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Plant Mucilages. IX.[1] The Location of the *O*-Acetyl Groups and the Nature of the Branches in Bletilla-glucomannan*

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The mucous polysaccharide from the tubers of *Bletilla striata* Reichenbach fil. named Bletilla-glucomannan was isolated and investigated in this laboratory.[1] The substance is composed of D-mannose and D-glucose in the molar ratio of 3 : 1. The measurement of osmotic pressure gave the value of 182000 as its molecular weight. Partial acid hydrolysis of it elucidated the structure to be mainly composed of β-1 $\rightarrow$ 4 linked aldohexopyranose residues. Periodate oxidation study also supported this conclusion, but both the value of formic acid liberation and the yield of mannose by Smith degradation suggested that the polysaccharide contains six aldohexose units per one end group on the average and a part of mannose residues occupies branching positions.

The present work was undertaken to identify and determine the acyl groups, and the location of them was elucidated. This paper is also concerned with the structural features of the polysaccharide, particularly the nature of the branches, revealed by methylation analysis.

The infrared spectrum of Bletilla-glucomannan has the absorption bands of 1735 and 1250 cm^{-1} suggesting the presence of ester linkages in addition to the absorption of 890 cm^{-1} being due to β-glycosidic linkages. The acid hydrolysate of the polysaccharide was analyzed directly by gas-liquid chromatography (GLC) using 20% tetramethyl cyclobutanediol adipate $-$ 4% phosphoric acid column. It gave one peak, whose retention time was precisely equal to that of authentic sample of acetic acid, and the possibility of presence of other acids was eliminated. The acetyl content of the polysaccharide was determined to be 4.2% by GLC. This result corresponds to one acetyl group for every five aldohexose residues.

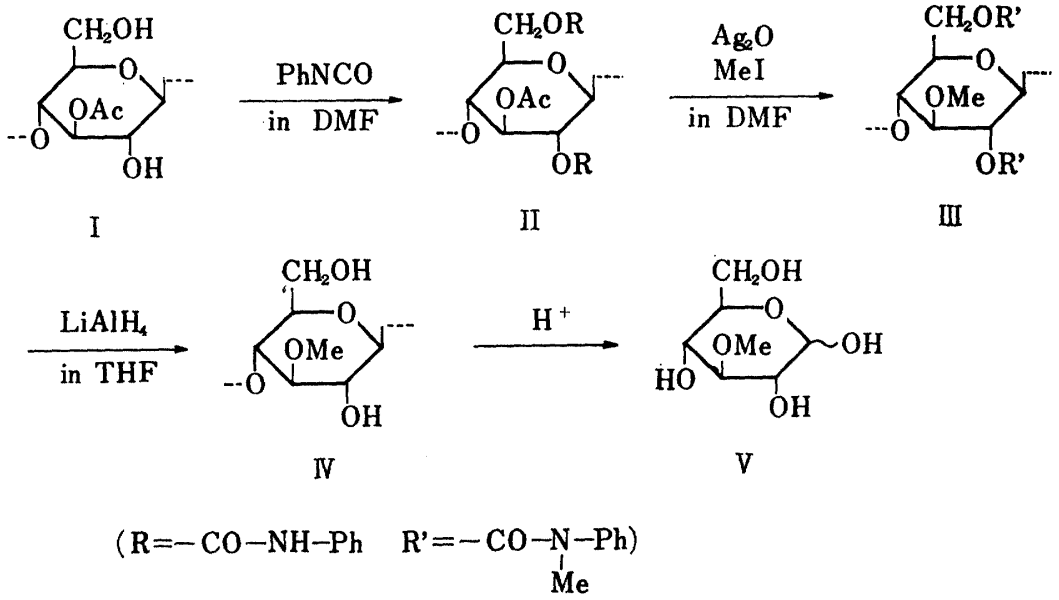
The presence of *O*-acetyl groups in glucomannans obtained from coniferous woods has already been known in literatures.[2-7] On the other hand, although

* 本報告は *Chem. Pharm. Bull.* (Tokyo), **22**, 2710 (1974) に発表

1) Rart VIII : M. Tomoda, S. Nakatsuka, M. Tamai and M. Nagata, *Chem. Pharm. Bull.* (Tokyo), **21**, 2667 (1973).
2) H. Meier, *Acta Chem. Scand.*, **15**, 1381 (1961).
3) E. Katz, *Tappi*, **48**, 34 (1965).
4) W. S. Linnell, N. S. Thompson and H. A. Swenson, *Tappi*, **49**, 491 (1966).
5) H. A. Swenson, *Tappi*, **51**, 141 (1968).
6) T. Koshijima and R. Tanaka, *Mokuzai Gakkaishi*, **16**, 399 (1970).
7) R. Tanaka and T. Koshijima, *Mokuzai Gakkaishi*, **18**, 403 (1972).

there was a report[8] on acetylated salep glucomannan, very little was known about the nature of acetylated glucomannan isolated from the tubers of higher plants.

In order to make clear the location of *O*-acetyl groups in Bletilla-glucomannan, the method originally developed by Bouveng[9] was employed. The sequence of reactions is illustrated in Chart 1. The polysaccharide (I) was swollen in dimethylformamide and treated with an excess of phenylisocyanate for conversion of the free hydroxyl groups to phenylcarbamate esters (II). Then the phenylcarbamate (II) was methylated with methyl iodide and silver oxide in dimethylformamide. The *O*-acetyl groups were replaced by *O*-methyl groups and the phenylcarbamoyl groups were *N*-methylated to give the partially-*O*-methyl-glucomannan *N*-methylphenylcarbamate (III) by this procedure. After removal of *N*-methylphenylcarbamoyl groups by reduction with lithium aluminium hydride in tetrahydrofuran, the resulting *O*-methyl derivative (IV) was hydrolyzed and the products were analyzed by paper partition chromatography (PPC), and by GLC of the alditol acetate after reduction and acetylation of the hydrolysate. Besides mannose and glucose, a hexose methyl ether was detected and identified as 3-*O*-methyl-D-glucopyranose (V) by comparison with the synthetic specimen.



(Chart 1)

Owing to this result, it is able to conclude that the *O*-acetyl groups are attached to position 3 of the most of D-glucopyranose units in Bletilla-glucomannan. The value of quantitative analysis indicated that about 80% of glucose residues

8) D. H. Juers, H. A. Swenson and S. F. Kurath, *J. Polym. Sci.*, Part A-2, **5**, 361 (1967) [*C. A.*, **66**, 115912d].

9) H. O. Bouveng, *Acta Chem. Scand.*, **15**, 96 (1961).

in the polysaccharide possess 3-*O*-acetyl groups.

The methylation of Bletilla-glucomannan was performed with sodium methylsulfinylcarbanion and methyl iodide in dimethylsulfoxide. The fully methylated product was hydrolyzed with formic acid and dilute sulfuric acid. The products were separated by PPC, then analyzed by GLC after conversion to alditol acetates. As the hydrolysis products of the methylated polysaccharide, 2, 3, 4, 6-tetra-*O*-methyl-D-mannose, 2, 3, 6-tri-*O*-methyl-D-mannose, 2, 3, 6-tri-*O*-methyl-D-glucose and 3, 6-di-*O*-methyl-D-mannose were obtained in a molar ratio of 1.7 : 5.1 : 3.0 : 2.2. These methyl derivatives of component sugars were also identified as their methyl glycosides by GLC.

The results of the methylation analysis provided the evidences that the polysaccharide has a main chain of β-1→4 linked aldohexopyranose residues, as already suggested by partial acid hydrolysis study. The isolation of 3, 6-di-*O*-methyl-D-mannose showed the branched structure with 1→2 branch point at a part of mannose residues, occurring with an average repeating unit of six component sugar residues. And this value is in good agreement with the results of periodate oxidation and Smith degradation.

Detailed investigations by partial enzymic hydrolysis and by partial acetolysis are now under progress to reveal the sequences of linkages of component sugar residues in the whole molecule.