[CONTRIBUTION FROM THE DIVISION OF PLANT BIOCHEMISTRY, COLLEGE OF AGRICULTURE, UNIVERSITY OF CALIFORNIA]

Starch. V. The Uniformity of the Degree of Branching in Amylopectin

BY A. L. POTTER AND W. Z. HASSID

Tapioca and corn amylopectins were subfractionated by fractional precipitation with methyl alcohol into three and four subfractions, respectively. End-group determinations by the periodate oxidation method showed that each of the three tapioca and each of the four corn subfractions possessed approximately the same degree of branching as its original parent amylopectin material. These data indicate that all the amylopectin molecules of a particular starch possess the same average number of glucose residues per terminal glucose unit.

Amylopectin is known to be a heterogeneous mixture of branched molecules having different molecular weights.[1] However, there is no definite information concerning the heterogeneity of the degree of branching in amylopectin, that is, it is not known whether or not the number of glucose residues per terminal glucose unit varies from molecule to molecule. Analysis of unfractionated amylopectins from seven different plant sources showed that their average number of glucose residues per terminal glucose unit varies from 22 to 27.[2]

Hassid and McCready[3] isolated a fraction from Golden Bantam sweet corn which existed in close proximity with starch and which, similar to glycogen, had 12 glucose residues per terminal glucose unit. This indicated that the degree of branching in amylopectin may not be uniform.

In view of these results, it was of interest to examine subfractionated amylopectin samples by the end-group assay method and to ascertain whether or not the molecules of the subfractions contain the same number of glucose residues per terminal glucose unit. For this purpose tapioca and corn amylopectins were separated into a number of subfractions by fractional precipitation with methyl alcohol, and their end-group assays determined by the periodate oxidation method as reported in a previous paper.[2a]

The percentage yields of the subfractions are given in Table I. Also listed are the "inherent" viscosities and the end-group assays of the original amylopectins and their subfractions. As shown in this table, all of the fractions from tapioca and corn amylopectin give approximately the same end-group values as the original amylopectins. The results show that the amylopectin molecules of the two starches possess the same average number of glucose residues per terminal glucose unit. However, this does not exclude the possibility that within a single molecule, the length of the chains may vary considerably.

TABLE I

CHAIN LENGTH OF AMYLOPECTIN SUBFRACTIONS

Source	Subfract.	Yield, %	Concn., C, g./100 ml.	Inherent viscosity, $[\eta]$	Glucose residues per terminal glucose unit
Tapioca amylopectin	...	..	0.120	1.68	26.6
Tapioca subfraction	I	25.0	.120	0.80	26.5
Tapioca subfraction	II	46.4	.120	1.62	26.5
Tapioca subfraction	III	28.6	.120	2.04	26.4
Corn amylopectin	...	..	.160	1.51	25.2
Corn subfraction	I	10.4	.160	0.44	26.2
Corn subfraction	II	39.8	.160	1.26	25.0
Corn subfraction	III	37.4	.160	1.47	25.8
Corn subfraction	IV	12.4	.160	1.57	26.1

Experimental

Fractionation of Amylopectin.—The amylopectin samples were prepared from the "Pentasol" non-precipitable fractions according to the method of Schoch.[4] The amylopectins were subfractionated by a modification of the method of Kerr.[5]

Twenty grams of tapioca amylopectin was dissolved in 2 l. of hot water to which 1 g. of potassium acetate, 200 ml. of *n*-butyl alcohol and 680 ml. of methyl alcohol were added. The mixture was cooled slowly to room temperature and allowed to stand until the precipitated starch settled. The supernatant liquid was carefully decanted and the material in the solution precipitated with an excess of methyl alcohol (Fraction I). The residue was then dissolved in 1500 ml. of hot water to which 1 g. of potassium acetate, 150 ml. *n*-butyl alcohol and 420 ml. of methyl alcohol were added. The supernatant liquid was decanted and the material precipitated as before (Fraction II). The residue (Fraction III) and Fractions I and II were dehydrated with methyl alcohol and dried in a vacuum oven at 80°. By a similar procedure 25 g. of corn amylopectin was fractionated into four subfractions.

Inherent Viscosities.—The viscosities were determined by the method described by Lansky, Kooi and Schoch.[6] The inherent viscosities were calculated by using Wagner's formula,[7] which takes into account the "kinetic energy" correction. The term "inherent viscosity," $\{\eta\}$, represents the value of the quantity $\ln \eta r/C$, at finite value of C, where ηr is the relative viscosity and C is the concentration of grams per 100 ml. This is to be distinguished from the intrinsic viscosity, $[\eta]$, which is the infinite dilution value of the inherent viscosity

$$\{\eta\} \equiv \ln \eta r/C = f(c)$$

$$[\eta] \equiv \lim \{\eta\} \neq f(c)$$

$$C \to 0$$

The intrinsic viscosities of the original corn amylopectin and corn amylopectin subfraction IV were determined by extrapolating the inherent viscosities at various concentrations to zero concentration. The intrinsic viscosities and the inherent viscosities determined when 0.160 g. of starch per 100 ml. solution of the corn amylopectin were used, are 1.48 and 1.51, respectively; those of the corn subfraction IV, using the same concentration, are 1.53 and 1.57, respectively. The inherent viscosities of the other amylopectin samples were determined in duplicate at a single concentration. The values and the concentrations at which they were determined are given in Table I.

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Acknowledgment.—The authors are grateful to the Corn Industries Research Foundation for their support of this work.

BERKELEY 4, CALIFORNIA RECEIVED SEPTEMBER 1, 1950

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE STATE UNIVERSITY OF IOWA]

The Addition of Bromine Chloride to Carbon–Carbon Double Bonds[1]

BY ROBERT E. BUCKLES AND JOHN W. LONG

A mixture of N-bromoacetamide and hydrogen chloride has been used as a source of the elements of bromine chloride for addition to ethylene, styrene, cinnamic acid, *cis*-stilbene and *trans*-stilbene. The structures of the chlorobromides which were obtained support the polar mechanism of addition in which positive bromine is transferred to the double bond to form a complex, *e.g.*, the bromonium ion, which is attacked by chloride ion to give the product. The direction of addition to unsymmetrically substituted double bonds is that predicted by this mechanism. The expected *trans*-addition has also been observed. The chlorobromides derived from styrene and *trans*-stilbene have also been prepared by the reactions of anhydrous stannic chloride with the corresponding dibromides. These displacement reactions presumably take place either by way of intermediates similar to those proposed for the addition reactions or by way of carbonium ion intermediates.

The physical evidence for the existence of bromine chloride is based mainly on thermodynamic and kinetic studies of the equilibrium of formation from bromine and chlorine.[2] Attempts to measure such properties as m.p.[3] and b.p.[3b] have led to the conclusion that bromine chloride is not a stable compound. The preparation of a sample of bromine chloride with a definite m.p. (−54°) and a characteristic vapor-pressure *vs.* temperature curve has been reported,[4] however.

Chemical evidence for the existence of bromine chloride in an equimolar mixture of bromine and chlorine is to be found in its action on ethylene to yield mostly 1-bromo-2-chloroethane[5] and in its action on diazodesoxybenzoin to give mostly α-bromo-α-chlorodesoxybenzoin.[6]

In the present work it has been found convenient to use a mixture of N-bromoacetamide and hydrogen chloride in a suitable solvent as a source of the elements of bromine chloride for addition to olefins. When aqueous hydrochloric acid was treated portionwise with N-bromo-acetamide while ethylene was bubbled through the solution, the product was 1-bromo-2-chloroethane just as when mixtures of chlorine and bromine were used.[5]

It has previously been shown[7] that even in relatively non-polar solvents N-bromoacetamide in the presence of an acid acts as a source of positive bromine for the polar mechanism of addition to double bonds. In this mechanism the positive bromine is transferred to the double bond to form a positively charged complex, *e.g.*, the bromonium ion, which is capable of maintaining its steric identity. The reaction of this complex with chloride ion leads to the bromochloride. The attack of chloride ion on the complex would be at the more positive carbon atom of an unsymmetrically substituted double bond so that Markovnikov's rule would hold for hydrocarbons. Also the attack would be on the side opposite to the attachment of the bromine in the complex; *i.e.*, *trans* addition would prevail.

Styrene, when treated with N-bromoacetamide and hydrochloric acid in water, yielded (36–44%) 2-bromo-1-chloro-1-phenylethane along with some dibromide. A similar addition reaction of *trans*-cinnamic acid in dimethylcellosolve gave a 56% yield of α-bromo-β-chlorohydrocinnamic acid. The structures of these products were established by dehydrohalogenation to known compounds. In either case the product is that predicted as the result of the addition of positive bromine and negative chlorine to the partially polarized double bond.

When an addition of bromine chloride in the reverse order (*i.e.*, N-chloroacetamide in the presence of hydrogen bromide) was carried out on styrene, the dibromide was the major product. When the reaction was carried out in water with excess hydrogen bromide, no bromochloride was isolated. With ether as a solvent and with a limited amount of hydrobromic acid, the reaction gave a 30% yield of impure 2-bromo-1-chloro-1-phenylethane. None of the isomer, 1-bromo-2-chloro-1-phenylethane, was obtained.

Although N-chloroacetamide would be considered as a source of positive chlorine the greater electronegativity of chlorine as compared to bromine would be expected to lead to an exchange of electrons so that a source of positive bromine would rapidly be formed.

$$AcNHCl + HBr \rightleftharpoons AcNH_2Cl^+ + Br^-$$
$$AcNH_2Cl^+ + Br^- \rightleftharpoons AcNH_2Br^+ + Cl^-$$
$$AcNH_2Cl^+ + Br^- \rightleftharpoons AcNH_2 + BrCl$$

Such reactions are well known.[8] With such a source of positive bromine present the formation of dibromide with excess hydrobromic acid and the formation of 2-bromo-1-chloro-1-phenylethane with limited hydrobromic acid would be the results expected.

The prevalence of *trans* addition was demonstrated by the addition of bromine chloride to *cis*- and *trans*-stilbene in dimethylcellosolve. A 67% yield of *erythro*-α-bromo-α′-chlorobibenzyl (III)

(1) Taken from the Ph.D. thesis of John W. Long. Presented before the Organic Division of the American Chemical Society, Chicago, Illinois, September, 1950.

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