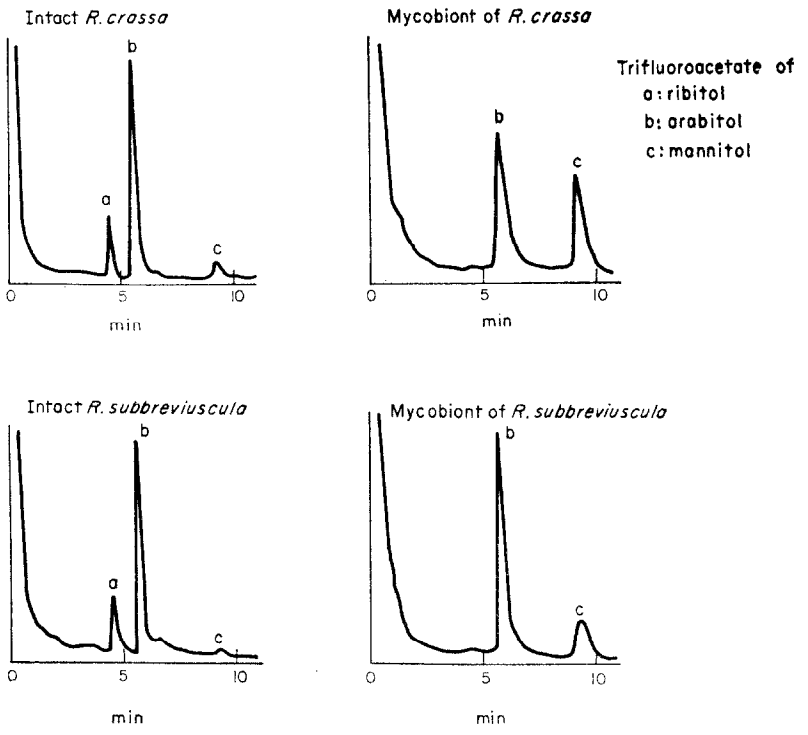

FIG. 2.

[6] cf. V. AHMADJIAN, *The Lichen Symbiosis*, pp. 119–120, Blaisdell, Toronto (1967).

GLC of Carbohydrates of Lichen Composites and Mycobionts

The trifluoroacetylated carbohydrate fractions were analyzed by GLC (Fig. 2) and both the lichens and their mycobionts gave very similar patterns. Three polyols were detected in both lichens, ribitol coming from the phycobiont, and arabitol and mannitol from the mycobiont. As shown by Smith,[7] ribitol of *Trebouxia* phycobiont would be transferred into the mycobiont and converted into arabitol and mannitol. The carbohydrate metabolism of *Ramalina crassa* and *R. subbreviuscula* is obviously the same as in the other lichens having *Trebouxia* as the phycobiont.

The analysis of carbohydrates of lichen symbionts has been reported by Maruo *et al.*[8] and Smith *et al.*[9] Our results agree then except that Smith's observation[10] that the mycobiont of *Xanthoria aureola* could form arabitol only when it was cultivated on a medium with ribitol did not hold for the *Ramalina* mycobionts, which formed arabitol on a medium free from ribitol.

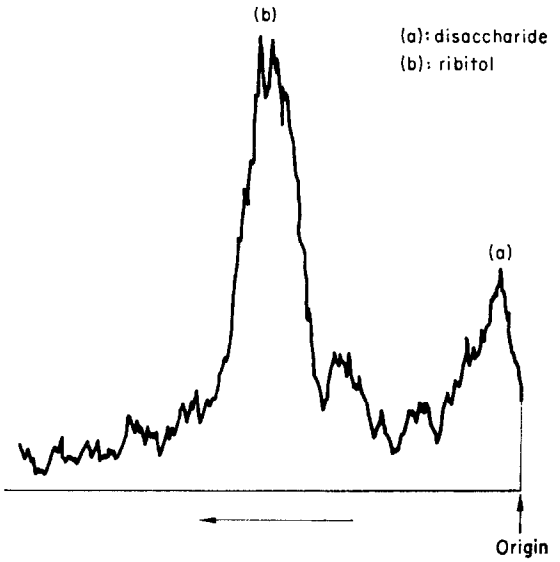

FIG. 3.

The Photosynthetic Product of Phycobiont isolated from Ramalina crassa

The isolated phycobiont of *Ramalina crassa* was cultivated under illumination of light in an atmosphere of $^{14}CO_2$, when ribitol-^{14}C was obtained as a characteristic product of the photosynthesis.

$^{14}CO_2$ was taken up by the phycobiont for 4·5 hr under illumination of light. The soluble carbohydrate fraction of the phycobiont was chromatographed on paper in methyl ethyl ketone–acetic acid–water saturated boric acid (9:1:1) (Fig. 3). The peaks (a) and (b) in the above scanning chart correspond to a disaccharide and ribitol, respectively. Thus ^{14}C was mainly incorporated into ribitol.

[7] D. H. S. RICHARDSON and D. C. SMITH, *New Phytologist* **67**, 469 (1968).
[8] B. MARUO, T. HATTORI and H. TAKAHASHI, *Agri. Biol. Chem. Tokyo* **29**, 1084 (1965).
[9] D. H. S. RICHARDSON and D. C. SMITH, *Lichenologist* **3**, 202 (1966).
[10] D. H. S. RICHARDSON and D. C. SMITH, *New Phytologist* **67**, 69 (1968).

Richardson and Smith reported that *Trebouxia* directly isolated from the thallus fragments of *Xanthoria aureola* by centrifugation afforded ribitol^{14}C after 1 hr photosynthesis on a solution of $NaH^{14}CO_3$, whereas cultured *Trebouxia* of the same lichen under the same conditions showed distribution of ^{14}C in other compounds including amino acids, organic acids and sugar phosphates. Now we find that the cultured *Trebouxia* of *Ramalina crassa* affords ribitol-^{14}C after photosynthesis in $^{14}CO_2$.

(a) *Administration of photosynthetic products of the phycobiont of* Ramalina crassa *to the mycobiont of the same lichen.* The ^{14}C-labelled soluble carbohydrate fraction of *R. crassa* phycobiont was administered to the cultured mycobiont of the same lichen. After 24 hr cultivation the fungus was extracted with EtOH, and the extract was chromatographed. The chromatogram was scanned to measure the distribution of ^{14}C to show that ^{14}C was incorporated into D-mannitol and D-arabitol whereas the peak of ribitol [^{14}C] was not observed (Fig. 4). The incorporation ratio of ^{14}C into the ethanolic extracts of mycobiont was 23·6 per cent.

(b) *Administration of ribitol-*[U^{14}C] *prepared from ribose-*[U^{14}C] *to the mycobiont of* Ramalina crassa. After 24 hr cultivation the mycobiont fed with ribitol-[U^{14}C] was extracted

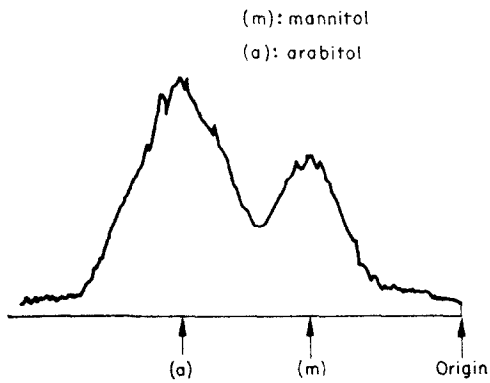

FIG. 4.

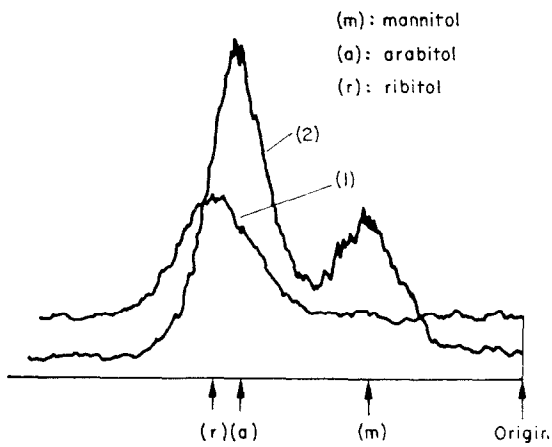

FIG. 5.

with EtOH. The distribution of ^{14}C in the ethanolic extracts was analyzed by paper chromatography, showing that ribitol was completely converted into D-mannitol and D-arabitol. The incorporation ratio of ^{14}C into the ethanolic extracts was 40·3 per cent. We observed good separation of ribitol and arabitol on paper (Fig. 5).

EXPERIMENTAL

Preparations of Materials

The lichens and their symbionts used for the present study were prepared as follows:

Lichens. Ramalina crassa (Del.) Mot. and *R. subbreviuscula* Asahina were collected at Tsumekizaki, Izu and Tanezashi beach, Hachinohe, Japan, respectively, and kept in a deep freezer.

Mycobionts. The mycobiont of *R. crassa* (culture No. 33) was cultivated by shaking method for 3 months at 20° in malt-yeast extract medium,[6] and that of *R. subbreviuscula* (culture No. 155) was cultivated for 6 months at 20° on malt-yeast extract agar medium.

Phycobionts. The phycobionts of both lichens were cultivated in Bold's mineral solution added with 2% glucose and 1% peptone (Trebouxia organic nutrient medium I)[6] or Bold's mineral solution bubbled with air containing 2% CO_2 at 20° with an illumination of light at an intensity of 2000 lx for 4 weeks.

Isolation of Carbohydrates

Intact lichens, mycobionts and phycobionts prepared as above were extracted 8–10 times by refluxing with 80% EtOH for 2–3 hr. The ethanolic extracts were concentrated and added with ether to extract pigments. The residue was dissolved in water and the aqueous portion was treated with IR 120, and IR 4B exchange resin to prepare the sample. The sample was trifluoroacetylated by Tamura's method[5] and injected to a gas chromatography using 2% XF 1105 column at 140°.

In order to distinguish the peaks of monosaccharides from those of polyols, the sample was treated with $NaBH_4$ before trifluoroacetylation to examine if any peaks were shifted by this treatment.

Carbon Dioxide [^{14}C] *Uptake of Cultivated Phycobiont*

Phycobiont cells of *Ramalina crassa* cultivated in 10 tubes (50 ml medium each) were transferred into a beaker (500 ml) which was placed in a desiccator in which $^{14}CO_2$ (0·4 mc) was introduced. The experiment was carried out at 15° for 4·5 hr under illumination of light at an intensity of 2000 lx.

Isolation of Soluble Carbohydrate from the Phycobiont

The phycobiont cells which were taken by filtration immediately after $^{14}CO_2$-uptake were refluxed 3 times with 80% EtOH (150 ml each). The extract was evaporated below 50° and washed with ether to remove pigment. The residue was dissolved in water (50 ml) and purified by chromatography through IR-120 column and then IR 4B column. The soluble carbohydrate thus obtained showed total activity $1·0 \times 10^6$ dis/min.

Preparation of Ribitol ($U^{14}C$)

D-Ribose ($U^{14}C$) was reduced with $NaBH_4$ to yield ribitol [$U^{14}C$] which was purified by paper partition chromatography.

Administration of Soluble Carbohydrate and Ribitol [$U^{14}C$] *to the Mycobiont*

The fungus (fr. wt. *ca.* 1·5 g) prepared in 6 malt-yeast extract agar slant tubes was transferred into Monod's tubes (50 ml each) containing Bold's mineral solution (pH 5·5) (30 ml) added with the solution of algal ^{14}C-labelled soluble carbohydrate (total activity $7·20 \times 10^5$ dis/min) or ribitol [$U^{14}C$] (total activity $1·04 \times 10^7$ dis/min) filtered through Seitz's filter. All the treatments were made under aseptic condition and the fungus was incubated at 20° for 24 hr.

Acknowledgements—The authors wish to thank Dr. S. Kurokawa, National Science Museum, Tokyo for his kind advice in lichenology, and Dr. T. Imanari of this Faculty for his valuable technical advice on GLC.