Synthetic Polysaccharides. VI. Preparation of Basic Derivatives of Polyglucose[1]

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Preparation of highly substituted polycationic derivatives of synthetic polyglucose is reported. Of the various methods investigated for the introduction of the basic amine groups, the most successful was the reaction of 1-diethylamino-2,3-epoxypropane with polyglucose in aqueous sodium carbonate, which gave the corresponding diethylaminohydroxypropyl ethers with a maximum substitution of 1.6 groups per anhydroglucose unit. Such derivatives were converted to the corresponding quaternary ammonium hydroxides or chlorides. Methods of preparation and physical data such as pK' values are presented for the different derivatives. These polycations have a highly branched structure, are soluble in water and in certain organic solvents, and are useful tools in studies of interaction of charged macromolecules.

Synthesis of water-soluble polyelectrolytes of branched compact structure containing a large number of cationic groups in a small space was the object of the experiments reported here. Effect of polyelectrolytes on enzyme action[2,3] and on virus inhibition[4,5] demonstrated the need for these derivatives.

For the synthetic studies we used the chemically synthesized polyglucose[6] prepared with various degrees of branching and molecular weight.[7] It has a compact, spherical structure[8] and is highly water-soluble.

We attempted to introduce the cationic groups into the synthetic polyglucose by substituting various percentages of the available hydroxyl groups on the anhydroglucose units with different amine groups. We hoped to obtain polymers with graded variations in molecular properties, such as molecular weight, degree of branching, number of the substituting cationic groups per molecule, and strength (pK') of cationic groups.

In most instances, the published methods led only to a low degree of substitution and caused excessive breakdown of the natural polysaccharides. For our purpose, it was important to select a method which would provide controlled degrees of substitution up to very high degrees of substitution, and reaction conditions which would not cause polymer breakdown.

Results and Discussion

Diethylaminoethyl Ether of Polyglucose.—Following a modified procedure for the preparation of diethylaminoethyl (DEAE) ethers of cellulose,[9] we obtained basic products from polyglucose which indicated 0.1 DEAE group for each anhydroglucose residue on the basis of nitrogen content. This degree of substitution (D.S.) is comparable to the DEAE ion exchange cellulose used in chromatography.[9] Currently a modification of the same reaction using 2-chlorotriethylamine and sodium hydroxide was reported to give dextrans with as high as 0.8 degree of substitution by using drastically increased concentrations of alkali and of other reactants.[10] Since we expected that strong base would break down the polyglucose, this method was not used.

Reaction of Polyglucose with Ethylenimine.—This reaction was expected to give the 2-aminoethyl ether of polyglucose. Using essentially the same procedure as for starch,[11] we obtained two basic derivatives; one having 0.1 and the other 0.2 degree of substitution. This was about the same range as was obtained on starch.[11] Contrary to expectation, when we used more vigorous reaction conditions the polyglucose derivative had a lower degree of substitution. It is possible that at the higher temperature and longer reaction time the ethylenimine tends to polymerize with itself at the expense of the addition reaction with the polyglucose.

Treatment of Tosyl Derivative of Polyglucose with Ethylenediamine.—Employing another approach we attempted to replace the tosyl grops in a polyglucose tosylate with 2-aminoethylamino group by using ethylenediamine under conditions similar to those previously employed for the partial replacement of sulfonyl groups of various sulfonated starches.[12] Our product, which showed 0.75

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[3] P. T. Mora, B. G. Young, and M. J. Shear, *Makromol. Chem.*, **37**, 212 (1960).

[4] B. G. Young and P. T. Mora, *Virology*, **12**, 493 (1960).

[5] P. T. Mora, B. G. Young, and S. Rizvi, *J. Biol. Chem.*, in press.

[6] P. T. Mora and J. W. Wood, *J. Am. Chem. Soc.*, **80**, 685 (1958).

[7] P. T. Mora, J. W. Wood, P. Maury, and B. G. Young, *ibid.*, **80**, 693 (1958).

[8] P. T. Mora, *J. Polymer Sci.*, **23**, 345 (1957).

[9] E. A. Peterson and H. A. Sober, *J. Am. Chem. Soc.*, **78**, 751 (1956).

[10] W. McKernan and C. R. Ricketts, *Chem. Ind.* (London), 1490 (1959).

[11] R. W. Kerr and H. Neukom, *Stärke*, **4**, 255 (1952).

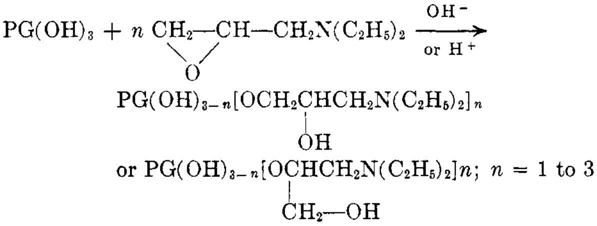
[12] A. A. Houghton, U. S. Patent 2,206,354.

aminoethylamino groups and 1.0 remaining tosyl groups per anhydroglucose unit on the basis of the nitrogen and sulfur content, was insoluble in water and in the organic solvents which were tried. This insolubility indicates a high degree of cross-linking due to the bi-functionality of the diamine substituent.[13]

Reduction of the Cyanoethyl Ethers of Polyglucose.—In treating polyglucose with acrylonitrile in dilute aqueous alkali the 2-cyanoethyl ether of polyglucose was readily obtained in good yield and in high degree of substitution (2 to 2.6 D.S. per monomeric unit). By one modification (experiment A) of a general procedure recommended for monomeric aliphatic and aromatic nitriles[14] it was possible to reduce the cyanoethyl derivative of the polyglucose with lithium aluminum hydride, using a one to one ratio of hydride to nitrile group.[15] The 3-aminopropyl derivative thus obtained behaved as a strong base, and contained 0.8 of a 3-aminopropyl group per anhydroglucose unit. The yield of the desired product was somewhat low, however, probably because of the difficulty of recovery from the inorganic by-products. Tetrahydrofuran was used in experiment B, since it is a better solvent than diglyme for the hydride (the diglyme is a better solvent for the nitrile), but the reduction was only partially completed, yielding a product with 0.1 of a 3-aminopropyl group and 0.9 of a 2-cyanoethyl group per A.G.U. (anhydroglucose units) as indicated by titration, and the infrared spectra. In a third experiment an attempt was made to reduce the nitrile with 3:1 molar complex of sodium borohydride and anhydrous aluminum chloride[16]; however, the reaction product was completely soluble in alcohol and was lost upon dialysis, indicating that extensive degradation had occurred, probably through cleavage and reduction of the hemiacetal linkages between the anhydroglucose units. All of these reduction experiments of the cyanoethyl ethers of polyglucose with the metal hydrides were carried out under an atmosphere of dry nitrogen, because of the oxygen-sensitivity of the intermediate hydride complexes.

Following a slight modification of a method used by Freifelder[17] for conversion of aliphatic nitriles to the amines by catalytic hydrogenation with 5% rhodium-on-alumina, an unsuccessful attempt was made to reduce the 2-cyanoethyl ether of polyglucose in ammoniacal Methyl Cellosolve solution.

Reaction of Polyglucose with 1-Diethylamino-2,3-epoxypropane.—The most useful procedure we found for preparation of cationic tertiary amine derivatives with high and controlled degree of substitution was treatment of polyglucose with epoxyamines, following a method previously employed on alkali cellulose[18] For example, 1-diethylamino-2,3-epoxypropane is expected to react with polyglucose to give a mixture of the isomeric 3-diethylamino-2-hydroxy-1-propyl) and (3-diethylamino-1-hydroxy-2-propyl) ethers, depending on how the epoxide ring is opened:



$$PG(OH)_3 + n\ CH_2{-}CH{-}CH_2N(C_2H_5)_2 \xrightarrow[\text{or } H^+]{OH^-} PG(OH)_{3-n}[OCH_2CH(OH)CH_2N(C_2H_5)_2]_n \text{ or } PG(OH)_{3-n}[OCH(CH_2{-}OH)CH_2N(C_2H_5)_2]n;\ n = 1 \text{ to } 3$$

In this scheme $PG(OH_3)$ represents the starting polyglucose with an average of three free hydroxyls per anhydroglucose units.[6–8]

Using various concentrations of aqueous sodium carbonate solution in place of the sodium hydroxide,[18] we succeeded in synthesizing amino derivatives with various degrees of substitution without apparent degradation of the macromolecular structure. The reaction was also carried out in the presence of hydrochloric acid, but this resulted in lower degrees of substitution.

It can be seen from Table I that the highest degree of substitution (1.6) was obtained when the molar ratios of epoxyamine to polyglucose, were between 7:1 and 8:1, and the molar ratios of epoxyamine to carbonate were between 3.5:1 and 4:1 (expt. IX and XI). The slight differences in the concentrations of the sodium carbonate (25 to 28%) were probably not significant when comparing the molar ratios of the reactants under otherwise similar conditions (expt. III–XII). Aqueous bicarbonate failed to bring about a satisfactory reaction of the epoxyamine with polyglucose (XIII). Using polyglucose and epoxyamine only, the product had 0.3 degree of substitution (XIV). Carrying out a similar reaction under aqueous conditions, a product with 0.6 D.S. was obtained in good yield (XV). When hydrochloric acid concentrations were used which only partially neutralized the amine group of the epoxyamine (XVI and XVII), and when a lower ratio of epoxyamine to polyglucose (2 to 1) was used, the products had substitution in the range of 0.2–0.4. A complete neutralization of the amine group of the intermediate with hydrochloric acid

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(13) Recently Prof. E. Husemann (personal communication) has informed us that she has been able to obtain similar derivatives of amylose which were soluble in water, by heating tosylated amylose with 10–20-fold amounts of anhydrous ethylenediamine at 100° for four to five hours. She also stated, that tosylates with higher degrees of substitution and with tosyl groups on the secondary hydroxyls may require heating for ten to fifteen hours to complete the reaction with ethylenediamine.

(14) W. G. Brown, *Org. Reactions*, **6**, 480 (1951).

(15) *Cf.*, L. H. Amundsen and L. S. Nelson, *J. Am. Chem. Soc.*, **73**, 242 (1951).

(16) H. C. Brown and B. C. Subba Roa, *J. Am. Chem. Soc.*, **78**, 2582 (1956).

(17) M. Freifelder, *J. Am. Chem. Soc.*, **82**, 2386 (1960).

(18) G. Montégudet, *Peintures, Pigments, Vernis*, **34**, 204, 271, 311 (1958).

TABLE I

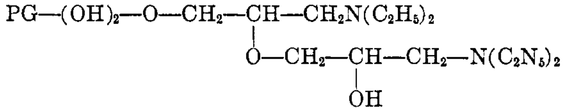
SYNTHESIS OF 3-DIETHYLAMINO-2-(& 1)-HYDROXY-1-(& 2)-PROPYL ETHERS OF POLYGLUCOSE

Expt. No.	Polyglucose,[a] A.G.U.	Epoxyamine		Inorganic Reagent				Reaction		Product			
										Yield[b]			
		Mole	Ratio[f]	Formula	Mole	Ratio[f]	Aqueous concn., %[g]	Temp.	Time, hr.	g.	%[h]	Nitrogen[c] fd., %	D.S.[d,e]
I	0.015	0.060	(4)	Na_2CO_3	0.060	(4)	13.7	66 ± 1	4	(C)1.4	53.2	0.87	0.1
II	.015	.060	(4)	Na_2CO_3	.060	(4)	28.0	66 ± 1	4	(C)0.78	27.7	1.42	.2
III	.015	.060	(4)	Na_2CO_3	.060	(4)	28.0	100 ± 1	3	(D)0.88	26.2	3.02	.5
IV	.015	.060	(4)	Na_2CO_3	.060	(4)	28.0	100 ± 1	4	(D)1.02	25.6	4.30	.8
V	.015	.060	(4)	Na_2CO_3	.045	(3)	28.0	100 ± 1	4	(D)1.21	30.4	4.33	.8
VI	.006	.039	(6.5)	Na_2CO_3	.012	(2)	24.6	100 ± 1	2.5	(D)0.7	44.9	3.78	.7
VII	.031	.193	(6.2)	Na_2CO_3	.062	(2)	25.8	100 ± 1	3	(A)3.50	40.7	4.50	0.9
										(B)0.79	8.2	6.24	1.7
VIII	.031	.193	(6.2)	Na_2CO_3	.062	(2)	28	100 ± 1	3	(D)3.77	41.9	4.83	1.0
IX	.077	.54	(7.0)	Na_2CO_3	.154	(2)	28	100 ± 1	4	(A)2.1	9.3	4.90	1.0
										(B)14.2	49.7	6.11	1.6
X	.031	.248	(8)	Na_2CO_3	.062	(2)	28	100 ± 1	3	(A)1.78	21.8	4.18	0.8
										(B)2.57	25.3	5.51	1.3
XI	.031	.248	(8)	Na_2CO_3	.062	(2)	28	100 ± 1	4	(A)1.47	15.5	5.16	1.1
										(B)1.86	16.3	6.13	1.6
XII	.006	.06	(10)	Na_2CO_3	.06	(10)	28	100 ± 1	4	(A)0.28	17.1	4.27	0.8
										(B)0.23	11.1	5.69	1.4
XIII	.015	.060	(4)	$NaHCO_3$	.060	(4)	9.2	Room temp.	20	(C)1.8	..	0.23	.1
XIV	.015	.083	(5.5)	None				101 ± 1	4	(D)1.65	53.2	2.13	.3
XV	.015	.083	(5.5)	H_2O (21 ml.)				97 ± 1	4	(C)2.9	80.8	3.36	.6
XVI	.031	.062	(2)	HCl	.0005	(0.016)	0.01 *N*	99 ± 1	2	(C)0.6	9.3	1.68	.2
										(D)3.0	46.2	2.38	.4
XVII	.031	.062	(2)	HCl	.004	(0.16)	0.08 *N*	100 ± 1	2	(C)2.9	56.3	1.85	.3
										(D)0.8	14.7	1.94	.3
XVIII	.031	.062	(2)	HCl	.065	(2.1)	0.74 *N*	100 ± 1	1	(C)3.5	..	0.25	.1

[a] Polyglucose, sample No. 10-98A (I), fractionated within 0–60% ethanolic limits, with a mol. wt. (number average) $\bar{M}_n$ = 26,200, was used in expts. I, IX, XIV, XV, XVI, XVII, and XVIII while sample No. 4-104-2, fractionated within 0–90% ethanolic limits, with $\bar{M}_n$ = 6600, was used in the remaining experiments. [b] (C) Insoluble both in 90% C_2H_5OH and in $(C_2H_5)_2O$: 90% C_2H_5OH (5:1); (D) soluble in 90% C_2H_5OH and insoluble in $(C_2H_5)_2O$: 90% C_2H_5OH; (A) a fraction, obtained as outlined in Fig. 2, with similar solubility to (D); (B) a fraction, obtained as outlined in Fig. 2, soluble in both the above solvent mixtures. [c] Calculated nitrogen content for 1 D.S. was 4.81%. [d] All products, with exception of those from expt. I, XIII, and XVIII, gave a precipitate with polyglucose sulfuric acid ester (containing three SO_4H groups/A.G.U.). [e] D.S. = degrees of substitution of the diethylaminohydroxypropyl group per anhydroglucose unit of polyglucose. [f] Molar ratio of reactant in respect to anhydroglucose unit taken as one. [g] W./w. basis. [h] Calculated on the basis of what the maximum theoretical yield would be if all the polyglucose had reacted to give the derivative having the D.S. indicated below.

failed to result in any significant substitution (XVIII).

The diethylaminohydroxypropyl ethers of a relatively low degree of substitution were soluble in water only, while those of an intermediate D.S. were soluble in water and in 90% ethanol. On further increase in the D.S. the products became soluble in a 5:1 mixture of ethyl ether and 90% ethanol, while retaining solubility in the previously mentioned solvents. Infrared analysis, however, failed to show any significant differences between the spectra of the fractions which were insoluble and soluble, respectively, in 90% ethanol (fractions C and D, expt. XVI and XVII). From another preparation (IX), the ether–90% ethanolic soluble fraction B had the same infrared spectrum as the fraction A, the latter being insoluble in this mixture. It is possible that the ether-soluble derivative has a second substituting group on the same side chain, since a new hydroxyl is formed on the propyl chain after the opening of the epoxy ring, and this hydroxyl might now be a site for attack by a second epoxyamine, which would result in two substituting groups on a single hydroxyl of the polyglucose:



$$\begin{array}{l} \text{PG—(OH)}_2\text{—O—CH}_2\text{—CH—CH}_2\text{N(C}_2\text{H}_5)_2 \\ \qquad\qquad\qquad\quad | \\ \qquad\qquad\qquad\quad \text{O—CH}_2\text{—CH—CH}_2\text{—N(C}_2\text{N}_5)_2 \\ \qquad\qquad\qquad\qquad\qquad\quad | \\ \qquad\qquad\qquad\qquad\qquad\quad \text{OH} \end{array}$$

Such substitution might continue on the hydroxyl of the propyl groups, and this might increase the ether solubility of the product, while it would not lead to any difference in the elemental analysis or in infrared absorption, as compared with the derivative when two different hydroxyls on the glucose unit react with two molecules of epoxyamine.

Preparation of the Quaternary Ammonium Derivative.—The diethylaminohydroxypropyl ether of polyglucose, obtained by the above reaction, was treated with ethyl iodide to obtain the corresponding quaternary ammonium iodide. The iodide was then converted to the free base by treatment with anion exchange resin. A titration curve of the strongly basic quaternary derivative is shown in Fig. 1, indicating two cationic groups, one with a p*K*′ of 7.4 and a stronger one with p*K*′ 9.5.

The quaternary ammonium hydroxide could not be prepared conveniently in the usual manner by shaking the iodide with an aqueous or ethanolic suspension of silver oxide, since the hydroxide became contaminated with dissolved or colloidal silver iodide, probably through a complex ion formation or sequesterization by the polyglucose derivative. Attempts to precipitate the silver iodide from this solubilized state as the sulfide, dichromate of ferrocyanide also failed. However, upon addition of concentrated nitric acid the silver did precipitate as the iodide.

The reaction of polyglucose with 1-diethylamino-2,3-epoxypropane and the ready conversion of the resulting tertiary amine to the quaternary ammonium compound thus gave a convenient method for preparing in good yield a series of high molecular weight basic polyglucose derivatives with graded substitution up to 1.6 cationic groups per glucose residue. It is expected that similar methods can be applied to other synthetic polysaccharides, prepared by the polycondensation of aldoses.[19]

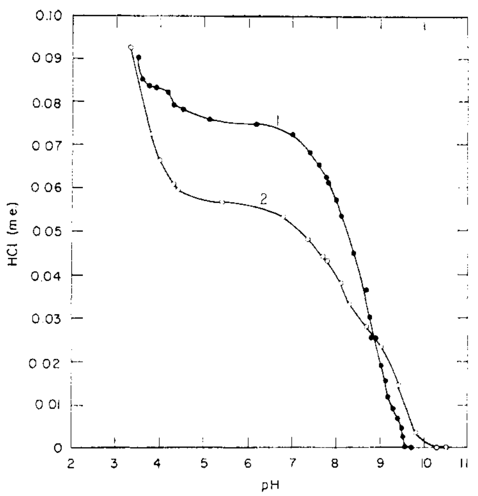


Fig. 1.—Titration curves of (1) 3-diethylamino-2-hydroxyl-1-propyl ether of polyglucose (Table I, expt. IX, product B), and (2) the corresponding poly-{[3-(α-D-glucopyranosyl)-2-hydroxy-1-propyl]triethylammonium hydroxide.} In general, the titrations of the various polyglucose amines were carried out automatically using the Metrohm potentiograph, as follows: The aqueous solutions of the derivatives were made acidic with 1 *N* hydrochloric acid and were boiled 1 min. to decompose any carbonate salts present. The solutions were then cooled to room temperature and the pH readjusted to 11–11.5 with 1 *N* sodium hydroxide. All operations were carried out under nitrogen. After correcting for water blank, the *pK*′ values were estimated from the center of the symmetrical portion of each curve. These values were then checked for agreement with inflections in the first derivative curve as determined by the above instrument during a duplicate titration.

The resulting polycations are unique in several respects. Because of the highly branched polymeric core of the carbohydrate residues, large numbers of cationic groups are concentrated in a small space, and are held together rigidly through the covalent linkages. Because of the hydrophilic nature of the carbohydrate core, most of the derivatives are highly water-soluble. The remaining carbohydrate hydroxyls are available for substitution to yield different derivatives (*e.g.*, methyl, ethyl, benzyl, carboxymethyl, nitrate, etc.). For example, a graded substitution of a cationic polyglucose derivative with increasing amounts of methyl groups would yield a series of polymers suitable for study of the relative importance of

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[19] P. T. Mora and J. W. Wood, *J. Am. Chem. Soc.*, **82**, 3418 (1960).

electrostatic forces to hydrophobic bonds[20] in macromolecular interactions.

Experimental

Polyglucose.—In the majority of the synthetic studies sample A was used as starting product, unless otherwise noted. This sample A was prepared by an acid-catalyzed (0.164% phosphorous acid) polymerization of glucose, first at 140–141° for 1 hr. then at 148–150° for 26.5 hr., in a glass resin flask under conditions similar to those of the first stage of the "two-stage" polymerization method, published previously (expt. 5, Table I, ref. 6). Fractionation of the crude polymer within 0–90% limits of ethyl alcohol followed by freeze-drying of the resulting sirup gave a white powdery product, having a number average molecular weight by reducing end-group method $\bar{M}_n = 6600$, and intrinsic viscosity $[\eta] = 0.05$.

A second sample of polyglucose, sample B, used in the remaining experiments, was prepared by heating glucose under similar conditions to those described above, except that polymerization was carried out without stirring in shallow layers on stainless steel trays in an oven under vacuum at 100–130° for 10.75 hr., then at 130–156° for 8.5 hr. The crude polymer thus obtained was fractionated within 0–60% limits of ethyl alcohol, followed by exhaustive dialysis for 48 hr. in Visking cellophane against running distilled water. The residue upon freeze-drying gave a white powdery polyglucose which was of higher molecular weight: $\bar{M}_n = 26{,}200$, $[\eta] = 0.1$. sedimentation constant $S_{20,w} = 1.2$ (Svedberg units). Sedimentation was carried out in the analytical ultracentrifuge in phosphate buffer $\tau/2$, pH 7.5, and corrections were made to water at 20°.

Reagents.—The 2 chlorotriethylamine hydrochloride and 2-aminoethyl hydrogen sulfate were Eastman Kodak practical grade and were used without further purification. The following reagents were Eastman Kodak pure grade: 2-bromoethylamine hydrobromide, *p*-toluenesulfonyl chloride, ethylenediamine. The diethylamine and epichlorhydrin were Fisher reagent grade. Dimethyl ether of diethylene glycol (Eastman practical grade) was passed through a column of activated alumina (Alcoa, F-20 grade) to remove peroxides and excess moisture. It was further dried by storage over calcium hydride for several days and then distilled from the dehydrating agent; b.p. 160–162°. Tetrahydrofuran (Eastman pure grade) was purified by refluxing over solid potassium hydroxide; the solvent was then decanted and distilled from sodium ribbon, followed by a second distillation from lithium aluminum hydride; b.p. 65–66°. Acrylonitrile (Eastman) was redistilled, b.p. 76.5–77°. The lithium aluminum hydride and sodium borohydride were obtained from Metal Hydrides, Inc. The anhydrous aluminum chloride was Baker and Adamson, reagent grade. The rhodium catalyst, 5% on alumina, was obtained from Baker and Co. Ethylenimine,[21] b.p. 55–57° (ref. 21, 56–58°), yield 35.5%, and 1-diethylamino-2,3-epoxypropane,[22] b.p. 60–65°/20–21 mm. (ref. 22, 62–65°/20 mm.), yield 47%, were prepared according to published procedures.

Diethylaminoethyl Ether of Polyglucose.—To a solution of 14.4 g. (0.36 mole) of sodium hydroxide in 65 ml. of water in a round bottom flask which had been cooled in an ice bath was added 5 g. (0.03 anhydroglucose units = A.G.U.) of polyglucose sample A. When the temperature of this solution had dropped to 3°, there was added in a thin stream with mechanical stirring a solution of 15.6 g. (0.09 mole) of 2-chlorotriethylamine hydrochloride in 40 ml. of water. The temperature rose to 8°, then dropped to 1°, while the stirring was continued for 15 min. The ice bath was replaced with an oil bath and the reaction mixture was heated with stirring to 80° for 35 min. After being cooled to room temperature, the reaction mixture was poured in a thin stream into 600 ml. of cold well stirred absolute ethanol. The precipitate was collected by centrifugation, stirred up with 90% ethanol, and centrifuged again. An aqueous solution of the collected precipitate was then dialyzed in cellophane for 1.5 days against running distilled water. Freeze-drying of the basic (pH 8.3) dialyzed solution gave 2.3 g. of a fluffy, light tan solid, containing 0.8% nitrogen. An aqueous solution of this product did not give a precipitate at room temperature with an aqueous solution of the anionic polyglucose sulfuric acid ester[23] (10 mg./ml.) which had three sulfate groups per anhydroglucose unit (sample H in ref. 23).

A similar experiment in which 2-bromoethylamine hydrobromide was substituted for 2-chlorotriethylamine hydrochloride resulted only in the recovery of the polyglucose.

2-Aminoethyl, Ether of Polyglucose. Method A.—Polyglucose sample A, 20.3 g. (containing 8–10% moisture; 0.11 A.G.U.) and 21.5 g. (0.5 mole) of ethylenimine were placed in a pear-shaped, heavy-walled Pyrex pressure bottle which was equipped with a wired porcelain stopper with a silicon rubber gasket, and the mixture was allowed to stand about 1 hr. at room temperature. The bottle was then heated in an oil bath to 90 ± 1° for 3.75 hr. The bottle was then removed from the bath and allowed to stand overnight. The resulting dark red viscous sirup was dissolved in 75 ml. of water, and the solution was added in a thin stream to 10 times its volume of well stirred, cold, absolute ethanol. The resulting tan precipitate was separated and washed twice with 90% ethanol, centrifuging between each step. After a second precipitation (using a minimum amount of water and 10 times more absolute ethanol in the manner described), the product was dissolved in 100 ml. of water and the resulting solution dialyzed for 2 hr. as described previously. Freeze-drying of the dialyzed solution gave 19.0 g. of a light tan, fluffy solid; yield: 88.8%. (Yields were calculated in all instances on the basis of what the theoretical yield would be of a product having the D.S. indicated by nitrogen content.)

Anal. Calcd. for $[C_6H_9O_5(-CH_2CH_2NH_2)]_n$: C, 46.82; H, 7.37; N, 6.83. Found: C, 44.53; H, 7.13; N, 1.84.

The purified derivative had a degree of substitution of 0.2 2-aminoethyl groups per A.G.U., calculated on the basis of the nitrogen analysis. Sedimentation constants $S_{20,w} = 0.7$. Titration of a 0.1 *N* solution of the base indicated a pK' value of 9.5.

An aqueous solution of the 2-aminoethylpolyglucose derivative gave only a slight precipitate with a solution of polyglucose sulfuric acid ester.

Method B.—Polyglucose sample B, 3 g. (0.019 A.G.U.) and 6 g. (0.139 mole) of ethylenimine were sealed in a heavy-walled, Pyrex tube and allowed to stand overnight. The reaction mixture became viscous and homogeneous, and the tube was then placed in a Carius furnace for 6 hr. at 100–105°. After cooling, the tube was opened, and the viscous, reddish syrup was removed by dissolving it in 50 ml. of water. The solution was added in a thin stream to 600 ml. of cold, well stirred, absolute ethanol. A precipitate separated which was collected by centrifugation and washed with 90% ethanol. The damp product was reprecipitated from aqueous solution with alcohol twice more in the same manner and finally dialyzed. After being dried in a vacuum over phosphorus pentoxide, the aminoethyl ether of polyglucose was obtained as a tan powder weighing 2.4 g. (80% yield); a nitrogen content of 0.61% indicated a 0.1 D.S, per A.G.U. An aqueous solution of the material was basic.

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[20] *Cf.*, W. Kauzmann, *Advan. Protein Chem.*, **14**, 1–63 (1959).

[21] C. F. H. Allen, F. W. Spangler, and E. R. Webster, *Org. Syntheses*, **30**, 38 (1950).

[22] H. Gilman, C. S. Sherman, C. C. Price, R. C. Elderfield, J. T. Maynard, R. H. Reitsema, L. Tolman, S. P. Massie, Jr., F. J. Marshall, and L. Goldman, *J. Am. Chem. Soc.*, **68**, 1291 (1946).

[23] J. W. Wood and P. T. Mora, *J. Am. Chem. Soc.*, **80**, 3700 (1958).

but did not give a precipitate with polyglucose sulfuric acid ester.

Polyglucose Tosylate.—Polyglucose sample A, 11.4 g. (0.07 A.G.U.), *p*-toluenesulfonyl chloride, 54.2 g. (0.28 mole, a 33% excess) and 200 ml. of dry pyridine was stirred and maintained at 48–52° for 12 hr. and 23 min. The clear reddish-brown reaction mixture was cooled and then poured into an ice-water mixture. A tan, creamy, finely divided product separated which was collected by centrifugation, and purified by alternate washing with water and centrifugation until the crude product was neutral to litmus. The material was collected by filtration. After being dried in a vacuum oven (50–60° at 0.1 mm.), the yield was 28.6 g. of a light cream-colored powder, m.p. 141.8–148.6° dec., $[\alpha]^{23.2}\text{D}$ +72.43° (*c* 1.05, $CHCl_3$). The tosylate was soluble in acetone and dimethyl sulfoxide, insoluble in ethanol, ethyl ether, and water.

Anal. Calcd. for $[C_6H_7O_5\cdot(O_2SC_6H_4CH_3)_3]_n$: C, 51.91; H, 4.52; S, 15.40. Found: C, 50.12; H, 4.81; S, 12.9.

The derivative contains 1.5 tosyl groups per anhydroglucose unit on the basis of the sulfur analysis.

Reaction of Polyglucose Tosylate with Ethylenediamine.—Polyglucose tosylate, 2 g., and 5 g. of ethylenediamine were ground to a fine slurry in a mortar. The slurry was then transferred to a small flask with the aid of several more portions of ethylenediamine totaling 15 g., and the mixture was heated in an oil bath to 100° for 10 min. After being cooled to room temperature, the reaction mixture was precipitated by pouring it into 100 ml. of water. The solid was centrifuged off and was washed exhaustively with water. After drying the precipitate in vacuum over phosphorus pentoxide, a brownish horn-like product (1.2 g.) was obtained. The material was insoluble in alcohol and water.

Anal. Calcd. for $[C_6H_8O_5\cdot(NHCH_2CH_2NH_2)(SO_2C_6H_4CH_3)]_n$: N, 7.82; S, 8.94. Found: N, 5.59; S, 8.05.

Polyglucose 2-Cyanoethyl Ether.—A mixture of 10 g. (0.062 A.G.U.) of polyglucose sample A, 12 ml. of 1% (w./v.) aqueous sodium hydroxide, and 70 ml. (55.8 g., 1.05 moles) of freshly distilled (b.p. 76.5–77°) acrylonitrile was stirred and heated under reflux. During the active reflux period of 55 min., the temperature of the reaction mixture was 66–73°. At the end of this period the refluxing had ceased, and it did not resume when the temperature of the bath was raised several degrees. The cooled reaction mixture was poured into 400 ml. of ice-cold 50% ethanol. The supernatant was decanted from the gummy residue and the latter was triturated, first with an excess of water, then with 10% (w./v.) hydrochloric acid until it became acid to Bromthymol Blue indicator. The residue was finally triturated with water until it became neutral to litmus. Drying the gum in vacuum over phosphorus pentoxide for 1 week gave 15 g. of an amber-colored, brittle resin which still had an odor resembling that of a methacrylate ester. An additional precipitation was performed by adding a solution of this crude nitrile in 35 ml. of acetone in a thin stream to a well stirred, ice-cold mixture of 350 ml. each of absolute ethanol and absolute ether. The precipitate was separated by centrifugation, washed with absolute ether, recentrifuged, and dried in vacuum over calcium chloride. The purified nitrile was obtained as a fine white powder weighing 6.4 g.; $[\alpha]^{25.4}\text{D}$ +34.58° (*c* 1.0, acetone).

Anal. Calcd. for $[C_6H_7O_5(-CH_2CH_2CN)_3]_n$: C, 56.06; H, 5.96; N, 13.08. Found: C, 54.21; H, 6.05; N, 12.01.

On the basis of the nitrogen analysis, the product had a degree of substitution of 2.6 cyanoethyl groups per anhydroglucose unit.

In a larger scale preparation in which 30 g. (0.19 A.G.U.) of polyglucose, 36 ml. of 1% sodium hydroxide solution, and 200 ml. (159.5 g., 3 moles) of acrylonitrile were heated to reflux for 98 min. at 69–82°, a yield of 41.3 g. of purified nitrile was obtained.

Anal. Found: C, 54.22; H, 6.16; N, 10.57.

This analysis indicates a D.S. of 2.0 cyanoethyl groups per anhydroglucose unit for this preparation.

Reduction of Polyglucose Cyanoethyl Ether. A. With Lithium Aluminum Hydride Using Diglyme as Solvent.—A solution of 3 g. (0.01 equiv. wt. units) of polyglucose cyanoethyl ether (2.6 D.S.) in 30 ml. dimethyl ether of diethylene glycol (diglyme) was added to a suspension of 1.9 g. (0.05 mole) of lithium aluminum hydride in 75 ml. of diglyme over a period of 10 min. The temperature of the reaction mixture rose gradually to 43°. With continued stirring the reaction mixture was slowly heated in an oil bath from 43° to 66° over a period of 10 min., and finally from 66° to 71° over 18 min. more. The reaction mixture was then cooled externally and 25 ml. of an aqueous 25% sodium hydroxide solution was added dropwise with stirring. Because of the difficulty of separating the resulting gelatinous mixture, it was diluted with 125 ml. of water and acidified to pH 4.6 with concd. hydrochloric acid. After standing several hours, the aluminum hydroxide had partly separated, leaving a clear supernatant layer. The precipitate was then centrifuged off, washed with water, and after being recentrifuged it was discarded. The combined supernatant layer and washings were adjusted to pH 8 with concd. ammonium hydroxide, and the aluminum hydroxide which separated was removed by centrifugation and discarded. After being adjusted to pH 11 with concd. ammonium hydroxide, the solution was dialyzed overnight against running distilled water. A small amount of aluminum hydroxide which had again separated was removed by filtration, and the filtrate (pH 9.3) was freeze-dried, giving 0.7 g. of a fluffy white derivative. The material contained 0.8 3-aminopropyl groups per A.G.U., based on a nitrogen content of 5.43%. This derivative was essentially free of any unreduced nitrile as indicated by its infrared spectrum.[24] The derivative gave a strong precipitate in an aqueous solution with polyglucose sulfuric acid ester.

B. Partial Reduction with Lithium Aluminum Hydride Using Tetrahydrofuran as Solvent.—A solution of 5.6 g. of dry polyglucose 2-cyanoethyl ether (2.6 D.S., 0.019 equiv. wt. unit) in 100 ml. of tetrahydrofuran was added dropwise to a well stirred slurry of 4.0 g. (0.11 mole) of lithium aluminum hydride in 100 ml. of tetrahydrofuran under an atmosphere of dry nitrogen, over a 30-min. period. The temperature rose slowly to 40° where it was maintained by means of a water bath for an additional 25 min., while the stirring was continued. The reaction mixture was cooled to room temperature and was decomposed cautiously by the dropwise addition of 4 ml. of water followed by the addition of a solution of 40 g. of sodium potassium tartrate in 160 ml. of water. After standing for some time the aqueous layer was separated and filtered to remove suspended solid material. The basic filtrate was made acidic (Congo Red) with 10% hydrochloric acid, and the resulting solution was dialyzed for 24 hr. in cellophane against running distilled water. After being filtered to remove a white precipitate of aluminum hydroxide, the dialyzed solution was passed through a column of Amberlite #400 ion exchange resin in the hydroxyl form. The combined eluates and washings which had a pH of 9.4 were freeze-dried to give 0.5 g. of a fluffy white powder.

This derivative contained 6.36% (Dumas) of total nitrogen. Of this value, 0.86% was titratable nitrogen, leaving a difference of 5.5% attributable to unreduced nitrile. On the basis of these values the derivative contained approximately one-tenth of a 3-aminopropyl group and approximately nine-tenth of a 2-cyanoethyl group per A.G.U. respectively. This rather high value for the nitrile group was confirmed by the fact that the infrared spectrum in potassium bromide of this compound gave an absorption maxima at 2250 cm.$^{-1}$, and the height of the peak was approximately one-half that

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[24] We thank Mrs. Katherine Warren of the National Heart Institute for these and other infrared analyses.

given by the 2-cyanoethyl ether of polyglucose which had a D.S. of 2.

Preparation of Polyglucose 3-Diethylamino-2(or 1)-hydroxy-1-(or 2)-propyl Ethers.—A summary of the different reaction conditions which were used to prepare this derivative with different degrees of substitution is presented in Table I. The following examples illustrate the procedures.

Experiment VII. A solution of 5 g. (0.03 A.G.U.) of polyglucose sample A, 6.6 g. (0.06 mole) of sodium carbonate, and 25 g. (0.19 mole) of 1-diethylamino-2,3-epoxypropane in 19 ml. of water was heated to reflux (internal temp. 102°) in an oil bath for 3 hr. The mixture which had separated into two dark reddish clear liquid phases was allowed to cool to room temperature and poured into 10 vol. of absolute ethanol with stirring. The mixture was centrifuged to remove sodium carbonate. To the supernatant was added 5 vol. of absolute ether with stirring. A precipitate separated, and the supernatant was set aside. The precipitate was collected by centrifuging. The product was reprecipitated twice more by dissolving it in 90% ethanol and adding 5 vol. of ethyl ether, followed by solution in water and dialysis in cellophane against running distilled water for 23 hr. On being freeze-dried, the solution gave 3.5 g. (41% yield) of a light tan fluffy powder containing 4.5% nitrogen, which represents a D.S. of 0.9 diethylaminohydroxypropyl groups per A.G.U. (See product A, expt. VII). An aqueous solution of the material was strongly basic and gave a precipitate with polyglucose sulfuric acid ester. The supernatant, containing the ether-soluble fraction, on being concentrated to dryness *in vacuo* on the steam bath, gave a dark red resin. Exhaustive dialysis of an aqueous solution of this material followed by freeze-drying of the dialyzed solution gave 0.79 g. of a fluffy tan powder containing 6.24% nitrogen (1.7 D.S.) (see expt. VII, product B). An aqueous solution of this substance was basic and gave a precipitate with polyglucose sulfuric acid solutions.

Under more drastic reaction conditions, and in larger scale preparations the yields of the ether soluble fractions increased (see expt. IX, X, and XI). This may be because of multiple substitutions on the same carbohydrate hydroxyls (see discussion).

A titration curve of product B expt. IX is shown in Fig. 1 (curve 1). It is estimated that one of the four titratable groups has a pK' value of about 3.5, while the remaining three groups have an average pK' value of 8.5–9.

In experiments I through XII the samples were prepared in a manner similar to that of VII. Sedimentation constant of the product from experiment VIII (ether-insoluble) had a sedimentation constant of $S_{20,w} = 1.2$, as determined in the ultracentrifuge.

Conditions and results are summarized in Table I.

Experiment XVI. A solution of 5 g. (0.031 A.G.U.) of polyglucose sample B and 8 g. (0.062 mole) of 1-diethylamino-2,3-epoxypropane in 50 ml. (0.5 meq.) of 0.01 *N* hydrochloric acid was heated to reflux at 99° for 2 hr. The resulting dark reddish solution was cooled to room temperature and added in a thin stream to 500 ml. of ice-cold well stirred absolute ethanol. The flocculent precipitate was separated by centrifugation, washed with 90% ethanol, followed by recentrifugation. The pooled supernatant and ethanolic wash were set aside for later recovery of the alcohol-soluble fraction. The gummy alcohol-insoluble fraction was reprecipitated a second and then a third time from a solution in 30–33 ml. of water (containing 0.2% w./v. sodium chloride) using 10 vol. of absolute ethanol to 1 vol. of the aqueous solution. The supernatant layers and washes from the latter precipitations were discarded as they did not give any precipitate on being added to 5 vol. of absolute ethyl ether. The final precipitate from alcohol was dissolved in water and dialyzed in cellophane against running distilled water for 4.75 hr. The basic solution (pH 9.5) on being freeze-dried gave 0.55 g. (9.3% yield) of a fluffy white powder. The alcohol-insoluble derivative contained 0.2 diethylaminohydroxypropyl groups per A.G.U. based on a nitrogen content of 1.68%. An aqueous solution of this material gave a precipitate with polyglucose sulfuric acid ester.

The pooled alcoholic supernatants and the wash from the first precipitation was added to 5 times its volume of absolute ethyl ether. A precipitate appeared, which was collected by centrifugation; the supernatant layer was discarded. Two more precipitations of the alcohol-soluble fraction were carried out by adding a solution of the material in 100–135 ml. of 90% ethanol to twice its volume of absolute ethyl ether and separating the residue each time by centrifugation. The latter supernatants were discarded since they gave no more precipitates with an excess of ether. The final precipitated gummy residue was dissolved in water and dialyzed in cellophane against running distilled water for 4.75 hr. After dialysis the basic solution (pH 10.3) was freeze-dried and it gave 3.0 g. (46.2% yield) of a fluffy cream-colored powder. The alcohol-soluble derivative contained 2.38% nitrogen which indicates a D.S. of 0.4 diethylaminohydroxypropyl groups per A.G.U.; an aqueous solution of this product gave a precipitate with polyglucose sulfuric acid ester.

For the summary of the results on preparation XVI, and for preparations XVII and XVIII which were obtained in a similar manner, see Table I.

A Summary of the Purification Methods for the Various Basic Derivatives of Polyglucose.—The aminoalkyl ethers of polyglucose and the lower substituted diethylaminohydroxypropyl ethers of polyglucose which were insoluble in alcohol were purified by repeated precipitation from aqueous solutions followed by exhaustive dialysis of the aqueous solutions of the derivatives. The products were then recovered by freeze-drying the dialyzed solutions. The higher substituted diethylaminohydroxypropyl ethers of polyglucose which were soluble in 90% ethanol, and in mixtures of 90% ethanol and ethyl ether were purified according to Fig. 2.

Preparation of the Quaternary Ammonium Derivative from the Diethylaminohydroxypropyl Ether of Polyglucose.—Poly{[3-(α-D-glucopyranosyl)-2-hydroxy-1-propyl]-triethylammoniumhydroxide}: A solution consisting of 2 g. (0.0054 equiv. wt. unit) of 3-diethylamino-2-hydroxy-1-propyl ether of polyglucose (1.6 D.S., fraction B, expt. IX) and 2.5 g. (0.016 mole) of ethyl iodide in 30 ml. of absolute ethanol was heated to reflux for 3 hr. The dark amber-colored reaction mixture was poured into 300 ml. of well stirred ice-cold absolute ether. A precipitate separated which was collected by centrifugation, and was washed several times with ether, followed by recentrifugation each time. After being freed of ether in a vacuum desiccator, the product was obtained as a brown, horny material. This material, without further purification was converted directly to the free base by passing a solution of it in 50 ml. water through a column of Amberlite resin #IRA-401 (OH^- form) and washing the column with water. The strongly basic amber-colored eluate on freeze-drying gave 2 g. (84.0% yield) of a cream-colored fluffy product having an ammoniacal odor.

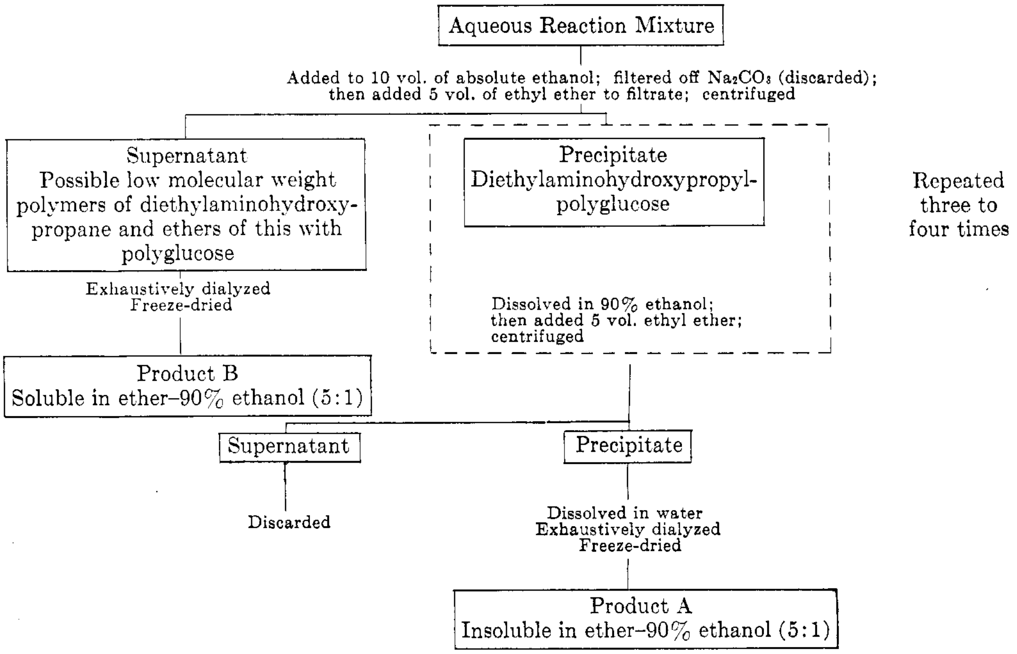
Anal. Calcd. for $\{C_6H_{8.4}O_5[CH_2CH(OH)CH_2—N^+(C_2H_5)_3\ OH^-]_{1.6}\}_n$: N, 5.09%. Found: N, 5.06%.

The quaternary ammonium hydroxide derivatives behaves as a strong base, having two basic pK' values, one 7.4, the other 9.5 (see Fig. 1). An aqueous solution of the material absorbs carbon dioxide quite readily, and gives precipitates both with aqueous solutions of polyglucose sulfuric acid ester and of Reinecke salt.

The corresponding chloride was prepared by neutralizing 0.5 g. of the free base to pH 6.7 with 0.1 *N* hydrochloric acid and dialyzing the resulting solution for a short period in cellophane against distilled water. The dialyzed solution

FIGURE 2

PURIFICATION OF THE ALCOHOL-SOLUBLE DIETHYLAMINOHYDROXYPROPYL ETHERS OF POLYGLUCOSE



when freeze-dried gave 0.44 g. of a light tan, fluffy powder.

Anal. Calcd. for $\{C_6H_{8.4}O_5[\text{—}CH_2CH(OH)CH_2N^+(C_2H_5)_3Cl^-]_{1.6}\}_n$: N, 4.75; Cl, 12.02. Found: N, 4.49; Cl, 10.38.

Attempted Reductions of Polyglucose Nitrate Ester.—Polyglucose nitrate ester (D.S. = 2.2)[25] was treated with sodamide in liquid ammonia. Only highly degraded low molecular weight products resulted, in line with similar experience on reduction attempts of cellulose dinitrate.[26]

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2-Fluoroprednisone

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2-Fluoroprednisone (V) has been synthesized in four steps from cortisone bismethylenedioxy derivative (I) *via* alkoxalylation, treatment with perchloryl fluoride, removal of the blocking group, and 1,2-dehydrogenation by *Nocardia corallina.*

The synthesis of 2α-fluorohydrocortisone has recently been reported from this laboratory.[1] Despite the apparent lowering of glucocorticoid activity caused by the introduction of the 2α-fluorine atom,[1] it was of interest to prepare a Δ1,2-dehydro derivative, since the fluorine atom would then become essentially coplanar with ring A and perhaps confer enhanced biological activity. The electronic interrelationship between the electronegative fluorine atom and the biologically important C_1-C_2 double bond might also be important in this regard. It was decided that our initial effort would be directed to the preparation of 2-fluoroprednisone (V) from 2α-fluorocortisone (IV).

A pathway to 2α-fluorocortisone (IV) which did not involve 2α-fluorohydrocortisone as an intermediate was desirable, since the latter compound is somewhat tedious to prepare in quantity. Introduction of a fluorine atom at the 2α-position of a Δ^4-3-keto steroid is conveniently achieved *via* the reaction of perchloryl fluoride with a 2-alkoxalyl derivative.[1] For this purpose the bismethylene-

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[1] H. M. Kissman, A. M. Small, and M. J. Weiss, *J. Am. Chem. Soc.*, **82**, 2312 (1960).