EFFECT OF BASE CONCENTRATION UPON THE REACTIVITIES OF THE HYDROXYL GROUPS IN METHYL D-GLUCOPYRANOSIDES

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ABSTRACT

The anomeric methyl D-glucopyranosides were treated with *N,N*-diethylaziridinium chloride in various molarities of aqueous sodium hydroxide at variable ionic strength and constant ionic strength. It was found that the extent of reaction of the hydroxyl groups at C-2, C-3, and C-4 decreased with increasing concentration of base. Reaction at the C-6 hydroxyl group remained essentially constant with increasing concentration of base. Neither varying the ionic strength nor the concentration of reagent affected the distribution of substituents at any given concentration of base.

INTRODUCTION

In connection with previous studies concerned with the distribution of substituent linkages to the D-glycopyranosyl residues of cotton cellulose[1], it was observed that the relative reactivities of the hydroxyl groups at C-2, C-3, and C-6 of both the α and β anomers of methyl D-glucopyranoside were dependent upon the concentration of the base in which the reaction was conducted.

It had generally been assumed by investigators in this field that the relative reactivities of the various hydroxyl groups in glucosides and other carbohydrates (and the resulting distribution of substituents in kinetically controlled, substitution-reactions) were independent of the reaction medium and conditions, and were dependent only upon the nature of the chemical reagent involved. However, other, preceding studies have shown that the relative reactivities of the hydroxyl groups in the methyl 4,6-*O*-benzylidene-D-glucopyranosides (and in starch and cellulose) are substantially different for reactions conducted in aqueous and nonaqueous media[2].

The distributions of methyl groups resulting from the partial methylation of methyl glucosides have been reported by several investigators[3,4]. However, in all of these cases the methylations were carried out in relatively concentrated, basic solutions. To our knowledge, no study has been reported on the effect of base concentration upon the reactivity of the hydroxyl groups in methyl glucosides or carbohydrates other than cotton.

This paper describes the results of a study of the effects of base concentration on the reactivities of the hydroxyl groups of simple glucosides.

RESULTS

The effect of concentration of base upon the reaction of *N,N*-diethylaziridinium chloride with methyl β-D-glucopyranoside is summarized in Table I. Very similar results were obtained with the α anomer.

TABLE I

REACTION OF METHYL β-D-GLUCOPYRANOSIDE WITH *N,N*-DIETHYLAZIRIDINIUM CHLORIDE

Molarity of NaOH	*N*, %	*D.S.*	*Distribution of substituents*			
			2-O-	*3-O-*	*4-O-*	*6-O-*
0.1	0.84	0.124	2.08	1.11	0.75	1.00
0.5	0.72	0.105	1.58	0.75	0.48	1.00
1.0	0.58	0.084	1.29	0.61	0.33	1.00
2.0	0.52	0.075	1.09	0.49	0.26	1.00
4.0	0.50	0.072	0.72	0.33	0.11	1.00
6.0	0.38	0.054	0.60	0.44	0.14	1.00

In order to determine whether or not the decrease in reactivity of the hydroxyl groups at C-2, C-3, and C-4, relative to that at C-6, is due to changes in activity coefficients of the individual hydroxyl groups, the ionic strengths of the reaction mixtures were altered. The reaction of *N,N*-diethylaziridinium chloride with methyl β-D-glucopyranoside was carried out in various concentrations of sodium hydroxide together with sufficient sodium chloride to keep the ionic strength constant (6M in Na^+). The resulting distributions of substituents in the 2-*O*-, 3-*O*-, and 4-*O*-positions (relative to that at the 6-*O*-position) in the methyl β-D-glucopyranoside derivative, were essentially the same as those found when the reaction was carried out at variable ionic strenght. The results are shown in Fig. 1. The reactions of *N,N*-diethylaziridinium chloride with methyl α-D-glucopyranoside at variable and constant ionic strength were also carried out; however, the 3-*O*- and 4-*O*-substituted glucosides were not well separated by gas–liquid chromatography in the case of the α-D-derivatives and quantitative determinations could not readily be made for these isomers. The ratio of 2-*O*- to 6-*O*-substitution is shown in Fig. 1. It is clear from the curves in Fig. 1 that the reaction of the methyl D-glucopyranosides with *N,N*-diethylaziridinium chloride is, within limits of experimental error, independent of the ionic strength.

In preceding studies[1], it was found that the ratio of monosubstitution at the 2-*O*-, 3-*O*-, and 6-*O*-positions of the D-glucopyranosyl residues of cotton cellulose was independent of the number of treatments of the cellulose with *N,N*-diethylaziridinium chloride and base, at least up to 5 treatments. The distribution of (2-diethylaminoethyl) substituents in the monosubstituted methyl β-D-glucopyranoside was likewise unchanged from that described in Table I when the reactions were conducted with double the concentration of *N,N*-diethylaziridinium chloride, and the extent of reaction (as indicated by nitrogen content) was substantially higher than those

different *A. vinelandii* strains which had galacturonic acid as the main constituent but which also contained smaller proportions of glucose, rhamnose, and possibly mannuronic acid. Galacturonic acid was identified by colour reactions which distinguish between galacturonic acid and glucuronic acid, and the possibility that these polysaccharides may also consist of mannuronic and guluronic acid residues should not be completely excluded. Based upon a positive reaction with thiobarbituric acid, the authors also reported that one of the strains possibly produces a sialic acid-like component. The possibility that the positive reaction with thiobarbituric acid may be due to degradation products of the polyuronide should, however, be considered. Claus[20] reported that *A. vinelandii* Strain 1484 (Göttingen) produced an extracellular polysaccharide containing L-rhamnose as the main component, but also containing considerable amounts of a component, which he identified as "2-keto-3-deoxygalactonic acid", giving the thiobarbituric acid reaction. The possibility that different polysaccharides are produced by different *A. vinelandii* strains must, therefore, be taken into account, but our results indicate that both yield and composition of extracellular polysaccharide vary significantly with variation of the composition of the medium, and careful studies should be made before differences between strains can be established. Variations in the gross composition of polysaccharides in *A. vinelandii* and other *Azotobacter* species, with variation of the medium, have also been reported by Zaitseva *et al.*[21], who, however, did not identify the uronic acids.

The results indicate that the sequence of the monomers in the *Azotobacter* alginate is of the same type as observed for algal alginates, *i.e.*, that the monomers are distributed in a blockwise fashion along the chain. It is remarkable that such completely unrelated organisms as brown algae and *Azotobacter vinelandii* produce polysaccharides having fine-structures as similar as these results indicate, and it is probable that the mechanism of the biosynthesis in the two types of organisms must be closely related. We have previously suggested that alginate can be described as a penultimate copolymer[22], where the probability of a monomer being mannuronic or guluronic acid is determined by the identity of the next-nearest neighbour.

The uronic acid composition of the alginate shows a remarkable variation, and it is evident that the calcium content of the medium is of importance. Both the results from the experiments with different amounts of calcium in the medium and the change in composition of the alginate produced after different times of incubation indicate that, when the calcium content is low, the alginate produced contains only a small proportion of guluronic acid. The results for a high content of calcium in the medium are less clear, because the alginate that is richest in guluronic acid is produced at an intermediate concentration of calcium in the solution.

An increase in the calcium concentration of the medium, after a certain amount of the alginate is produced, leads to a change in the composition of the alginate, even if the cells have been removed by centrifugation. This observation suggests that the culture medium contains an enzyme which is capable of epimerizing mannuronic acid residues in the alginate molecule to guluronic acid residues in the presence of calcium. The observed variation of the composition of the alginate may, therefore, be

study, the substitution at the 4-*O*-position was not measured. The work described in this paper confirms the earlier conclusions.

The change in the extent of formation of each monosubstituted glucoside with changes in the base concentration of the reaction medium was calculated from the total D.S. and the fraction of each isomer in the mixture. This change is shown in Fig. 2. These curves show that the yields of 2-*O*-, 3-*O*-, and 4-*O*-monosubstituted

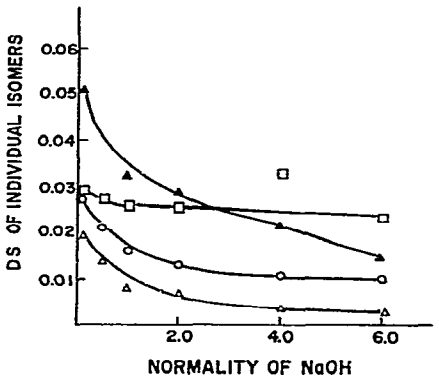

Fig. 2. Extent of formation of the isomeric monosubstituted glucosides as a function of the molarity of sodium hydroxide in the reaction medium: ▲, 2-*O*-(2-diethylaminoethyl)-D-glucopyranoside; ●, 3-*O*-(2-diethylaminoethyl)-D-glucopyranoside; △, 4-*O*-(2-diethylaminoethyl)-D-glucopyranoside; □, 6-*O*-(2-diethylaminoethyl-D-glucopyranoside.

glucosides decrease with increasing concentration of base, whereas the yield of the 6-*O*-monosubstituted glucosides remains essentially constant through the range of base concentration.

DISCUSSION

The suppressing effects that increasing concentrations of sodium hydroxide have upon the reactivities of hydroxyl groups at C-2, C-3, and C-4, versus very little change in that at C-6, are evident from the preceding section and from Fig. 2. In addition to the obvious structural difference between secondary and primary hydroxyl groups, there are substantial differences in acidity among the hydroxyl groups[4,7,8] and the hydroxyl group at C-6 is less sterically restricted[9]. It is known that carbohydrates form hydrates[10] in which water is bound to the hydroxyl groups. The hydroxyl group at C-6 in glucoses or methyl glucosides would be expected to be more highly hydrated (more strongly hydrogen-bonded to water) than the other hydroxyl groups, since primary alcoholic groups are generally considered to be more hydrophilic than secondary ones.

Many complexes between carbohydrates and alkali, alkaline earth salts, and organic bases have been reported[9,10,11]. Rendleman[11] has pointed out that carbohydrates form two types of compounds in alkaline solution; alcoholates (alkoxides) and adducts. The adducts form at vicinal hydroxyl groups.

On the basis of preferential reaction with the hydroxyl group at C-2 in basic media, it has been pointed out by several investigators[3,4] that this hydroxyl group in glucoses (and polysaccharides) is the most acidic. This is rationalized as being because of its proximity to, and by an inductive effect from, the hemiacetal group at C-1. Decreasing acidities of the secondary hydroxyl groups in the order C-2 > C-3 > C-4 are predicted and are consistent with the observed relative order of substitution of 2-diethylaminoethyl groups at these positions. However, it has never been adequately explained why the secondary group at C-2 should be more acidic than the primary hydroxyl group at C-6.

It is proposed that the reactivity of the hydroxyl group at C-6 is repressed by a strong solvent sheath around the oxyanion, whereas the reactivity of the hydroxyl group at C-2 is accentuated by its proximity to the hemiacetal group at C-1 and by hydrogen bonding of the hydroxyl group at C-3 to the oxyanion at C-2.

In dilute base the hydroxyl group at C-2 would then be expected to form alkoxide ions readily. This tendency would account for the high reactivity of the hydroxyl group at C-2 toward *N,N*-diethylaziridinium chloride, since this reagent reacts through the formation of an alkoxide ion[1]. Under these conditions, the alkoxide ion at C-6 is relatively unreactive compared with that at C-2, because of immerion of the former in a larger solvent sheath to which it is interconnected by hydrogen bonds[12].

Increasing the base concentration would promote the formation of adducts between the base and the vicinal hydroxyl groups at C-2, C-3, and C-4. The stability of the sodium hydroxide adducts at the hydroxyl groups at C-2, C-3, and C-4 is sufficiently high to reduce the degree of oxyanion formation at these sites; the result is a decrease in reaction occurring at these hydroxyl groups. The hydroxyl group at C-6 is not vicinal to another hydroxyl group and cannot form this type of adduct; thus, there is very little change in the reactivity of this hydroxyl group with increasing alkali concentration.

It is proposed that the same factors operate in the reactions of fibrous cotton cellulose. However, other factors are also involved in reactions of cellulose because of the heterogeneous nature of the reaction, the occurrence of reactions on solid surfaces, and the selective accessibilities of hydroxyl groups on the surfaces of the crystalline regions.

EXPERIMENTAL

N,N-*Diethylaziridinium chloride.* — To 17.2 g (100 mmoles) of 2-chloroethyldiethylamine hydrochloride in 50 ml of water was added 50 ml of 10% sodium hydroxide solution. From the upper layer (2-chloroethyldiethylamine) that separated, 7.5 g of material was removed and placed in a 100-ml volumetric flask. About 75 ml of water was added and the mixture was shaken until the 2-chloroethyldiethylamine was converted into the soluble *N,N*-diethylaziridinium chloride (10–15 min)[1]. The volume was then adjusted to 100 ml with distilled water.

Reactions of glucosides with N,N-*diethylaziridinium chloride.* — *A. In various molarities of sodium hydroxide.* The glucoside, 1.94 g, (10 mmoles) was dissolved in 25 ml of sodium hydroxide solution of the desired final molarity in a 50-ml volumetric flask. To this solution was added 5 ml (2.50 mmoles) of the *N,N*-diethylaziridinium chloride solution. Then 5.0 ml of sodium hydroxide solution of double the final desired molarity was added and the volume was adjusted to 50 ml with sodium hydroxide of the final desired molarity. The solutions were 0.20 molar in glucoside and 0.05 molar in *N,N*-dimethylaziridinium chloride, and ranged from 0.10 to 6.0 molar in sodium hydroxide. The solution was transferred to a 100-ml round-bottomed flask and shaken for 16 h at 25°. Carbon dioxide was passed through the solution until the minimum pH was reached (about 8.0). Analytical filter-aid (2 g) was added to the neutralized solution to promote friability after freeze-drying. The solution was concentrated and freeze-dried. The residue (consisting of glucoside, substituted glucoside, sodium hydrogen carbonate, and filter aid) was placed in a Soxhlet extractor and extracted for 16 h with dry *p*-dioxane. The product, consisting of glucoside plus substituted glucoside, was dissolved in 10–15 ml of water and freeze-dried to an amorphous solid.

B. In various molarities of sodium hydroxide at constant ionic strength. These reactions were conducted and the products isolated as described under *A*, except that sufficient sodium chloride was added to each reaction mixture so that the total concentration of sodium ions was 6M in each case.

C. Identification and determination of byproduct, (2-hydroxyethyl)diethylamine. A reaction mixture prepared as described in *A* was shaken with ether before neutralization with carbon dioxide. A sample of the ether extract was subjected to g.l.c. analysis with a column of SE-30 operated isothermally at 75°. The chromatogram showed only one peak, which corresponded to that produced by an authentic sample of (2-hydroxyethyl)diethylamine. Neither the substituted glucosides, *N,N*-diethylaziridinium chloride, nor its dimer (tetraethylpiperazinium dichloride), are extracted by ether.

The reaction products prepared as described in *A* were extracted with ether for 8 h in a continuous liquid–liquid extractor before neutralization with carbon dioxide. The boiling flasks each contained 1.0 g of benzoic acid to prevent volatilization of the amino alcohol during evaporation of the ether. The ether was evaporated and nitrogen was determined in the residue by the Kjeldahl method.

*D. Identification of the 2-*O-*, 3-*O-*, 4-*O-*, and 6-*O-*substituted glucosides.* The four peaks obtained by g.l.c. analysis of the reaction products of *N,N*-diethylaziridinium chloride and methyl β-D-glucopyranoside were identified as the *2-O-*, *3-O-*, *4-O-*, and 6-*O*-(2-diethylaminoethyl)-D-glucopyranoses by hydrolytic removal of the aglycon and relating the chromatographic characteristics of the products with those of authentic (2-diethylaminoethyl) derivatives[5]. After hydrolysis, the two peaks representing α and β anomers of the 4-*O*-substituted derivative were observed beneath the first peak of the 3-*O*-, and the second peak of the 2-*O*-substituted derivative, respectively.

*Analysis for 2-*O-*, 3-*O-*, 4-*O-*, and 6-*O-*substituted glucosides.* — The distribution

of substituents among the 2-*O*-, 3-*O*-, 4-*O*, and 6-*O*-positions was determined in the freeze-dried residue (from extraction with *p*-dioxane) by g.l.c. with an Aerograph model 1520* instrument. The column used was a 1/8 in. (o.d.) stainless-steel tube, 5 ft long, packed with 5% SE-30 on Chromosorb W (80–100 mesh). The column was operated isothermally at 180°. All samples were trimethylsilylated by the method of Sweeley *et al.*[6] before chromatographic analysis.

The area of each peak was determined by triangulation, and the distribution of substituent groups was calculated relative to the 6-*O*-derivative as unity, and also as the fraction of the total reaction-product.

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