Distribution of Hydroxyethyl Groups in Hydroxyethyl Starch[1]

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ABSTRACT

By isolation of the products of acidic hydrolysis of hydroxyethyl starch (0.6 molar substitution), the distribution of hydroxyethyl groups on the C-2, C-3, and C-6 hydroxyl groups of the anhydroglucose units is established as $k_2:k_6:k_3 = 5.6:1.0:0.2$. There is little polysubstitution (e.g. 2,6-, 2,3-, etc.) and little polyoxyethylene substituent. A minor product, *cis*-1,2-*O*-ethylene-D-glucofuranose, formed by dehydration of 2-*O*-hydroxyethyl-D-glucose, has been fully characterized.

Hydroxyethyl starches, produced by reacting starch with ethylene oxide in the presence of an alkaline catalyst, are well-established products of use in the textile and paper industries (1). In recent years, the use of hydroxyethyl starch as a blood volume expander (2) and as a cryophylactic agent for erythrocytes (3) has been suggested. Hydroxyethyl starch having about 0.6 hydroxyethyl groups per anhydroglucose unit is effective for use as a blood volume expander (4), and in this paper we describe an investigation of the distribution of substituent groups in this material. Our procedure entailed hydrolysis with acid of the hydroxyethyl starch, followed by isolation of the resulting hydroxyethyl-D-glucoses with cellulose chromatography.

MATERIALS AND METHODS

Preparations of Hydroxyethyl-D-Glucoses

The 2-, 3-, and 6-*O*-hydroxyethyl-D-glucoses (5) were prepared from the corresponding carboxymethyl-D-glucose methyl esters (6) by reduction with lithium aluminum hydride, and had physical properties in agreement with those reported previously (5,7).

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Hydrolysis of Hydroxyethyl Starch

The starting material used in this investigation was prepared in the conventional manner by treating a solution of starch in sodium hydroxide with ethylene oxide, and had a molar substitution (MS) of 0.6 when measured by a modified Zeisel method (8). Hydroxyethyl starch (96.5% dry substance, 1.034 g.) was hydrolyzed in 0.75*M* sulfuric acid (50 ml.) at 100°C. for 3 hr., after which the acid was removed by treatment with Duolite A-4 (OH) weak-base, ion-exchange resin.

Fractionation of Hydrolysate

The solution obtained when the resin was filtered off was concentrated to a syrup and this was applied to Whatman 3MM paper. Development with ethyl acetate:acetic acid:water (9:2:2 v./v.) for 24 hr. effected a separation of D-glucose (493 mg.) from hydroxyethyl-D-glucoses (540 mg.); sugars were located by spraying guide strips with alkaline silver nitrate reagent. The combined weights represent 93% recovery of starting material. The mixture of hydroxyethyl-D-glucoses was rechromatographed for 30 hr. on five sheets of Whatman 3MM paper (46 cm. wide) in 1-butanol:1-propanol:water (6:9:5 v./v.) and the separated components had R_g 1.21 (6-*O*-hydroxyethyl-D-glucose), 1.57 (2-*O*-hydroxyethyl-D-glucose), and 1.86 (3-*O*-hydroxyethyl-D-glucose). The major product was 2-*O*-hydroxyethyl-D-glucose; only a small amount of 3-*O*-hydroxyethyl-D-glucose was apparent.

A minor product having R_g 2.4 in 1-butanol:1-propanol:water (6:9:5 v./v.) could be detected on paper chromatograms with alkaline silver nitrate or sodium metaperiodate-benzidine used as reagent, but not with reagents (e.g. aniline oxalate or benzidine) used for the detection of reducing sugars. This product, obtained in 4% yield, crystallized readily from aqueous ethanol, and its identification as *cis*-1,2-*O*-ethylene-D-glucofuranose and its probable mode of formation are described later.

The sugars were eluted with water, evaporated to dryness under reduced pressure, and weighed.

Analytical Methods

Molecular weight determinations on aqueous solutions of D-glucose derivatives were made with a Mechrolab osmometer. Oxidations with lead tetraacetate (9), sodium metaperiodate (10,11,12), or sodium hypoiodite (13) were performed by published procedures. Hydroxyl content was determined by the procedure of Fritz and Schenk (14), involving acetylation in pyridine with acetic anhydride at 50°C. for 5 to 10 min. Acetyl content was determined spectrophotometrically by a ferric hydroxamate method (15). Melting points were determined with a Thomas-Hoover capillary melting-point apparatus, and optical rotations were measured with an ETL-NPL automatic polarimeter. Infrared spectra were measured in potassium bromide discs with a Perkin-Elmer 137 instrument, and nuclear magnetic resonance spectra of D-glucose derivatives in deuterium oxide were measured with a Varian A60 spectrometer. Acceptable elemental analyses were obtained for all compounds described.

RESULTS AND DISCUSSION

Separation, Identification and Properties of the Hydrolysis Products

The 2-, 3-, and 6-*O*-hydroxyethyl-D-glucoses isolated by preparative paper chromatography from the hydroxyethyl starch hydrolysate were obtained in crystalline form, and were identified by comparison of their physical properties with those of authentic compounds prepared by the method of Shyluk and Timell (5) (Table I). Molecular weight determinations on these three compounds with a Mechrolab osmometer gave values of 220 to 228, consistent with a mono-*O*-hydroxyethyl-D-glucose structure (molecular weight 224). The three mono-ethers show markedly different behavior toward alkaline copper reagents (Schoorl or Somogyi-Nelson) used for the determination of reducing sugars. 2-*O*-Hydroxyethyl-D-glucose does not reduce these reagents; this behavior is typical of 2-substituted D-glucoses, such as 2-*O*-methyl-D-glucose, sophorose, and kojibiose. In addition, 3-*O*-hydroxyethyl-D-glucose and 6-*O*-hydroxyethyl-D-glucose show about one-third and four-fifths of the reducing power of D-glucose with alkaline copper reagents. However, all three D-glucose derivatives can be accurately determined by the use of sodium hypoiodite (13), with D-glucose used as a standard.

TABLE I. COMPONENTS OF HYDROLYSATE OF HYDROXYETHYL STARCH (1.034 g.)

Compound	Weight mg.	M.P. °C.	$[\alpha]_D^{25a}$
D-Glucose	493	...	+52.5°
2-O-Hydroxyethyl-D-glucose	424	152	+25°→+59°
6-O-Hydroxyethyl-D-glucose	83	107	+85°→+49°
3-O-Hydroxyethyl-D-glucose	15	126	+88°→+52°
cis-1,2-O-Ethylene-D-glucofuranose	38	223	−58°
Unidentified compounds	42	...	...

[a] Measured on 1% aqueous solutions. Where mutarotation data are shown, the initial value was obtained at 2 min. and the final value at 3 hr.

An alternative procedure for isolation of hydroxyethyl-D-glucoses from the hydrolysate of hydroxyethyl starch was tried. This comprised removing D-glucose by fermentation with baker's yeast, and separating the unfermentable hydroxyethyl-D-glucoses by chromatography on Pittsburgh granular charcoal, with aqueous ethanol used as eluant. The latter procedure was used by Croon and Lindberg (7) in their study of the distribution of substituents in hydroxyethyl cellulose, and is useful for the isolation of hydroxyethyl-D-glucoses on a large scale. However, for analytical use we prefer the paper-chromatographic procedure to that of charcoal chromatography.

Distribution of the Hydroxyethyl Substituent

Molar substitution is a measure of moles of hydroxyethyl group per anhydroglucose unit; a hydroxyethoxyethyl substituent ($HOCH_2CH_2OCH_2CH_2$-) is measured by the Zeisel method (8) as two hydroxyethyl substituents. Degree of substitution (DS) is a measure of the substitution of an anhydroglucose unit without regard to the molecular size of the substituent. It follows that MS $\geq$ DS. The distribution of hydroxyethyl substituent among the three hydroxyl groups of the anhydroglucose units of the 0.6-MS hydroxyethyl starch was determined from the weights of the individual components iso-

lated from the hydrolysate as $k_2:k_6:k_3 = 5.6:1.0:0.2$ (Table I). From the weight of D-glucose isolated from the hydrolysate and the observed low level of di-substitution products, the DS of this hydroxyethyl starch is estimated at 0.5, in good agreement with the value of 0.6 MS determined on the hydroxyethyl starch.

Since our work was completed, Srivastava and Ramalingam (16) have reported on the use of a periodate oxidation technique to determine the distribution of hydroxyethyl groups in 0.1-DS hydroxyethyl starch. They find that "about 84% of the hydroxyethyl groups are present at C-2 positions, the remaining ether groups residing at the C-6 position, with only negligible substitution at C-3." These results obtained with 0.1-DS hydroxyethyl starch are in accord with our results on material having 0.6 MS, and with the results obtained by Husemann and Kafka (17) for hydroxyethyl amylose having up to 1.1 DS, but not with those obtained by Lott and Brobst (18) for hydroxyethyl amylose having 0.23 and 0.52 MS. The latter workers (18), using a gas-chromatographic technique (19), found that C-2 is the most reactive position in etherification, and that "complex derivatives" comprised less than 10% by weight of the hydrolysates of hydroxyethyl amylose having 0.23 and 0.52 MS. However, the C-3 hydroxyl group was found more reactive than that at C-6, the relative distribution of hydroxyethyl substituent being $k_2:k_6:k_3 = 3.4:1:1.5$ for hydroxyethyl amylose having 0.5 MS. We would not have expected to find this difference between starch and amylose in the relative reactivities of hydroxyl groups on etherification with ethylene oxide, and have no explanation at present for the observed difference.

It is of interest to compare the distribution of substituent in 0.6-MS hydroxyethyl starch with that in hydroxyethyl cellulose of similar MS. The latter material, when prepared by heterogeneous reaction, shows $k_2:k_6:k_3 = 0.3:1.0:0.1$ with about 25% of the ethylene oxide units in the form of polyoxyethylene substituent (7).

Identification of cis-1,2-O-Ethylene-D-Glucofuranose

The minor product isolated from the hydroxyethyl starch hydrolysate had m.p. 222° to 223°, $[\alpha]^{25}_D$ −58° (*c* 1.0, water), molecular weight 205, and an elemental analysis in agreement with the formula $C_8H_{14}O_6$: calculated for C, 46.60; H, 6.85; O, 46.60; found: C, 46.64; H, 6.86; O, 46.47. On oxidation with aqueous sodium metaperiodate solution, the compound consumed 1.12 moles of periodate (10) and 1.05 moles of formaldehyde (12) were formed; no formic acid (11) was produced (Table II). Control oxidations were also performed on known carbohydrates.

TABLE II. OXIDATION VALUES OF VARIOUS CARBOHYDRATES AFTER 24 HR.
(Figures in parentheses are expected values)

Compound	$NaIO_4$	HCO_2H	CH_2O	$Pb(OAc)_4$
Erythritol	3.25 (3)	2.00 (2)	2.00 (2)	3.16 (3)
Methyl α-D-glucopyranoside	2.12 (2)	0.96 (1)	0.02 (0)	..
2-O-Hydroxyethyl-D-glucose	3.24 (3)	1.36 (2)	1.00 (1)	2.90 (3)
cis-1,2-O-Ethylene-D-glucofuranose	1.12 (1)	0.00 (0)	1.05 (1)	1.06 (1)

The absence of a reducing group in the compound was indicated by its failure to react with sodium hypoiodite (13). Application of the acetylation procedure of Fritz and Schenk (14) afforded a crystalline di-*O*-acetate, m.p. 205° and $[\alpha]^{25}_{D}$ + 1.7° (*c* 2, ethanol); the acetyl content (15) was determined as 2.07 acetyl groups per mole, and infrared analysis showed the presence of a hydroxyl group.

On the basis of this evidence, the structure of the compound can only be formulated as 1,2-*O*-ethylene-D-glucofuranose, a bicyclic compound containing a dioxan ring fused to a furan ring. By inspection of molecular models, *cis*-fused rings are considered to be favored; the possibility of forming the alternative *trans*-fused system is sterically questionable but not impossible. An unequivocal determination of the nature of the ring fusion could not be made from an examination of the nuclear magnetic spectrum. The anomeric proton occurs as a doublet at 0.7 p.p.m. downfield from the DOH peak, and the coupling constant for H-1 and H-2 ($J_{1,2}$) is 2.9 c.p.s. Abraham and coworkers (20) have examined the nuclear magnetic resonance spectra of 1,2-*O*-isopropylidene-alpha-D-*xylo*-hexofuranose derivatives containing two *cis*-fused five-membered rings, and have calculated the projected valency angle between the bridgehead hydrogens to be about 50°. Similar calculations (20) for 1,2-*O*-ethylene-D-glucofuranose with the observed $J_{1,2}$ value of 2.9 c.p.s. give angles of 54° and 123° for *cis*- and *trans*-fused rings, respectively. Examination of molecular models shows that a conformation having a dihedral angle of 54° is possible but that one having 123° is questionable.

However, synthesis of *cis*-1,2-*O*-ethylene-D-glucofuranose has been effected by cyclizing 2-*O*-chloroethyl-alpha-D-glucofuranoside with alkali, details of which are being reported elsewhere; the synthetic material was identical with that isolated from the hydroxyethyl starch hydrolysate on the basis of a comparison of melting points, specific rotations, infrared spectra, and nuclear magnetic resonance spectra.

Interrelation of 2-O-Hydroxyethyl-D-Glucose and cis-1,2-O-Ethylene-D-Glucofuranose

These two compounds were treated individually with 0.75*M* sulfuric acid at 100°C., the course of the reaction being followed polarimetrically. The 2-*O*-hydroxyethyl-D-glucose, $[\alpha]^{25}_{D}$ + 25°, and *cis*-1,2-*O*-ethylene-D-glucofuranose, $[\alpha]^{25}_{D}$ −58°, attained the same equilibrium value of $[\alpha]^{25}_{D}$ + 47° within 2 and 1 hr., respectively. After removal of acid, paper chromatography permitted isolation of both 2-*O*-hydroxyethyl-D-glucose in about 90% yield and *cis*-1,2-*O*-ethylene-D-glucofuranose in about 5% yield from each acid-treated solution. Only trace amounts of other compounds were observed. On this evidence we consider the occurrence of *cis*-1,2-*O*-ethylene-D-glucofuranose in the hydrolysate of hydroxyethyl starch to arise by dehydration of 2-*O*-hydroxyethyl-D-glucose.

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Literature Cited

1. HJERMSTAD, E. T. *In* Industrial gums, R. L. Whistler, ed., p. 727. Academic Press: New York (1959).
2. BALLINGER, W. F., MURRAY, G. F., and MORSE, E. E. Preliminary report on the use of hydroxyethyl starch solution in man. J. Surg. Res. 6: 180 (1966).
3. KNORPP, C. T., MERCHANT, W. R., GIKAS, P. W., SPENCER, H. H., and THOMPSON, N. W. Hydroxyethyl starch. Extracellular cryophylactic agent for erythrocytes. Science 157: 1312-1313 (1967).
4. SCHOCH, T. J. *In* Report to committee on plasma substitutes. Nat. Acad. Sci.-Nat. Res. Council Publ. (1965).
5. SHYLUK, W. P., and TIMELL, T. E. A new method of preparing hydroxyethyl ethers of glucose. Can. J. Chem. 34: 571-574 (1956).
6. SHYLUK, W. P., and TIMELL, T. E. Synthesis of four carboxymethyl esters of glucose. Can. J. Chem. 34: 575-582 (1956).
7. CROON, I., and LINDBERG, B. Distribution of substituents in hydroxyethyl cellulose. Svensk. Papperstid. 59: 794-799 (1956).
8. HOFFMAN, D. O., and WOLFROM, M. L. Determination of methoxyl and ethoxyl groups in acetals and easily volatile alcohols. Anal. Chem. 19: 225-228 (1947).
9. PERLIN, A. S. Structure of reducing disaccharides by lead tetraacetate oxidation. Anal. Chem. 27: 396-406 (1955).
10. ASPINALL, G. O., and FERRIER, R. J. A spectrophotometric method for the determination of periodate consumed during the oxidation of carbohydrates. Chem. Ind. (London) 1216 (1957).
11. SLOANE-STANLEY, G. H. Aspects of spectrophotometric iodometry. Biochem. J. 73: 8P (1959).
12. FRISELL, W. R., MEECH, L. A., and MacKENZIE, C. G. A simplified photometric analysis for serine and formaldehyde. J. Biol. Chem. 207: 709-716 (1954).
13. MILLER, G. L., and BURTON, A. L. Spectrophotometric determination of aldoses by an iodometric procedure. Anal. Chem. 31: 1790-1793 (1959).
14. FRITZ, J. S., and SCHENK, G. H. Acid-catalyzed acetylation of organic hydroxyl groups. Anal. Chem. 31: 1808-1812 (1959).
15. HESTRIN, S. Reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine, and its analytical applications. J. Biol. Chem. 180: 249-261 (1949).
16. SRIVASTAVA, H. C., and RAMALINGAM, K. V. Distribution of hydroxyethyl groups in commercial hydroxyethyl starch. Staerke 19: 295-300 (1967).
17. HUSEMANN, E., and KAFKA, M. Distribution of substituents in water-soluble amylose ethers. Makromol. Chem. 41: 208 (1960).
18. LOTT, C. E., and BROBST, K. M. Gas-chromatographic investigation of hydroxyethyl amylose hydrolyzates. Anal. Chem. 38: 1767-1770 (1966).
19. SWEELEY, C. C., BENTLEY, R., MAKITA, M., and WELLS, W. W. Gas-liquid chromatography of trimethylsilyl derivatives of sugars and related substances. J. Am. Chem. Soc. 85: 2497-2507 (1963).
20. ABRAHAM, R. J., HALL, L. D., HOUGH, L., and McLAUGHLAN, K. A. A proton resonance study of the conformations of carbohydrates in solution. J. Chem. Soc. 3699-3705 (1962).

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