GENERAL METHODS AND TECHNIQUES

1. **Evaporation**
   
   Unless otherwise stated, solutions were concentrated in vacuo at temperatures below 50°.

2. **Melting Points**
   
   Melting points, determined in thin-walled capillaries, are uncorrected.

3. **Optical Rotation**
   
   Optical rotations, determined at room temperature (25 - 30°) in 0.5 dm tube with a Hilger polarimeter, are equilibrium values, unless otherwise mentioned.

4. **Paper Chromatography**
   
   Routine qualitative separations were made by descending technique on Whatman No.1 filter paper. The separated materials were detected on the paper by one of the following reagents: (a) periodate-benzidine\textsuperscript{12}, (b) p-anisidine hydrochloride\textsuperscript{13}, (c) acetonic silver nitrate-alcoholic sodium hydroxide\textsuperscript{14}, and (d) aniline phosphate.
Quantitative separations were carried out either on Whatman No.1 or No.3 MM papers, the components being located by spraying marginal strips with reagents a or b and extracting them from the unsprayed central portion of the chromatogram with water, followed by 50% aqueous ethyl alcohol. The aqueous as well as alcohol extracts were mixed and evaporated to dryness.

5. Electrophoresis

Electrophoresis of the compounds was carried out on Whatman No.1 filter papers using 0.1 M borate buffer at an applied potential of 500 volts for 3 h. Spray reagents a and b were employed for locating the separated materials. Rg and Mg refer to rates of movement of sugars on paper relative to D-glucose and tetra-O-methyl-D-glucose respectively.

6. Thin-Layer Chromatography (TLC)

TLC was carried out on silica gel, (National Chemical Laboratory, Poona) on (11 cm x 22 cm) glass plates. Detection of the sugars and their derivatives was effected by spraying the plates with conc. sulphuric acid followed by heating for 15 min. at 110-120°.
7. **Column Chromatography**

Column chromatography was performed with silica gel obtained from National Chemical Laboratory. Cellulose column chromatography was performed with cellulose powder (BDH, made in England) using appropriate solvent systems.

8. **Gas-Liquid Chromatography (GLC)**

Gas chromatography was carried out on Pye-Argon Chromatograph equipped with an ionization detector. A 4 ft. x 4 mm. glass column packed with 10% W/W SE-52 on silanized chromosorb-W (60-80 mesh) was used for separation. The column was maintained at 200°. Argon with a flow rate of 50 ml/min, was used as the carrier gas. The retention times (Rf) were calculated from the beginning of the solvent peak. The trimethyl silyl (TMS) derivatives used for GLC were prepared by the method of Lott and Brobst according to which 10 mg. of the dry sample was dissolved in absolute pyridine (1 ml.) followed by the addition of hexamethyl disilazane (1 ml) and trifluoroacetic acid (0.1 ml) and vigorous shaking.

9. **Infrared Spectroscopy**

I.r. spectra were taken on Perkin Elmer-Infrared 137, a double beam instrument. The samples
were examined in any of the forms mentioned below:

(i) As solutions in dry chloroform or dry carbon tetrachloride using 0.1 mm. thickness sealed cells.

(ii) As pellets of potassium chloride or potassium bromide.

(iii) As mulls in paraffin.

10. Acid Hydrolysis

Acid hydrolysis of the samples was carried out in 2N-sulphuric acid for 6 to 8 h on a boiling water bath. The hydrolysates, after deionization with Amberlite IR-120 (H⁺) and IR-45 (OH⁻) resins, were concentrated for further examination.

11. Determination of D.S.

Morgan's method16 for determination of ether and ethers of ethylene glycol, as modified by Lortz17, was used for estimating the D.S. of hydroxyethylated materials.

12. Purification of Solvents

The solvents were purified and dried by the recommended procedures. DMF was dried by successive distillations from sodium hydroxide,
calcium oxide and phosphorous pentoxide, and collecting the final distillate over calcium hydride. DMSO (super dry) was prepared by drying it over caustic soda followed by drying over calcium hydride.

MATERIALS

Hydroxyethyl Corn Starch

The product used in this investigation was a commercial sample of approximate D.S.(0.1) supplied through the courtesy of Dr. C.L. Mehlretter of the Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Illinois, U.S.A.