17.28-cc. sample of diborane during three days at 40°, 0.8 cc. of diborane was absorbed, producing 1.03 cc. of $CH_3NHB_2H_5$ and 0.7 cc. of hydrogen. Shorter reaction periods, typified by an experiment in which diborane under 100 mm. pressure was passed over polymeric CH_3NHBH_2 at 100°, produced no detectable amount of methylaminodiborane.

Discussion

The observed regular trend toward increased thermal stability as methyl groups are successively substituted for N-bonded hydrogen atoms in the aminodiborane series appears to be inversely related to the stability of the polymeric forms of the aminoborines from which these compounds are formally derived.

Thus it is noteworthy that NH_2BH_2, CH_3NHBH_2 and $(CH_3)_2NBH_2$ show volatility characteristics indicative of a progressively lower degree of polymerization, while the corresponding aminodiboranes exhibit a complementary increase in thermal stability. Formation of a stable polymer withdraws aminoborine units from equilibria such as $B_2H_6 + 2BH_2NH_2 \rightleftarrows 2B_2H_7N$, accounting for the relative instability of B_2H_7N. This effect is lessened in the case of methylaminodiborane, and becomes immeasurable in dimethylaminodiborane, due to the low free energy change in the association of $(CH_3)_2NBH_2$—approximately 1.8 kcal./mole at 100°, according to preliminary experiments in this Laboratory.

The decrease of polymerization energy with N-methylation of the aminoborines might be related to the electron-releasing (+I) effect of the methyl groups, permitting resonance-contribution by structures in which boron has a complete octet. However, steric effects may well be more important.

The regular increase in volatility from B_2H_7N to dimethylaminodiborane is most convincingly attributed to steric factors; thus the reverse trend of boiling points (76.2, 66.8 and 50.3°) with increasing molecular weights, is in harmony with the suggestion that more methyl groups force the molecular dipoles farther apart, sharply lowering the intermolecular attraction.

Acknowledgment.—The generous support of this work by the Office of Naval Research is gratefully acknowledged.

Summary

The new compounds $CH_3NHB_2H_5$ and $(CH_3)_2NB_2H_5$ have been prepared from diborane and methylamine or dimethylamine. These and the parent compound, B_2H_7N, are regarded as derivatives of diborane in which a bridging hydrogen atom is replaced by N. A second hydrogen atom can be replaced by chlorine, yielding the volatile, unstable and self-inflaming $(CH_3)_2NB_2H_4Cl$. Volatility, stability and ease of preparation increase in the order $NH_2B_2H_5$, $CH_3NHB_2H_5$, $(CH_3)_2NB_2H_5$; the last can be stored permanently at ordinary temperatures.

LOS ANGELES 7, CALIF. RECEIVED JANUARY 17, 1949

[CONTRIBUTION FROM THE GEORGE M. MOFFETT RESEARCH LABORATORIES, CORN PRODUCTS REFINING COMPANY]

The Molecular Weight of the β-Amylase Limit Dextrin from Corn Starch

BY RALPH W. KERR AND FRANK C. CLEVELAND

Difficulty is experienced in the determination of the molecular weight of starch molecules by physical measurements on dispersions in appropriate solvents owing to the reluctance of these molecules or their derivatives to dissociate, or to become disentangled from each other. It is presumed that this difficulty arises because of the linear or long, thread-like structure of the amyloses, or because of the many linear branches on the large amylopectin molecules. On occasion, some branched starch derivatives are found to be so highly associated that they swell but do not dissolve to any very great extent in any neutral solvent. Accordingly, although molecular weight determinations on the amyloses have been reported frequently,[1-5] examinations of the amylopectins have either been approached with considerable caution, or the results have been given with the qualification that the error may be very large.[1,2,6] However, it appeared that if the linear terminal branches were removed, such as, by hydrolysis with β-amylase, association effects would be minimized when observations were made on the residual portion of the amylopectin molecules. Furthermore, inasmuch as branched starch molecules are thought to be of considerable size and measurements such as osmotic pressure determinations are of a very low order, it is obvious that by removing the end branches which constitute, on the average, nearly half the weight of amylopectin molecules, material would be provided the molecular weight of which could be estimated with very much greater accuracy. Lastly, since it has now generally been concluded that the action of β-amylase on amylopectin stops at a definite end-point, leaving the molecule intact behind points of branching, then it fol-

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lows that a determination of the molecular weight of this limit dextrin, divided by the fractional yield of this material from the hydrolysis, should provide a fairly accurate calculation for the molecular weight of the parent molecules.

The principal deterrent to this enzymic approach has been doubt concerning the reliability of β-amylase preparations. The isolation of crystalline β-amylase by Balls, Thompson and Walden[7] which may be highly purified by repeated crystallizations, has served to remove this limitation.

Experimental

Preparation of Corn Limit Dextrin.—Fifty grams, dry basis of corn B-fraction, prepared by the use of Pentasol precipitation on autoclaved, defatted corn starch according to the method of Schoch[8] was dissolved in 1500 ml. of hot water by stirring and bringing to a boil. The solution was cooled to 45° with stirring. The *p*H was 6.0. Then 3 drops of a saturated ammonium sulfate suspension of four times recrystallized β-amylase[9] was added and the hydrolysis mixture held at 45°. The reaction was followed by removal of 5-ml. aliquots (40 ml. in all) from time to time and oxidation with alkaline potassium ferricyanide according to the method of Gore and Steele.[10] Hydrolysis was apparently at an end after three hundred and twenty minutes, at which time 54.4% of the B-fraction had been hydrolyzed to maltose.

It would appear from the method used by Balls and co-workers[7] to prepare the enzyme, wherein the crude β-amylase was pretreated with hydrochloric acid at *p*H 3.25 to 3.30 and wherein the enzyme was exposed for long periods of time to *p*H levels of about 3.5, that freedom from α-amylase activity in the final product was assured. This fact was confirmed by Balls and co-workers. It seems reasonable to assume also that after 4 recrystallizations of the β-amylase, a high degree of chemical purity was attained as well as biochemical purity. However, to test the possibility that this crystalline enzyme sample was not free from traces of α-amylase activity, the limit dextrin which remained in the reaction mixture after three hundred and twenty minutes was treated with another drop of enzyme suspension and the reaction was allowed to proceed for an additional nineteen hours, under toluene. No further increase in reducing value, whatsoever, was observed, the final value being equal to 54.3% conversion to maltose. The solution was brought to a boil, evaporated under reduced pressure to 625 ml. and two volumes of methanol were added with stirring to precipitate the limit dextrin. It was obvious that the dextrin consisted of two fractions of about equal parts by weight. The first precipitated when the concentration of methanol reached about 50% by volume and the second in the region of 60–67%. The entire yield of dextrin was combined and further purified by dissolving in water, adding methanol with stirring until the alcohol content reached 67% and collecting the precipitate. The purification procedure was repeated until the supernatant liquor was substantially free from soluble reducing material. Then the dextrin in a concentrated water solution was poured into absolute methanol, filtered and washed 4 times over a period of four days with 200 ml. of methanol. The methanol was removed *in vacuo* at room temperature. The yield of dextrin was 19.98 g., dry basis, or 41.1%, when allowance is made for the samples removed for analysis during the hydrolysis. This value is 90% of the calculated yield. The ferricyanide reducing value[11] of the limit dextrin is 0.79. For comparison, other ferricyanide reducing values are corn starch, 1.00; corn A-fraction, 1.43; corn B-fraction, 0.46.

Acetylation of Corn Limit Dextrin.—Three grams of the dextrin, dry basis, was ground to a powder and dispersed in 60 ml. of formamide.[12] The mixture was heated with stirring to 85° in about fifteen minutes, cooled to room temperature and diluted with 100 ml. of pyridine. To the clear, limpid solution, 80 ml. of acetic anhydride was added dropwise with stirring and the reaction mixture was allowed to stand at room temperature overnight. The clear solution was added slowly to 1500 ml. of water which was stirred with a circular motion. Unexpectedly, the product showed a very pronounced tendency to precipitate in fibrous form. The acetate was washed three times, each with one liter of water, separated by filtration and dried in the air. The dry material was mixed with 75 ml. of pyridine until dispersed and then heated over a period of about fifteen minutes to 85°. The solution was cooled to room temperature, 60 ml. of acetic anhydride added and the balance of the acetylation procedure completed as in the first phase. The final product, which also was fibrous, was washed with 5 one-liter portions of water and the washing period extended over a period of five days. The yield of acetate was 4.84 g., dry basis, or approximately 90%; acetyl = 44.7%. Polarimeter readings on a 2% solution of the acetate in chloroform gave a value $[\alpha]^{25}D$ 166.5°.

Determination of Osmotic Pressures.—Osmotic pressures were determined at several concentrations in chloroform solution according to general procedures given previously.[11] Solution of the acetate in chloroform appeared to be rapid and complete at room temperature. However, the solutions were held at the boiling point for fifteen minutes and cooled to 30° before use. Static measurements were made at all concentrations, since the experimental error inherent in the dynamic method was considered to be too large in relation to the small actual pressures which were developed in this series of experiments. Moreover, although the actual temperature of the osmotic pressure cell is of some importance in all measurements, it was readily apparent that in the determination of very small pressures, variation in temperature during the period of observation, such as the normal variation, or periodic fluctuation of a well-regulated, thermostatically controlled bath, may set up disturbances which cause very large errors. Accordingly, in addition to using a large metal cell of high heat capacity, the cell was brought to 30° and then heavily insulated in addition to being placed in a cabinet thermostatically held at 30 ± 0.05°. Under these conditions, a thermometer fitted into a metal well on the cell showed a variation of only a few thousandths of a degree during periods of observations.

Permeability of the membrane in the osmometer to small fragments of carbohydrate molecules which might possibly be present in the limit dextrin sample was determined as follows: When the highest concentration of solute used was in the osmometer (2.0349 g. per 100 ml.), it was allowed to stand seventy-two hours, the osmotic pressure was determined as shown and then the liquid in the solvent side was drawn into a weighed evaporating dish. The volume was 12 ml., and the residual weight after evaporation was 0.0003 g. The volume in the solution side was 15 ml. and contained therefore 0.3052 g. Accordingly, less than one part per thousand of the acetate diffused through the membrane.

Several tests at lower concentrations gave residues within the experimental error of weighing.

Discussion

The results of osmotic pressure measurements for the triacetate of the β-amylase limit dextrin from corn B-fraction are shown in Fig. 1 and Table

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TABLE I

OSMOTIC PRESSURES OF THE ACETATE OF β-AMYLASE LIMIT DEXTRIN FROM CORN B-FRACTION IN CHLOROFORM

C, concn., g. per 100 ml.	π, g. per square cm.	π/C
0.1021	0.100	0.979
.1924	.147	.765
.3036	.228	.751
.4504	.280	.622
.6075	.426	.701
.8008	.648	.809
1.0130	.825	.815
1.4530	1.604	1.104
2.0349	3.003	1.480

I. It will be observed that the slope of the curve plotting π/C against C is negative at very low concentrations, then changes in slope and at relatively high concentrations assumes a positive slope. A similar shape has been observed by Steurer[13] for high molecular weight ethyl cellulose in toluene. This shape has also been observed by us previously in unreported studies on derivatives of hydrolyzed branched starch molecules although we were unable to observe a similar effect with the acetates of linear starch molecules,[5] at least using concentrations as low as 0.3 g. per 100 ml., which appeared to be the limit for reliable measurements using techniques which were available at that time. In studies on hydrolyzed, branched starch molecules, and using concentrations in the range of 0.2 to 0.8 g. per 100 ml., it was at first assumed that the osmotic pressure curves had very nearly zero slope. More extended observations by improved techniques on branched fractions from acid hydrolyzed starch of the order of DP_n 500–1000 (which will be the subject of a future report) have shown in this case also, that the slope of the curve becomes negative as infinite dilution is approached. This is presumed to be due to residual association between solute molecules; the effect vanishes at low orders of DP_n. At relatively high concentrations, the curve for branched starch molecules shows the usual anomalous osmotic pressure effects given generally by high polymers which has been discussed recently by Bawn.[14]

Extrapolation of curves of the shape shown is difficult and constitutes the principal element of uncertainty of the results reported. It is believed, however, that the extrapolation shown in Fig. 1 indicates at least the correct order of magnitude for the osmotic pressure of the limit dextrin acetate at infinite dilution. The corresponding DP_n value is 810.

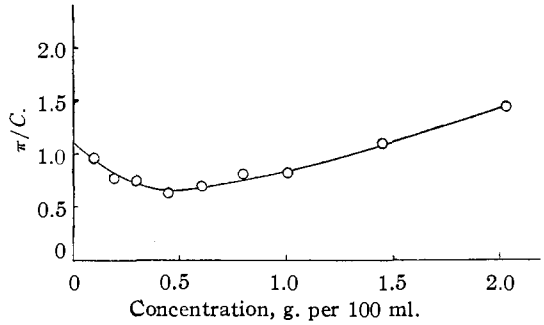

Fig. 1.—Osmotic pressure–concentration relationship for corn limit dextrin acetate in chloroform solution at 30°. The ordinate gives values for π/C as explained in Table I. The abscissa gives values for concentration in grams per 100 ml.

From this value and from the calculated yield of 46% limit dextrin, the indicated DP_n value for the parent amylopectin fraction is of the order of 1800.

In making this computation the assumption is made that each molecular weight group of corn B-fraction is made up of a variety of molecules which give the same average limit of hydrolysis as the total parent fraction. This may not be strictly true since a limited subfractionation of the butanol, non-precipitable fraction of corn starch by Kerr[15] showed that the subfraction of highest apparent molecular weight had a limit of conversion about 10% greater than the subfraction of lowest molecular weight. However, if the difference between various molecular weight groups is not substantially greater than 10%, then the calculation employed would be expected to give the correct order of magnitude for the DP_n of corn amylopectin.

Summary

A limit dextrin has been prepared in 90% of the calculated yield from corn B-fraction using crystalline β-amylase, and its ferricyanide reducing value determined.

The triacetate of the dextrin was found to be readily dispersible in chloroform, have a specific rotation of $[\alpha]^{25}D$ of 166.5° and an osmotic pressure at 30° equivalent to a DP_n of 800.

It was observed that the dextrin acetate tended to precipitate in fibrous form.

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