Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Note

A new method for determining the degree of hydrolysis in starch hydrolysates

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(Received December 4th, 1969; in revised form, March 23rd, 1970)

Although Painter[1] has devised a theoretical equation by which the degree of hydrolysis of homopolysaccharides may be obtained from the percentage of any one oligomer produced under random conditions, no absolute method appears to be available for determining the number of bonds broken in a polysaccharide undergoing non-random hydrolysis.

The investigations of Pasika and Cragg[2] with branched dextrans prompted us to examine the n.m.r. spectra of D-glucose, maltose, maltotriose, mixtures of these, and starch hydrolysates, in order to discover whether the signals due to the linked and free anomeric protons would offer a means for determining the degree of hydrolysis.

Spectra (60 MHz, Perkin–Elmer R-10 spectrometer) were obtained for solutions of samples (containing 80–100% by weight of solids, 0–20% of water) in D_2O, with the addition of a drop of D_2SO_4 for samples of high dextrose equivalent.

Fortuitously, the low-field doublet (L in Figs. 2 and 3) due to protons attached to *linked* anomeric carbon atoms occurs at τ 4.65 for maltose and all maltodextrins up to and including starch. Similarly, the doublets (α and β in Figs. 1–3) due to the protons attached to *unlinked* α- and β-D-anomeric carbon atoms also coincide for the entire maltodextrin series at τ 4.80 and 5.30, respectively, and all remaining ring-protons occur as a complex multiplet (R in Figs. 1–3) at τ 6.40. The spectra of D-glucose, maltose, and a commercial glucose syrup (starch hydrolysate) are shown in Figs. 1–3.

In Figs. 1–3, the signal due to the protons attached to *free* (unlinked) β-D-anomeric carbon-atoms is partly obscured by the HOD peak at τ 5.30. The latter, however, could be shifted to lower field by adding a drop of D_2SO_4, thereby revealing the doublet due to the protons attached to *free* β-D-anomeric carbon-atoms. When spectra were obtained at 100 MHz and 4°, and methyl sulphoxide was added to the sample solution in D_2O, the HOD signal was completely separated from all other signals. The areas of the three anomeric doublets, L, α, and β, and that of the ring-proton signals R could most accurately be obtained by excision from the spectra and weighing. The correct ratio of anomeric protons to ring protons (1:6) was thus obtained for the samples listed in Table I.

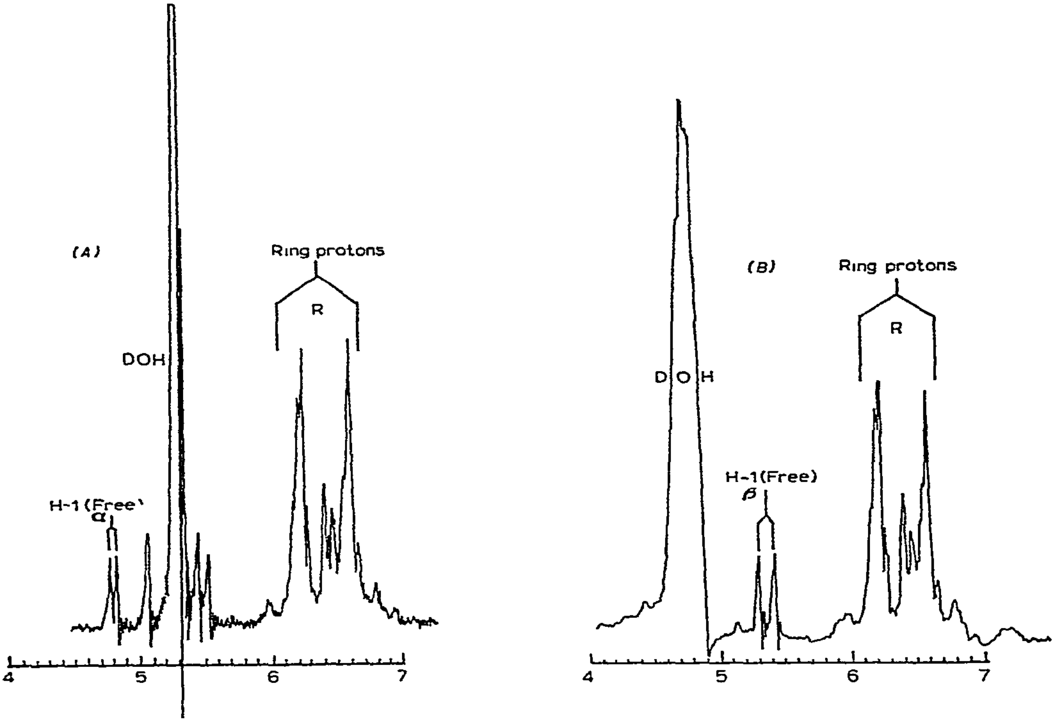

Fig. 1. N.m r. spectra of D-glucose: (*A*) D_2O, (*B*) D_2O/D_2SO_4.

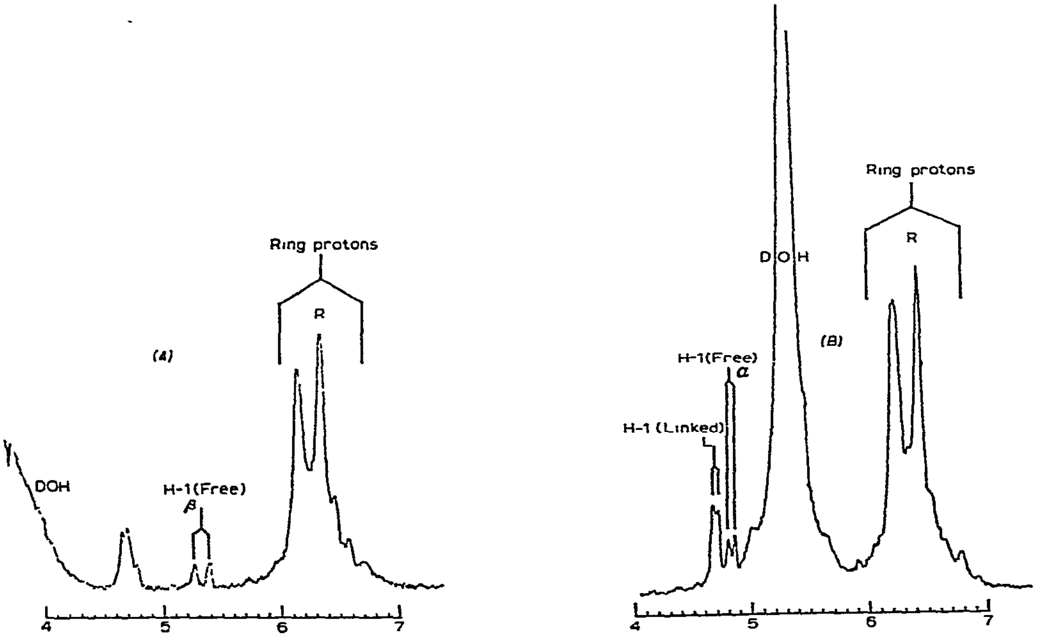

Fig. 2. N.m.r. spectra of maltose. (*A*) D_2O/D_2SO_4, (*B*) D_2O.

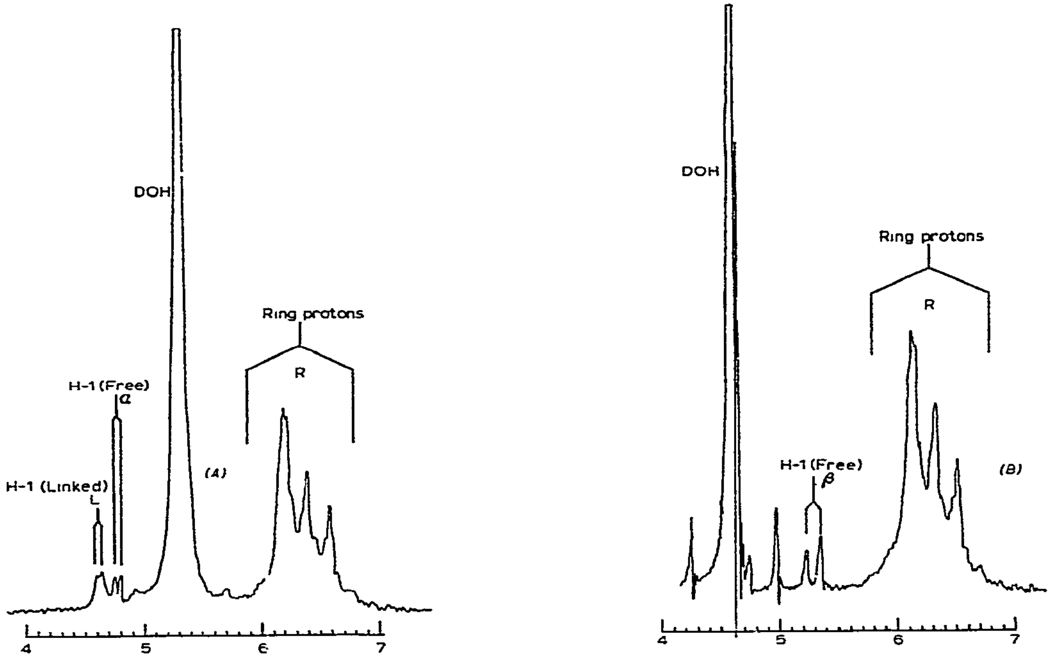

Fig. 3. N.m.r. spectra of glucose syrup. (*A*) D_2O, (*B*) D_2O/D_2SO_4.

TABLE I

RATIO OF SIGNAL AREAS FOR RING AND ANOMERIC PROTONS IN GLUCOSE SYRUPS AND THEIR CONSTITUENTS

Sample	*Ratio of areas* $(R/[L+\alpha+\beta])^a$
D-Glucose	6 00
Maltose	5.95
Maltotriose	6 05
D-Glucose–maltose (1:2)	6 05
Commercial, acid-converted glucose syrup (39 *D.E*)	5 97
Commercial, enzyme-converted glucose syrup (64 *D E.*)	6.15

[a] See Figs. 1–3.

Having established the validity of the method by the results in Table I, it is no longer necessary to expose the β-D anomeric signals in order to determine the degree of hydrolysis [100(fraction of bonds broken)]. Thus, the fraction of bonds unbroken is $L/(L+\alpha+\beta)$, but, since $(L+\alpha+\beta)/R = 1/6$, the fraction of bonds unbroken is $6L/R$. Therefore, 100(fraction of bonds broken), which is the degree of hydrolysis, is given by $100(1-[6L/R])$. By similar reasoning, the degree of hydrolysis may also be expressed as $600(\alpha+\beta)/R$.

We have therefore used these ratios to determine the degree of hydrolysis of a range of glucose syrups of known *dextrose equivalent* (*D.E.*) (*i.e.*, reducing power expressed as dextrose and calculated on a dry-weight basis). The results are shown in

TABLE II

DEXTROSE EQUIVALENTS AND DEGREES OF HYDROLYSIS IN GLUCOSE SYRUPS AND THEIR CONSTITUENTS

Sample	*Dextrose equivalent* (D.E.)	*Degree of hydrolysis*	*Average* D.P.	
			from degree of hydrolysis	*by reductive titration*
Laboratory, acid-converted glucose syrup	11.0	9.0	11.1	9.7
	15.0	15.0	6.7	6.4
	28	25.0	4.0	4.4
	33.0	31.2	3.2	3.0
	68.0	71.5	1.4	1.4
Commercial, acid-converted glucose syrup	36.0	36.0	2.8	—
	41.0	38.5	2.6	—
	41.0	38.0	2.6	—
	39.0	39.2	2.6	—
Commercial, enzyme-converted glucose syrup	41.2	43.0	2.3	—
Commercial, acid-converted glucose syrup	44.6	46.0	2.2	—
	56.5	55.8	1.8	—
D-Glucose–maltose (1·2)	72.0[a]	69.0 (calc.: 67%)	1.5	—
D-Glucose–maltose (1:1)	79.0[a]	72.2 (calc.: 75%)	1.4	—
Laboratory, acid-converted glucose syrup	82.0	79.2	1.3	—
	90.0	90.0	1.1	—

[a] Artificial compositions not met with in normal, starch hydrolysates.

Table II. The average *D.P.* (degree of polymerisation) of each syrup is also listed, and, in the case of five samples, this has been kindly checked by Professor G. N. Richards, University of Townsville, Australia, by a method involving reductive tritiation of the reducing group[3].

The results in Table II show a concise linearity between *D.E.* and degree of hydrolysis. It is well known that copper reduction methods for determining sugars are non-stoichiometric[4,5]. The amount of cupric ion reduced by one mole of glucose is, for example, *ca* 4–5 moles in the Lane and Eynon titration[6], but varies with the concentration of sugar. The molar reduction of the cupric ion is therefore clearly *not* a measure of the number of aldehyde groups, but a chemical property of the molecules to which the aldehyde groups are attached[7]. Nevertheless, it is possible that oxidation of a maltodextrin by cupric ion may be confined to the reducing residue, so that molar proportionality exists between members of the maltodextrin series and the amounts of cupric ion which they reduce. This result has been recognised for some time in Professor W. J. Whelan's laboratory and appears to be substantiated by the data in Table II.

The ease of application of n.m.r. spectroscopy and the close similarity of the results in Table II to the corresponding *D.E.* values suggest that this method may be potentially useful for in-line industrial control of starch-conversion processes.

ACKNOWLEDGMENT

We thank the Science Research Council, Tate and Lyle Ltd., Corn Products Ltd., Beecham Products (U.K.), and Tunnel Glucose Refineries for financial support, interest, and supply of samples for this work. We also thank Mr. E. Tarelli of Queen Elizabeth College, University of London, for his help with the spectroscopy, and Mrs. Lin of the Chemistry Department, University of Miami, for further help.

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