Phytochemistry, 1967, Vol. 6, pp. 1147 to 1148. Pergamon Press Ltd. Printed in England

SHORT COMMUNICATION

TREHALOSE IN THE CAMBIAL SAP OF *FAGUS SILVATICA* L.

FRANZ OESCH and HANS MEIER

Department of Botany, University of Fribourg, Switzerland

(*Received* 4 *February* 1967)

Abstract—Trehalose, previously not found in spermatophytes, has been isolated from the cambial sap of beech (*Fagus silvatica* L.) in amounts of 0·8–0·9 per cent of the neutral sugars. It was characterized by paper chromatography (before and after hydrolysis of the disaccharide), paper electrophoresis, i.r. spectroscopy, melting point and by demonstration of its non-reducing character.

INTRODUCTION

IN THE plant kingdom trehalose has hitherto been found in bacteria, algae, fungi and in a few members of the pteridophytes, e.g. in several species of *Selaginella*[1,2] and recently in *Botrychium lunaria*.[3]

The presence of trehalose in a member of the spermatophytes has, as far as we are aware, never unequivocally been proved. Berthelot[4] in 1858 isolated trehalose from "Trehalamanna", the cocoons of a beetle (*Larinus*) which is a parasite on *Echinops persica*, a species belonging to the composites. Lippmann[5] discovered trehalose in an exudate of *Carex brunescens*, but was unable to extract it from the plant itself. It must therefore be supposed that the compound had been formed in the exudate by microbiological transformation of exudated sugars.

EXPERIMENTAL AND RESULTS

The stem of an 80-yr-old beech tree (*Fagus silvatica* L.) was debarked immediately after felling in the beginning of July 1965, the remaining cells of the cambial zone and the youngest xylem cells were scraped off from the stem with glass knives and placed into boiling methanol. The material was stored in methanol at −15° and, after several months, a sample of the scrapings was filtered off, washed with methanol and the combined filtrates (*ca.* 500 ml) were evaporated to dryness (yield 872 mg). Water (50 ml) was added and the resulting colloidal solution was extracted ten times with ether (50 ml each time). The ether phase was discarded and the aqueous solution was passed first through a cation exchange column (100 cm³) of Cellex P (H^+ form) and then through an anion exchange column (150 cm³) of Cellex D (basic form). The neutral eluate was evaporated to dryness (yield 349 mg). Paper chromatography of the neutral fraction in the solvents ethyl acetate–pyridine–water 8:2:1 v/v, ethyl acetate–pyridine–water (upper phase) 2:1:2 v/v, ethyl acetate–acetic acid–water (upper phase) 3:1:3 v/v and n-butanol–acetic acid–water 3:3:2 v/v revealed the presence of glucose, fructose, galactose, saccharose, myoinositol, raffinose, stachyose and several spots which could not be attributed to any of the sugars usually present in cambial saps. One of these, however, showed the same R_f-value as α,α-trehalose in all the solvents used. Further it was revealed only when alkaline silver solution was used as a spray reagent, but did not react with anisidine hydrochloride. This indicates the non-reducing character of the sugar.

[1] O. ANSELMINO and E. GILG, *Ber. Deut. Pharm. Ges.* **23**, 326 (1913).
[2] T. YAMASHITO and F. SATO, *Chem. Abstr.* **24**, 75 (1930).
[3] O. KANDER and M. SENSER, *Z. Pflanzenphysiol.* **53**, 157 (1965).
[4] M. BERTHELOT, *Ann. Chim. Phys.* **55**, 272 (1858).
[5] E. v. LIPPMANN, *Ber. Deut. Chem. Ges.* **45**, 3431 (1912).

The whole neutral fraction was then applied to a carbon–Celite column (450 cm^3) which was first eluted with water (2 l.) and then with increasing concentrations of ethanol (0–20 per cent, 2·5 l.; 20–40 per cent, 2 l.). The eluate was collected in fractions of 25 ml. The sugar which corresponded to trehalose in its paper chromatographic behaviour was eluted from the column after the monosaccharides but just before sucrose at an ethanol concentration of about 18 per cent. The fractions in which trehalose was shown to be present were combined and evaporated to dryness (yield 3·1 mg). A sample was hydrolysed in 4 per cent sulphuric acid (6 hr at 100°) and only glucose was shown to be present in the hydrolysate. From another sample trehalose was crystallized as the dihydrate from 30% ethanol, m.p. and mixed m.p. 96°. Furthermore, after dehydration, the sample had the same i.r. spectrum as authentic dehydrated α,α-trehalose. Also the substance was indistinguishable from trehalose by paper electrophoresis (0·1 m borate puffer, pH 10.)

A second beech tree was felled at the end of May 1966 and extreme care was taken to prevent microbial activity during the whole extraction and fractionation procedure. The ion-exchange celluloses Cellex P and D were washed with absolute ethanol before application of the sample, and as an alternative Dowex ion-exchange resins were used. The eluate from the ion-exchange columns was directly dropped into ethanol. Also in this second isolation, trehalose was obtained in almost exactly the same quantity as in the first (0·8–0·9 per cent of the neutral sugar fraction). Furthermore, a small sample of the methanol extract of the scrapings after removal of the lipid substances and after desalting without the use of ion-exchange materials, showed the presence of trehalose chromatographically when different solvents were used.

DISCUSSION

As this is probably the first time trehalose has been isolated from a spermatophyte, the possibility that the substance had been produced as a result of microbial activity already in the tree or in the course of the extraction and fractionation procedure, had to be seriously considered and investigated. Since the investigation of the second tree, which seemed completely healthy and where special precautions against microbial activity during the extraction and fractionation procedure were taken, yielded the same results as those of the first, the formation of trehalose by microbial activity during the extraction and fractionation can be excluded.

Further, the similarity in the amounts found in the two trees felled at different times, almost excludes the possibility that they might have been infected in some part, for instance in the crown, and that trehalose of fungal origin could have been transported down the phloem. The trehalose found seems, therefore, to be a metabolic product of the tree itself.

Acknowledgement—The authors thank the "Schweizerischer Nationalfonds" for financial support.