the alcoholic solution was boiled to remove the excess methylamine. The sodium chloride which separated was removed by filtration and washed with alcohol; the filtrate was concentrated by evaporation and poured into cold water. Some of the free base, 2,4-dimethylaminoquinazoline (III), $\overline{N{=}C(NHCH_3)C_6H_4N{=}C}NHCH_3$, separated in white flakes while the remainder formed an oil which gradually solidified in the course of four or five days. The solid product thus obtained was filtered and crystallized from chloroform using a carbon dioxide snow–ether mixture as the cooling medium.

The yields obtained, the composition, and some of the physical properties of these two bases are tabulated in Table I. A number of salts of these bases were readily obtained from their alcoholic solutions by adding acid either alone or in alcoholic solution. The properties of these salts are collected in Table II.

Preparation of 2,4-Diacetaminoquinazoline, $\overline{N{=}C(NHCOCH_3)C_6H_4N{=}C}(NHCOCH_3)$.—A solution of 0.3 g. of 2,4-diaminoquinazoline and a small piece of fused sodium acetate in 10 cc. of acetic anhydride was refluxed on a water-bath for one hour. The colorless needles which separated were removed by filtration, washed with alcohol, then with ether, recrystallized from alcohol, and dried at 40°. A further quantity was obtained by pouring the acetic anhydride filtrate into 50 cc. of water, stirring until all was dissolved and adding dry sodium carbonate to the solution until effervescence ceased. The precipitate which formed was collected, washed with water, recrystallized from alcohol and dried at 40°. The total yield of 2,4-diacetaminoquinazoline was 0.2 g. It is soluble in hot water or hot alcohol, slightly soluble in acetone, and insoluble in ether; m. p. 230°.

Anal. Calcd. for $C_{12}H_{12}O_2N_4$: C, 58.99; H, 4.95; N, 22.95. Found: C, 58.68; H, 4.90; N, 23.08.

Summary

Ammonia or methylamine reacts with 2,4-dichloroquinazoline, splitting out two moles of hydrogen chloride and substituting the corresponding amino groups for the two halogens. The resulting diaminoquinazolines are monobasic. The following new compounds have been prepared: 2,4-dimethylaminoquinazoline and two of its salts, 2,4-diaminoquinazoline and six of its salts, and 2,4-diacetaminoquinazoline.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

The Properties of the Amyloses. Corn α-Amylose and Retrograded β-Amylose[1]

BY T. C. TAYLOR AND S. G. MORRIS

In a cereal starch such as that from corn, there remains after disorganization of the granule a certain amount of material which is very resistant to attempts at dispersion in water. In contrast the other and larger part may be dispersed readily. A further distinction may be made between the two parts by the fact that the insoluble residue carries combined with its carbohydrate certain high molecular weight fatty acid groups.[2] This insoluble fraction is called α-amylose and the soluble fatty-acid free is called β-amylose.[3]

On long standing, especially under conditions that lower the solubility of the dispersed or soluble β-amyloses such as freezing temperatures, addition of alcohol or other reagents,[4] a portion of this one-time soluble material will become insoluble. Even on raising the temperature of its aqueous suspension mixture, some of the material will not disperse again to give a clear solution, but remains behind as a residue which is similar in appearance to the α-amylose. This insoluble residue is called retrograded amylose.

The soluble portions of all the common starches so far studied which are not degraded hydrolytically too greatly, have the property of retrograding after the granule has been disorganized and the dispersion in water made. It is this type of retrograded material that is under discussion here. Retrogradation, however, may apparently also take place in the granule itself.

In certain starches, notably potato, all the amyloid material may be dispersed[5] and since there are apparently no fatty acids esterified with any part of this material, there is, according to our view, no α-amylose fraction in the sense that it exists in corn starch.

Because of rapid retrogradation, however, of the dispersed amylose material from potato starch, large amounts of amylopectin (α-amylose) are often reported to be present in this starch also,

(1) Taken from a dissertation presented by S. G. Morris to the Faculty of Pure Science of Columbia University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

(2) (a) Taylor and Nelson, THIS JOURNAL, **42**, 1726 (1920); (b) Taylor and Lehrman, *ibid.*, **48**, 1739 (1926).

(3) (a) Taylor and Iddles, *Ind. Eng. Chem.*, **18**, 713 (1926); (b) Taylor and Beckmann, THIS JOURNAL, **51**, 294 (1929).

(4) T. J. Schoch, Dissertation, Columbia University, 1933.

(5) Taylor and Schoch, THIS JOURNAL, **55**, 4248 (1933).

but its source is recognized by Hirst, Plant and Wilkinson[6] when they say of *potato starch* "free amylose cannot be kept as such, but gradually retrogrades through intermediate stages to the amylopectic condition."

While the tendency to retrograde does not seem to be as great in a water dispersion of corn β-amylose as in that from potato, yet it is definitely present. Indeed, insoluble material can be formed from a soluble corn β-amylose or any other amylose that has not been broken down hydrolytically, most simply by freezing the solution, if merely lowering the temperature does not succeed in causing relatively irreversible precipitation of some amylose.

Incidentally, a distinction must be made between what seems to be simple precipitation due to temperature variation where hydrated jelly-like masses form as the temperature is lowered but which jellies disperse to give clear solutions on reheating and relatively irreversible precipitation which is called retrogradation.

Insoluble materials from corn β-amylose formed through retrogradation, while containing no combined fatty acid as true corn α-amylose does, nevertheless appear in any fractionation process with the true α-amylose and in a sense dilute it.

This report concerns itself with an attempt to differentiate further between the two types of insoluble material in corn starch, namely, true fatty acid bearing α-amylose and retrograded β-amylose. The interpretation of the results rests on a working hypothesis which comes from some of the more recent concepts of the structure of high molecular weight compounds.

Briefly, "dissolved" β-amylose is looked upon as consisting principally of single chains of glucose units,[7] which during the process of dispersion become highly hydrated (associated with water) to give a relatively clear solution within certain limits (about 5 g. of dry material per 100 cc. at 20°). Under conditions that lower the solubility notably during the lowering of temperature or actually freezing, the chains begin to associate with one another to form sheaflike bundles. This association is more complete in some bundles than in others, so a retrograded paste, for that is what is formed by the reaction, according to this scheme, may consist of quite an assortment of bundles of loosely and tightly packed amylose chains with the inclusion in the micelles of some water.[8] The more highly the chains are associated with one another, the less the amount of combined water one may expect to find.

Experimentally, the retrograded material used here was produced by freezing a 3% clear dispersion of corn β-amylose, melting the mass at room temperature and boiling the suspension for ten minutes. Only the insoluble material that persisted through this treatment was taken somewhat arbitrarily as retrograded amylose. This residue represents, apparently, a relatively highly associated sheaflike bundle of amylose chains.

On repeated and prolonged treatment of the retrograded amylose with hot water, soluble material is formed. This is exemplified by the following experiment: 5-g. samples of dry retrograded amylose were suspended in 200 cc. of water and the suspension boiled under a reflux condenser for one hour. The mixture was cooled and placed in the electrophoretic cell where the solids migrated to the positive membrane leaving a clear supernatant solution which contained soluble amylose that gives the usual blue color with iodine test solution. After decantation of the solution the solids were removed from the membrane, resuspended in water, and boiled again as before. On repetition of this operation five times the residue from the membrane was dried by working with anhydrous methyl alcohol and subsequent heating. It weighed 1.3 g. When the operations are carried on further the residue is still less.

The soluble material coming from the reaction was in the main similar to ordinary β-amylose although there was some reducing carbohydrate coming, no doubt, from hydrolytic breakdown of some of the amylose chains. Presumably the treatment with boiling water causes principally a progressive hydration of the amylose chains associated together in the retrograded residue, the hydrated chains thereby becoming redispersed.

Both from the working hypothesis and from experimental experience it follows that there are different degrees of retrogradation and that a specific definition for a retrograded amylose would be difficult to set down.

In addition to boiling water there is another well-known method for dispersing, although somewhat incompletely, organized amyloses as they occur originally in the granule. That method employs cold aqueous alkali as the hydrating or disassociating agent and interestingly enough, retrograded starch (amylose), is according to Maquenne,[9] also dispersed by this treatment.

(6) Hirst, Plant and Wilkinson, *J. Chem. Soc.*, 2375 (1932).

(7) (a) Meyer and Mark, *Ber.*, **61**, 593 (1928); (b) Meyer and Mark, "Der Aufbau der hochpolymeren organischen Naturstoffe," Leipzig, 1930; (c) Staudinger and Schweitzer, *Ber.*, **63**, 2327 (1930); (d) Karrer, *Helv. Chim. Acta*, **12**, 1144 (1929), and **15**, 48 (1932); (e) Kolkmeijer and Favejee, *Z. Krist.*, **88**, 226 (1934).

(8) (a) Katz and Rientsma, *Z. physik. Chem.*, **150A**, 60, 67 (1930); (b) Katz and van Itallie, *ibid.*, **150A**, 90 (1930); (c) Katz and Derksen, *ibid.*, **150A**, 81, 90 (1930).

(9) Maquenne, *Compt. rend.*, **138**, 213 (1904); **137**, 797 (1903).

Acting on this suggestion, the two organized materials, insoluble retrograded β-amylose from once soluble β-amylose, and α-amylose which was never soluble, were treated with aqueous alkali. Both dispersed to give clear limpid solutions, indicating presumably rather complete disassociation and hydration of the chains.[10]

Now, however, a new observation was made, to wit, that on careful acidification in the cold, the retrograded β-amylose remains dispersed but the true α-amylose reprecipitates.

This striking difference, although qualitative, gave promise of a simple method for differentiating between retrograded β-amylose and corn α-amylose. A more detailed quantitative study was then made.

The weighed dry solid, either retrograded β- or true α-amylose, to be tested was added to water to make a 3% suspension and the mixture brought to the boiling point, cooled to room temperature and concentrated aqueous sodium hydroxide solution added until 2.5% was present. After stirring for a short time, the clear solution which always resulted, was neutralized and then slightly acidified with cold dilute hydrochloric acid. This concentration of amylose and alkali seemed to be the optimum one for the purpose. When corn α-amylose was used, a precipitate formed. This precipitate was separated and washed repeatedly by decantation in a centrifuge, then flocculated with alcohol, and the residue dried to constant weight *in vacuo* at 100°.

From three samples of α-amylose of 1.92, 2.00 and 9.00 g., there was recovered, respectively, 1.82, 1.80 and 8.30 g. on subsequent acidification.

On the other hand, there was no precipitate formed upon acidification of the aqueous alkaline dispersion of the retrograded β-amylose.

It is interesting to note that in every case the material recovered from the α-amylose (originally containing 4.6% combined fatty acids) after washing with ether to remove any extraneous fatty acids, contained still 4.8% combined fatty acids, an indication of the relative stability of this fatty-acid carbohydrate material.

Within the precision with which this type of measurement can be made, the recovered corn α-amylose may be said to be substantially unchanged by the treatment. Likewise, the α-amylose cannot be contaminated by any appreciable amount of retrograded β-amylose. Any loss of solids after precipitation of α-amylose seems to be due to the break-up of a small amount of the α-amylose, for what is recovered has the same amount of combined fatty acids as the original sample.

Next, synthetic mixtures of known amounts of corn α-amylose and retrograded β-amylose were made and analyzed by the method outlined above. From such a mixture, consisting of 4 g. of α-amylose and 4 g. of retrograded β-amylose, 3.8 g. of fatty-acid bearing α-amylose was recovered. In another instance from a mixture 1.8 g. of retrograded β-amylose and 2.00 g. of α-amylose, 1.95 g. of α-amylose was recovered. Retrograded material from potato starch dispersions when treated in the same manner gave on acidification no precipitate.

It is evident that corn α-amylose is not like the amylopectin of potato starch, that is, it is not simply retrograded β-amylose, nor is it admixed with any substantial amount of retrograded β-amylose as it comes from the electrophoretic cells in the separation procedure used in this Laboratory.

Conclusions

1. Retrogradation of an amylose has been discussed.

2. A method for differentiating between corn α-amylose and retrograded β-amylose is given.

3. The stability of the fatty acid–amylose complex in corn starch called α-amylose toward cold aqueous alkali has been demonstrated.

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(10) If disassociation is only partial, as it is when organized raw starch is treated with aqueous alkali, a jelly is formed.