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THE ISOLATION OF 1,2-*O*-ETHYLENE DERIVATIVES OF D-GLUCOSE FROM *O*-(2-HYDROXYETHYL)STARCH

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ABSTRACT

Acid hydrolysis of a commercial sample of *O*-(2-hydroxyethyl)starch (D.S., 0.1) gave, in addition to the expected 2-*O*-, 3-*O*-, and 6-*O*-(2-hydroxyethyl)-D-glucoses, three isomeric anhydro derivatives of 2-*O*-(2-hydroxyethyl)-D-glucose (**1**). These have been identified as 1,2-*O*-ethylene-α-D-glucofuranose (**2**), 1,2-*O*-ethylene-β-D-glucopyranose (**3**), and 1,2-*O*-ethylene-α-D-glucopyranose (**4**). The formation of 1,2-*O*-ethylene-D-glucose takes place by intramolecular glucosidation of **1**.

INTRODUCTION

By employing the Smith-degradation technique[1,2] for the structural analysis of polysaccharides, it has been shown[3] that, in a commercial sample of *O*-(2-hydroxyethyl) derivatives of maize starch (D.S., 0.1), O-2 is substituted to the extent of 84%; the remaining ether groups are located mainly at O-6 with only negligible substitution at O-3. To confirm these findings, another method of determining the distribution of *O*-(2-hydroxyethyl) groups, involving acid hydrolysis followed by quantitative chromatographic separation of the hydrolysis products, was investigated. In the course of this study, we have isolated, in addition to the expected 2-*O*-, 3-*O*-, and 6-*O*-(2-hydroxyethyl) derivatives of D-glucose, three isomeric anhydro derivatives of 2-*O*-(2-hydroxyethyl)-D-glucose (**1**) (*cf.* ref. 3*b*). We now report on the structural characterization of the anhydro derivatives of **1**.

RESULTS AND DISCUSSION

From the mixture of sugars produced on hydrolysis of *O*-(2-hydroxyethyl) derivatives of maize starch, D-glucose was removed by fermentation with baker's yeast. The residual mixture of non-fermentable sugars was fractionated by chromatography on a cellulose column to give four crystalline compounds (*A*, *B*, *C*, and *D*; Table I).

Compound *A* could not be located on the paper chromatogram by such spray reagents (for reducing sugars) as *p*-anisidine hydrochloride[4] or silver nitrate–sodium hydroxide[5], but was detected by the periodate–benzidine reagent[6]. The non-reducing character of *A* was confirmed by its lack of reaction with Fehling's solution. Molecular

TABLE I

PROPERTIES OF COMPOUNDS ISOLATED FROM THE HYDROLYSATE OF *O*-(2-HYDROXYETHYL)STARCH

Compound	R_G	*M.p.* (*degrees*)	$[\alpha]_D^{30}$ (*water*) (*degrees*)
A	2.3	215–216	−55
B	1.9	209–210	+54
C	1.9	134–135	+89.7
D	1.5	151–152	+57.2 (equilibrium value)

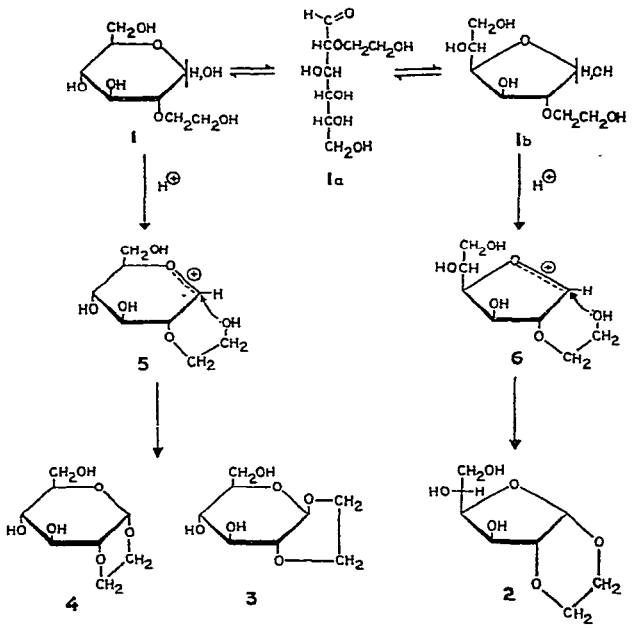
weight (M^+ 206, by mass spectrometry) and elemental analysis of the compound indicated that it was an anhydro derivative of a mono-*O*-(2-hydroxyethyl)hexose. Analysis of the fully acetylated derivative of compound *A* showed that the latter had three free hydroxyl groups. Hydrolysis of the compound with 0.5N sulphuric acid at 95° showed the following changes in specific optical rotation: −50.8 (initial)→ +61.8 (30 min)→ +66.5° (75 min, constant value). Chromatography of the hydrolysate showed the presence of **1**. Upon oxidation with sodium metaperiodate, *A* consumed one mole of the oxidant, with liberation of one mole of formaldehyde, per mole of the compound. No formic acid was produced. The periodate-oxidation data thus indicate an exocyclic, vicinal-diol grouping and a probable furanoid structure for *A*. The infrared spectrum of *A* contained bands of types A, B, C, and D (937, 876, 831, and 788 cm^{-1}, respectively) characteristic of compounds containing a furanoid ring[7]. In addition, strong bands appeared at 895, 946, 994, 1020, 1035, 1084, 1110, and 1150 cm^{-1}. Of these, the absorptions at 895 and those between 1020 and 1150 cm^{-1} are characteristic of the vibrations[8] of a dioxane ring.

On the basis of the foregoing evidence, compound *A* is designated 1,2-*O*-ethylene-D-glucofuranose. Although *A* has a negative optical rotation, it is assigned the α-D configuration **2** in view of the fact that its physical constants (Table I) are almost identical with those given[9] for synthetic 1,2-*O*-ethylene-α-D-glucofuranose (**2**).

As compared to compound *A*, compounds *B* and *C* were isolated only in small proportions. Both *B* and *C* were non-reducing, but could be detected on paper chromatograms by the periodate–benzidine reagent[6]. Hydrolysis of *B* or *C* with N sulphuric acid for 6 h gave **1** (chromatographic evidence). These results suggested that compounds *B* and *C* were also derivatives of **1**, in which the reducing group was blocked but could be regenerated on acid hydrolysis. Compound *B* has been identified as 1,2-*O*-ethylene-β-D-glucopyranose (**3**), which was synthesized by cyclization of 2-chloroethyl β-D-glucopyranoside under alkaline conditions[10,11]. Compound *C* has been tentatively identified as 1,2-*O*-ethylene-α-D-glucopyranose (**4**) from the fact that its physical constants (see Table I) are similar to those reported[12] for this compound.

Compound *D*, which was a major component of the mixture, was identical, in its physical constants and chromatographic and electrophoretic behaviour, to 2-*O*-(2-hydroxyethyl)-D-glucose (**1**)[13,14].

While this work was in progress, Ramnäs and Samuelson[15] reported the identification of **2**, **3**, and **4** in hydrolysates of *O*-(2-hydroxyethyl)cellulose. The major portion of 1,2-*O*-ethylene-D-glucose was found to be present in the α-D-pyranoid form. It is interesting to note that, in the present study, 1,2-*O*-ethylene-D-glucose, isolated from a hydrolysate of *O*-(2-hydroxyethyl)starch, was mainly present in the α-D-furanoid form.



Treatment of **1** with 1% methanolic hydrogen chloride for 8 h gave **2**. When the reaction was monitored by thin-layer chromatography, the conversion **1**→**2** was seen to be almost complete after 15 min, during which time the specific optical rotation had dropped from an initial value of +58.5 to −18.8°. On further heating, a third, as yet unidentified, component (*E*) having chromatographic mobility slightly slower than **4** was detected, and after 3 h, a fourth component appeared, which was probably a mixture of **3** and **4**. When **1** was heated with N sulphuric acid, the only compounds detected on the chromatogram were **1** and **2**, the latter being present in small proportion only. Further heating apparently resulted in the formation of a small proportion of a mixture of **3** and **4**. Compounds **3** and **4** are not very well separated by t.l.c., although **3** has a slightly higher R_F than **4**.

The foregoing results suggest that **2**, **3**, and **4** are all formed from **1**, produced during hydrolysis of *O*-(2-hydroxyethyl) starch, by intramolecular elimination of water between the hydroxyl group at C-1 and the hydroxyl group of the hydroxyethyl function at C-2. It is reasonable to assume that, under the acidic conditions, such cyclizations take place through the intermediacy of carbonium ions **5** and **6**. Höök and Lindberg[9] have shown that, whereas **2**, **3**, and **4** are all formed by treatment of **1**

with hydrogen chloride in *N*,*N*-dimethylformamide, treatment with M sulphuric acid for 18 h gives rise only to **2** in low yield.

It is noteworthy that treatment of **1** with acids in both aqueous and non-aqueous media gives predominantly **2**. Furthermore, from the hydrolysate of *O*-(2-hydroxyethyl)starch, **2** was isolated in relatively much greater quantities than **3** and **4**. These results indicate that, as in the case of the Fischer glycoside synthesis, the furanoid form (**1b**) of **1** reacts first to give the cyclic derivative **2**. However, in contrast with the formation of glycopyranosides from glycofuranosides, **2** is stabilized because of a fused-ring system, and its further conversion into **3** and **4** takes place rather slowly and to a lesser extent.

EXPERIMENTAL

General. — Melting points, determined in thin-walled capillaries, are uncorrected. Solutions were concentrated *in vacuo* at temperatures below 55°. Paper chromatography was performed on Whatman No. 1 paper with butyl alcohol–pyridine–water (6:4:3, v/v). R_G represents rate of movement on paper relative to D-glucose. Thin-layer chromatography (t.l.c.) was carried out on silica gel (National Chemical Lab., Poona) with acetone–hexane (4:1, v/v); detection was effected with conc. sulphuric acid at 120–130° for 15 min. Paper electrophoresis was carried out on Whatman No. 1 paper with 0.1M borate buffer at an applied potential of 500 volts.

Hydrolysis of the O-(2-hydroxyethyl) derivative of maize starch. — The derivative (D.S., 0.1; 125 g) was suspended in 72% (w/w) sulphuric acid (150 ml), and the mixture was kept for 3 h at 30°. The mixture was then diluted to 2.5 l with water, heated on a boiling-water bath for 6 h, neutralized with barium carbonate, and filtered. The filtrate was deionized by ion-exchange resins and evaporated to a syrup (125 g). D-Glucose in the hydrolysate was removed by fermentation with baker's yeast, to leave a syrupy mixture of non-fermentable sugars (12.0 g). A portion (6.22 g) of the mixture was chromatographed on a cellulose column (7 × 69 cm), with butyl alcohol saturated with water, to give the following fractions:

Fraction	*1*	*2*	*3*	*4*	*5*
Yield (g)	0.977	0.720	0.626	1.443	2.026
R_G	2.3	1.9	1.6, 1.5	1.5	1.5, 1.2

Examination of fractions. — (*a*) Fraction 1 crystallized on refrigeration. The crude product (0.6 g) was recrystallized from absolute ethanol to afford 1,2-*O*-ethylene-α-D-glucofuranose (**2**), m.p. 215–216°, $[\alpha]_D^{30}$ −55° (*c* 1, water) (Found: C, 46.2; H, 6.41. $C_8H_{14}O_6$ calc.: C, 46.6; H, 6.84%).

Two treatments of **2** (0.2 g) with pyridine (10 ml) and acetic anhydride (2 ml), in the usual manner, gave the acetate, m.p. 95–96° (from cyclohexane) (Found: C, 50.2; H, 5.97. $C_{14}H_{20}O_9$ calc.: C, 50.6; H, 6.06%).

A solution of **2** (0.1845 g) in sodium metaperiodate (250 ml, 0.02M) was kept in the dark at 10°, and the periodate consumption[16] and liberation of formic acid[17] were determined at intervals. The oxidation was complete in 2 h; 1 mole of the oxidant per mole of **2** was consumed, and no formic acid was produced.

To a solution of **2** (0.01025 g) in water (2 ml), 0.3M sodium metaperiodate (2 ml) and M sodium hydrogen carbonate (2 ml) were added, and the reaction mixture was kept in the dark for 1 h at room temperature (30°). N Sulphuric acid (15 ml) was added, followed by M sodium arsenite (5 ml), the volume was made up to 100 ml, and the formaldehyde content (1.04 moles per mole) was determined by the chromotropic acid method.

(*b*). The syrupy fraction *2* crystallized on storage for a few days, and the crude product (0.06 g) was recrystallized from absolute ethanol to give **3**, m.p. and mixed m.p. 209–210°, $[\alpha]_D^{30}$ +55° (*c* 1, water); lit.[10] m.p. 210–211°, $[\alpha]_D^{21}$ +56° (water). The X-ray diffraction pattern was identical with that of authentic **3**.

The mother liquor, after removal of **3**, from fraction *2* yielded 1,2-*O*-ethylene-α-D-glucopyranose (**4**) (0.12 g), which, after recrystallization, had m.p. 134–135°, $[\alpha]_D^{30}$ +89.7° (*c* 1, water); lit.[12] m.p. 131–132°, $[\alpha]_D^{24}$ +96.8° (water).

(*c*) Paper chromatography of fractions *3*, *4*, and *5* showed that all of them contained 2-*O*-(2-hydroxyethyl)-D-glucose (**1**) (R_G 1.5) as the major component. Fraction *4* crystallized from absolute ethanol to yield **1** (0.56 g, in 2 crops). Recrystallization from absolute ethanol gave **1** having m.p. 151–152°, $[\alpha]_D^{30}$ +57.2° (*c* 1, water, equilibrium value); lit.[13] m.p. 151–153°, $[\alpha]_D$ +55° (water). Compound **1** (0.3 g) was also isolated from fraction 5.

Identification of 3-O- *and* 6-O-(*2-hydroxyethyl*)-D-*glucose.* — The component having R_G 1.6 in fraction *3* was identified as 3-*O*-(2-hydroxyethyl)-D-glucose by comparing its chromatographic and electrophoretic mobility with that of an authentic sample. In a similar manner, the component having R_G 1.2 in fraction 5 was identified as 6-*O*-(2-hydroxyethyl)-D-glucose.

Treatment of 2-O-(*2-hydroxyethyl*)-D-*glucose* (**1**) *with methanolic hydrogen chloride.* — A solution of **1** (0.1 g) in 1% methanolic hydrogen chloride (5 ml) was refluxed for 8 h under anhydrous conditions. The solution was neutralized with silver carbonate and filtered through Celite, and the Celite bed was washed with methanol. The combined filtrate and washings were evaporated, and the residue was recrystallized from absolute ethanol to give compound **2**, m.p. and mixed m.p. 215–216°.

Treatment of 2-O-(*2-hydroxyethyl*)-D-*glucose* (**1**) *with sulphuric acid.* — A solution of **1** (0.1 g) in N aqueous sulphuric acid (5 ml) was heated on a boiling-water bath. Samples were withdrawn at intervals, neutralized by anion-exchange resin, and examined by t.l.c.

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