

Stable Dextrins and the Constitution of Starch.

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TWO fundamentally different theories have been advanced to explain the chemical constitution of starch. One of them considers starch as built up of low-molecular units joined together by forces other than normal valencies to "micelles" the size of which accounts for the conspicuous characteristics of starch.¹ According to the other theory starch is a macromolecular substance in which the units that determine colloidal and other properties are in fact the true molecules.

Some years ago, the first theory was predominant in spite of a pronounced resistance from certain investigators (Staudinger), but to-day it is practically abandoned. It might be said that several of the methods once used to demonstrate the existence of the small fundamental units cannot be regarded as conclusive, and most investigators agree that starch is a true macromolecular substance, the molecules of which are composed of a great number of hexoses united to chains of some sort. A corresponding theory is applicable to cellulose which, as a matter of fact, seems to be less complicated in chemical nature than starch.

The fact that starch may be in different ways rather completely converted into maltose led to the opinion that starch is in principle built up of maltose molecules. Haworth looks upon the starch molecule as a chain of glucose molecules linked together by glucosidic bonds, exclusively.² Much evidence is in agreement with this view, nevertheless various experimental results are suggestive of the belief that the molecules of native starch are constituted according to a more complicated scheme. The trimethyl starch, for instance, yields on acid hydrolysis much more tetramethyl glucose than could be expected from the probable molecular weight as calculated on the assumption of simple chains.³ Further are to be quoted the investigations of Staudinger who

has drawn the conclusion that the molecules of starch are branched chains.⁴

It is the author's opinion that the relation of starch to the starch-splitting enzymes is of the greatest importance for the clear understanding of its constitution. The chief product of the enzymatic degradation of starch is invariably maltose. In some cases, maltose might be the only product. It is, however, mostly accompanied by larger or smaller quantities of so-called stable dextrins (limit dextrins) which are not or in any case, only extremely slowly attacked by the enzyme in question. These stable dextrins have a higher molecular weight and a lower reducing power than maltose.

Some fractions or transformation products from native starch yield, when acted upon by certain amylases, nearly 100% of maltose. It might not be unnecessary to emphasise that the very probable presence of maltase in the enzyme preparations must be most carefully taken into account in all determinations of this kind. Among the breakdown products of native starch and soluble starch we have always been able to isolate dextrins, even after prolonged action of the enzyme. Contrary to certain investigators we never found complete decomposition to maltose of, say, potato starch by taka diastase. We could, in fact, always obtain considerable amounts of dextrins having a much higher molecular weight than maltose, although these dextrins as well give perfectly clear watery solutions of low viscosity.

The fact that certain fractions of starch give nearly 100% of maltose, is in the writer's opinion in favour of the idea that the stable dextrins are true residues from the partly decomposed starch molecules. Of course, the possibility must also be considered that the stable dextrins might be secondary products, formed through (perhaps spontaneous) union of primary products of lower molecular weight. This possibility cannot be dismissed without further notice,

¹ Karver, *Polymere Kohlehydrate*, Leipzig, 1925.

² Haworth, *The Constitution of Sugars*, London, 1929.

³ Haworth, *Chem. Soc.*, 1935, 1201, 1214, 1299; Staudinger and Eilers, *Ber.*, 1936, 69, 826.

⁴ Staudinger and Husemann, *Ann.*, 1937, 527, 195.

all the more as certain investigators have assumed that even maltose is a secondary product, formed in this way. Many facts are, however, not in accordance with this hypothesis. For instance, it should be anticipated that the quantity and the nature of the dextrins must vary *strongly* with the velocity of the enzyme action. This is, however, not the case. Pringsheim,⁵ maintaining the view that dextrins as well as maltose are secondary products, advances the argument, that identical dextrins always are formed by the amylases. This is not correct. Pringsheim seems to assume the existence of only two stable dextrins, di- and tri-hexosans, the former derived from amylose, the latter from amylopectin. Apart from the fact that Pringsheim wholly disregards the phosphorus content of starch and also other similar conditions, it must be emphasised that the stable dextrins always have a molecular weight rather higher than that of the above-mentioned substances. As a matter of fact, no dextrin so far isolated has a molecular weight lower than about 600. Furthermore, the nature of the stable dextrins varies strongly with the type of starch and enzyme used. All dextrins isolated by us also have a distinct reducing power roughly corresponding to one aldehyde group per molecule.

Many facts are in keeping with the view of Staudinger that native starches are composed of a great number of "polymer homologues". This alone renders it probable that most preparations of stable dextrins accordingly should be mixtures of dextrins differing in molecular weight and other qualities. The action of normal amylases consists, in the author's opinion, in the splitting off of maltose molecules from simple chains of glucose molecules, united by normal α -glucosidic bonds, *i.e.*, from chains possessing the Haworth formula. For instance, I assume that the β -amylase from plant material (ungerminated barley) attacks starch in the following way. Starting from the free ends of the chains or branches of chains the enzyme progressively sets free maltose molecules until it reaches some part of the molecule, differing in structure from the formula of Haworth. Starch is in the main constituted according to this formula, but at certain intervals there are in the

chains anomalies of one kind or other. The β -amylase consumes the chains, until such an anomaly puts a stop to its action. (Possibly the action ceases at a certain distance from the anomalies, the length of which depends on the type of the enzyme used.) In my opinion the fundamental difference between amylases is ascribable to the following condition. Some amylases as for instance barley β -amylase attacks starch exclusively from the free ends of the chains whereas others, such as malt amylase and taka diastase, have in addition the capacity of *breaking* the chains between the mentioned anomalies. Such circumstances are, in my opinion, the cause of the striking difference in the action of the so-called β - and α -amylases.

I have been led to this idea through studies on the action of the enzymes on starches of different origin but above all through the investigation of the stable dextrins formed. From what has already been said we are able to draw the very significant conclusion that during the action of the amylases on starch the less interesting parts of the molecules are first split off with the formation of maltose, whereas those details of the molecules diverging in some way from the Haworth formula remain unaltered. Thus, in the stable dextrins, the mentioned anomalies of the starch molecule will appear say 5-10 times more frequently than in the starch itself. Owing to the great mass of "polymaltose" present, an investigation of the native starch may, in many cases, hardly lead to the detection of the anomalies. In a corresponding study of the stable dextrins, however, it is quite conceivable that the "abnormal" structures may be brought to daylight. When converting starch into stable dextrins we concentrate the interesting parts of the starch molecule up to ten times, sometimes possibly more.

The question is now : what anomalies do appear in the starch molecules ?

1. Foreign elements or groups of atoms (non-carbohydrate constituents), above all phosphoric acid, but to a smaller degree also fatty acids and possibly other groupings. Amylases, free from phosphatases and other specific enzymes, do not liberate these foreign groups. A phosphoric acid group in a chain puts an end to the action of the amylase.

⁵ Pringsheim, *Polyasaccharide*, Berlin, 1931.

It might also be possible, that starch molecules, containing phosphoric acid, are not attacked by certain amylases at all. But in many instances we have been able to isolate stable dextrins of a very low molecular weight, possessing so much phosphoric acid as to justify the conclusion that the phosphorus-containing molecules of starch are, in a large measure, decomposed by the enzyme, leaving the phosphoric acid in the stable dextrins. A rather remote possibility is that the phosphoric acid itself does not prevent the amylase action but that it always appears in connection with some other anomaly in the constitution of the molecule.

2. Sugars other than glucose. Even in stable dextrins having a molecular weight corresponding to only 6 hexose molecules we have not been able to trace any other hexose than glucose.

3. Types of linkages between glucose molecules other than the typical maltose linkage. Two different types are conceivable: (a) Each glucose molecule is bound only to one or two others but the carbon atom No. 1 in the first one is linked to a carbon atom other than No. 4 in the second, presumably to Nos. 3 or 6. The chains are simple without branches. (b) Certain glucose molecules are attached to more than two others, i.e., the chains are branched. This corresponds to the hypothesis of Staudinger, arrived at through determinations of viscosity. Certain glucose molecules have not only the carbon atoms Nos. 1 and 4 but also Nos. 2 or 3 or 6 or possibly more of them occupied by other glucose molecules.

4. Whether the chains have branches—"side-chains"—or not, it is possible that in certain glucose molecules the oxygen-bridge is differently situated than in normal glucose.

Moreover, it seems highly probable that in starch more of these types of anomalies may occur simultaneously, so that in a preparation of stable dextrin produced enzymatically from a certain starch preparation different molecules may be formed for different reasons. This precludes the possibility that the stable dextrin formed from any native starch might be homogeneous, especially when realising that all native starches and all artificially produced types of starches

are themselves non-homogeneous. As a matter of fact, different molecules have not the same size and furthermore the constitutional anomalies are presumably not evenly distributed between all molecules and not situated similarly in different molecules. Accordingly, factors determining the nature of the stable dextrins are among others:

1. The individual magnitude of the starch molecules.
2. The occurrence of non-carbohydrate constituents and the situation of these foreign groups in the chains.
3. Side-chains and situation of their points of attachment.
4. Length of the side-chains.
5. Type of bonds between the glucose molecules in simple chains or at the points of attachment of side-chains.
6. Type of oxygen-bridge in certain glucose molecules.

Although all preparations of stable dextrins must be mixtures and all quantitative estimations of their qualities must be considered mean values, it might, of course, be possible to obtain homogeneous preparations by means of careful fractionation, especially when starting from a preparation of starch already fractionated.

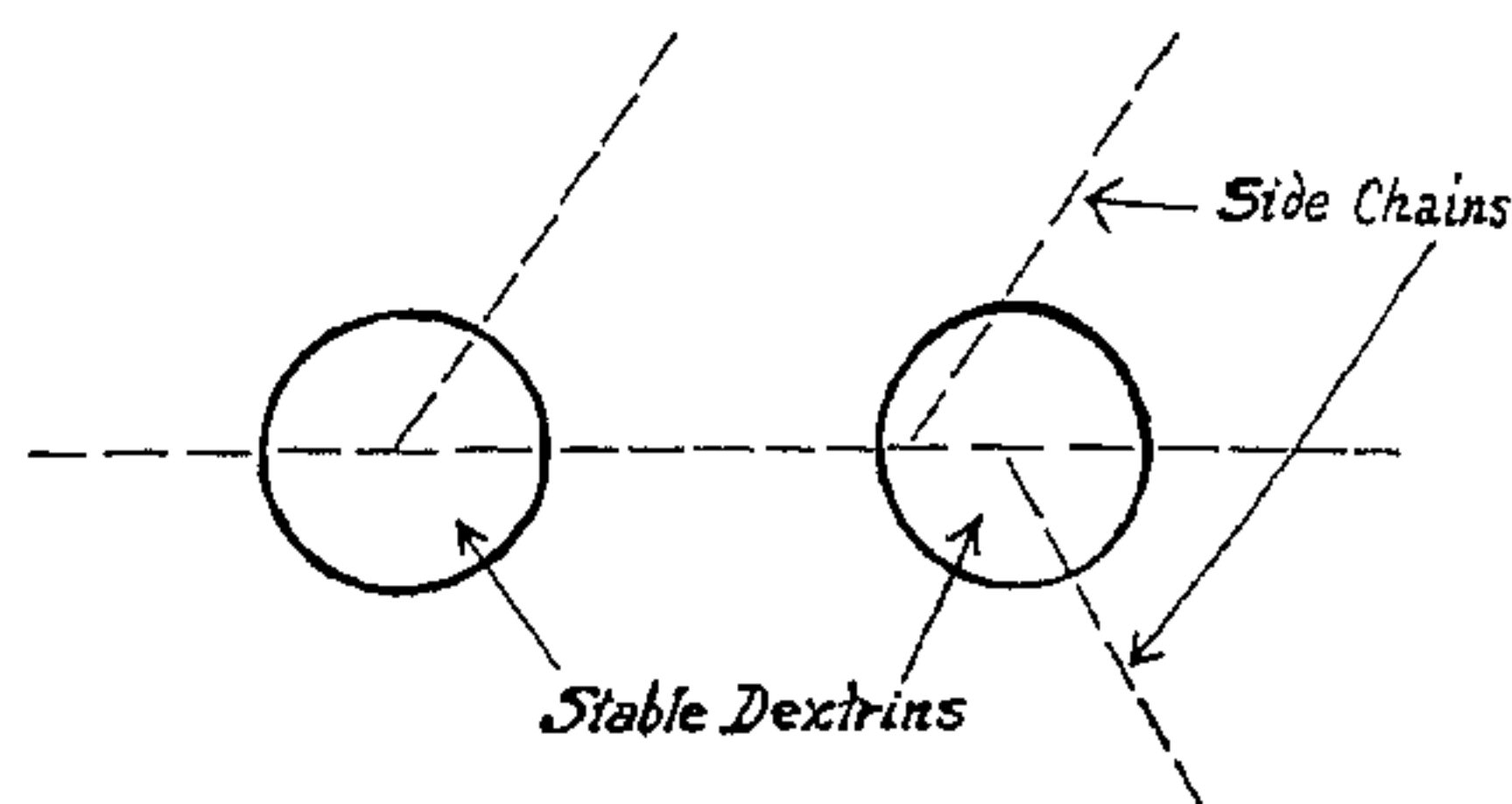
When investigating the limit dextrins it is obviously necessary to know their molecular size. For this purpose, we have applied the diffusion method being on various grounds an appropriate procedure. Hitherto, we have studied dextrins, secured from different starches by means of β -amylase from barley, α - + β -amylase from malt, taka diastase and saliva. In contrast to the dextrins produced by the three last-mentioned amylases, the β -dextrins are characterised by a high molecular weight or more adequately by a low diffusion constant. The values obtained with different β -dextrins vary between $k_{20} = 0,08 - 0,01$. According to Euler's empirical formula, these figures would correspond to molecular weights ranging from 7700 to ca. 500.000. Especially, marked dissimilarities exist between dextrins from different samples of starch. In some strongly degraded starch preparations, e.g., Zulkowski starch, etc., the average molecular weight of the β -dextrins approaches that of the initial starch. It has been found that

approximately 60% of maltose is formed at the same time as the limit dextrin from these starch preparations. This implies that the limit dextrins cannot arise as a result of the formation of 60% of maltose from *all* starch molecules. It is a much more natural assumption that particular molecules yield little if any maltose, whereas others give rise to more than 60% and many of them certainly to almost 100% of maltose.

In contradistinction to β -amylase, malt and taka amylases and saliva, produce from all the starches investigated, limit dextrins having a molecular weight of 600–3000. The difference between preparations obtained from various kinds of starch is not very striking and certainly no relation can be detected to the corresponding differences between the β -dextrins. The molecular weights which, referring to such low-molecular substances, are to be considered fairly reliable (although mean values), preclude naturally the concept of a di- or tri-hexosan nature of the substances; moreover, the possibility must be out of question that the stable dextrins may be in this particular instance unaltered components of the original starch. On the contrary, their formation must obviously be the result of a breakdown of all molecules, some indigestible remnants of the molecules being left. These fragments are identical with the abnormally constituted parts discussed above. Provided the β -amylase yields on the average 60% and, for instance, the β - + α -amylase yield 85% of maltose, the divergence between these enzymes concerning their mode of action upon starch cannot possibly be accounted for on the assumption that malt amylase attacks β -dextrins and splits off an additional number of maltose molecules from a continuous chain. If this be the case, the limit dextrins must evidently have a molecular weight, 35–40% of that of the corresponding β -dextrin. On the contrary, it must be assumed that the β -dextrin when acted upon by malt amylase is decomposed into a rather large number of minor parts, from which a certain amount of maltose is released. This tallies with the above assumption that malt

(taka) and salivary amylases have the power of attacking the chains *between* the abnormal parts of the starch molecules.

The estimation of the molecular weight of the limit dextrins must, of course, be accompanied by the determination of their constitution, *i.e.*, chiefly the nature of the glucosidic linkages between the glucose molecules. In this respect, our work proceeds so far along well-known lines. We have concentrated our researches on dextrins having the lowest molecular weight and the lowest P-content obtainable, this being done in the hope that such fractions may not be too unhomogeneous. In fact, much evidence supports our belief that we have succeeded in isolating some rather homogeneous fractions. According to my working hypothesis, the limit dextrins of lowest molecular weight (ca. 600) being practically P-free and containing ca. four glucose molecules represent those parts of the starch molecule, to which the side-chains are attached. Cf. the schematic figure, in which the dotted lines indicate chains of glucose molecules.



The first question is: do the four glucose molecules form a normal or a branched chain? (The normal reducing power of the substance precludes the idea of a ring form.) Of course, the methylation method of Haworth should enable us to answer this question quite satisfactorily, although it must be admitted that the procedure is rather laborious. When assuming the above theory to be correct it should be expected that the totally methylated dextrin would yield a much larger quantity of tetramethylglucose than would starch itself.