SUGARS IN THE ACID HYDROLYZATES OF POLYSACCHARIDES PART IV. ISOLATION OF LAMINARIBIOSE FROM HYDROL

SUGARS IN THE ACID HYDROLYZATES OF POLYSACCHARIDES
PART IV. ISOLATION OF LAMINARIBIOSE FROM HYDROL*

By

Akio SATO**, Kazuho WATANABE***, and the late Kiyoshi ASO

Department of Agricultural Chemistry, Faculty of Agriculture, Tohoku University, Sendai, Japan

(*Received Feb. 24, 1963*)

Hydrol, the residual mother liquor in the commercial production of crystalline D-glucose by acid hydrolysis of starch, is known to have undergone considerable "reversion". In recent years, application of chromatographic techniques to the separation of carbohydrate constituents of hydrol has resulted in the identification of principal glucose-disaccharides. Of the eleven possible disaccharides that can be formed by substitution of an α- or β-glucopyranosyl residue on the five available hydroxyl groups of the second D-glucopyranose, nine have been identified previously in hydrol (1—7). The two disaccharides, α,β-trehalose and laminaribiose, have not been established yet although their existence in hydrol is most likely. Anno *et al.* (8) reported on the isolation of laminaribiose from reversion products obtained by the action of cation exchange resin and mineral acid on D-glucose. The present report describes the isolation of laminaribiose in crystalline form from hydrol.

Hydrol was chromatographed on carbon-Celite column (9) using water and 5, 10 and 30 per cent aqueous ethanol as successive elution solvents. Laminaribiose was eluted together with a small amount of cellobiose and a considerable amount of higher oligosaccharides in the latter half of the 10 per cent ethanol effluent (Table 1). This sugar mixture was rechromatographed on carbon-Celite column in a similar manner, but no better separation was produced. The mixture was then fractionated on carbon-Celite column in the presence of borate buffer (10) and laminaribiose-borate complex was eluted as a single sugar component

* Published, in part, in Chem. and Ind., 1958, 887.

** Present address: Fermentation Research Institute, Agency of Industrial Science and Technology, Chiba.

*** Present address: Institute of Physical and Chemical Research, Tokyo.

from the column (Fig. 1). After removal of borate from the complex, crystalline α-laminaribiose was obtained from aqueous ethanol. Acetylation with sodium acetate and acetic anhydride yielded crystalline β-laminaribiose octaacetate.

Experimental

General Methods.

These are detailed in Part III of this series.

Carbon-Celite Column Chromatography.

A 250 g aliquot of the sample of hydrol described previously was diluted to 2500 *ml* with water and the solution was introduced onto a column (500 × 120 mm diam.) of carbon-Celite (1 : 1 by wt.). The column was washed with water (32 *l*) and 5% (22 *l*), 10% (14 *l*) and 30% (6 *l*) aqueous ethanol at a flow rate of ca. 1 *l*/hr. The effluent was collected in 2 *l* fractions and tested qualitatively for sugar content with the Molisch reagent. The Molisch-positive fractions were evaporated and analyzed by paper chromatography. After the first 6 *l* of 10% ethanol was passed down the column, the next 8 *l* contained substances corresponding to laminaribiose, cellobiose, and higher oligosaccharides (Table 1).

Table 1. Chromatography of Sugar Constituents of Hydrol on Carbon Column

Fraction No.	Developer			Sugar Composition
1	Water	2	1	No sugar
2— 5	Water	8	1	Glucose
6—16	Water	22	1	Isomaltose
17—18	5% EtOH	4	1	Isomaltose, kojibiose
19—21	5% EtOH	6	1	Isomaltose, kojibiose maltose, nigerose
22—24	5% EtOH	4	1	Gentiobiose, maltose, nigerose
25—28	5% EtOH	8	1	Gentiobiose, nigerose, sophorose
29—30	10% EtOH	4	1	Gentiobiose, nigerose, sophorose
31	10% EtOH	2	1	Gentiobiose, cellobiose
32	10% EtOH	2	1	Cellobiose laminaribiose
33—35	10% EtOH	6	1	Laminaribiose, higher oligosaccharides
36—38	30% EtOH	6	1	Higher oligosaccharides

Another unit of hydrol of the same quantity as above was chromatographed on a carbon-Celite column in the same way. The fractions containing laminaribiose from two columns were combined and evaporated to a syrup; yield, 10.0 g. The syrup was subjected to carbon-Celite rechromatography using aqueous ethanol of various concentrations but separation of laminaribiose from other sugar components was not achieved.

Carbon-Celite Column Chromatograhy in the Presence of Borate Buffer.

The above sugar mixture was then fractionated on a carbon-Celite column (450×60 mm diam.) with borate buffer (boric acid, 7.45 g/*l* and sodium hydroxide, 4.00 g/*l*; pH obtained 10.0) containing ethanol at various concentrations in place of the water; ethanol concentration in the buffer was increased stepwise (with an interval of 0.5%) from zero to 5.5 per cent, and 500 *ml* of the buffer of each concentration of ethanol was passed down the column. Each 500 *ml* of the effluent was treated with Amberlite IR-120 (H^+ form), the solution and washings were combined, and evaporated to dryness under reduced pressure. The residue was dissolved in methanol and the solution was distilled under reduced pressure to remove boric acid as its volatile methyl ester; the procedure being repeated three times. Elution behavior of each component from the column is shown in Fig. 1.

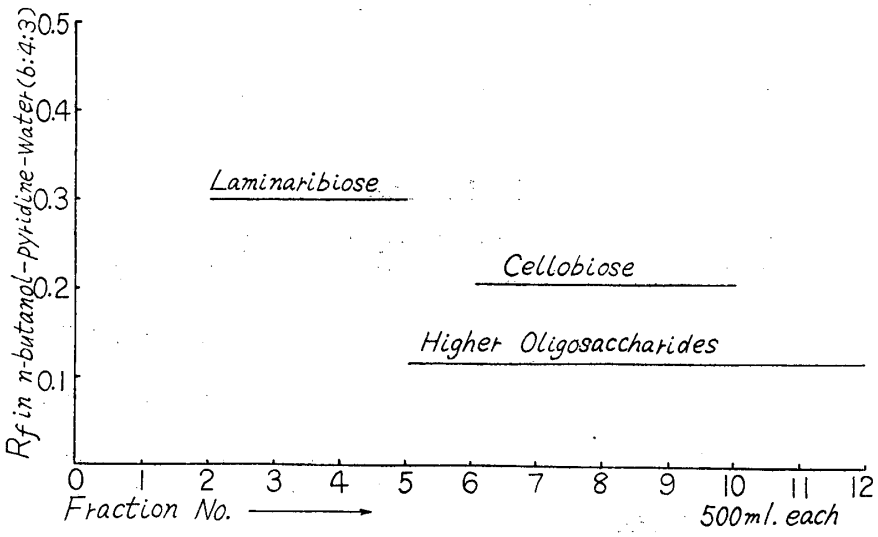

Fig. 1. Fractionation of Laminaribiose-fraction on Carbon Column in the Presence of Borate Buffer: Sugar sample, 10.0 g.; Column size, 450×60 mm. diam.

Fractions No. 2—4 containing a single component with the Rf value equivalent to that of laminaribiose were combined and evaporated to a syrup which was crystallized from aqueous ethanol; yield, 1.1 g, m.p. 196—198°C. Purification was effected by recrystallization from the same solvent: m.p. 205—206°C, $[\alpha]_D^{16}$ +24.1°→18.2° (*c* 3.6 in water). These values are in good agreement with those (11) published for α-laminaribiose. Mixed melting point with a known specimen showed no depression.

A portion (0.5 g) of the free sugar was acetylated with anhydrous sodium acetate and acetic anhydride. The syrupy acetylated product was crystallized from ethanol; yield, 0.5g, m.p. 156—158°C. Upon further recrystallization, the constants were: m.p. 161.5—162°C, $[\alpha]_D^{16}$—28.6° (*c* 3.4 in chloroform) (Found: C, 49.81; 5.63. Calcd. for $C_{12}H_{14}O_{11}(CH_3CO)_8$: C, 49.55; H, 5.64). These data

are in close agreement with the published values (11) for β-laminaribiose octaacetate. No change of melting point occurred on admixture with a known specimen.

Summary

A combined use of the usual carbon column chromatography and carbon column chromatography in the presence of borate buffer resulted in the isolation of laminaribiose as its crystalline free sugar and β-octaacetate from commercial hydrol.

Acknowledgment

The authors express their thanks to Mr. H. Tsuyama of the Sanmatsu Industrial Co. for the sample of hydrol used in this study. They are also much indebted to the Department of Agricultural Chemistry, Faculty of Agriculture, Tokyo University, for microanalysis and to the Laboratory of Biological Chemistry, Faculty of Science, Tohoku University, for measurement of optical rotation.

References

1) Berlin, H. (1926). *J. Am. Chem. Soc.*, **48**, 2627.
2) Wolfrom, M.L., A. Thompson, A.N. O'Neill, and T.T. Galkowski (1952). *idid.*, **74**, 1062.
3) Montgomery, E.M. and F.B. Weakley (1953). *J. Assoc. Offic. Agr. Chemists*, **34**, 457.
4) Sowden, J.C. and A.S. Spriggs (1954) *J. Am. Chem. Soc.* **76**, 3539.
5) Aso, K. and K. Shibasaki (1955), *J. Agr. Chem. Soc. Japan*, **29**, 855 (in Japanese); (1955). *Tohoku J. Agr. Res.*, **6**, 159.
6) Sowden, J.C. and A.S. Spriggs (1956). *J. Am. Chem. Soc.*, **78**, 2503.
7) Sato, A. and K. Aso (1957). *Nature*, **180**, 984.
8) Anno, K., N. Seno, E. Nakamura, H. Saito, and R. Hoshi (1959.) *Agr. Biol. Chem.*, **23**, 67.
9) Whistler, R.L. and D.F. Durso (1950). *J. Am. Chem. Soc.*, **72**, 677.
10) Barker, S.A., E.J. Bourne, and O. Theander (1955). *J. Chem. Soc.*, 4276.
11) Bächli, P. and E.G.V. Percival (1952). *ibid.*, 1242.