CCCLV.—*Polysaccharides. Part III. The Molecular Complexity of Inulin.*

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IT is now generally accepted that inulin is composed entirely of anhydro-γ-fructose or -fructofuranose units associated with water molecules, the relative proportion of water depending upon the method of drying. For instance, Tanret (*Bull. Soc. chim.*, 1893, **9**, 200, 227, 622) regards inulin as $(C_6H_{10}O_5)_6,H_2O$ at 130° and as $(C_6H_{10}O_5)_6,7H_2O$ in moist air at the ordinary temperature. Other attempts to determine the number of anhydrofructose units in the inulin molecule have given very conflicting results. Karrer and Lang (*Helv. Chim. Acta*, 1921, **4**, 249), from a determination of the molecular weight of trimethyl inulin in freezing phenol, find nine anhydrofructose units, in agreement with the molecular weight of triacetyl inulin in naphthalene, glacial acetic acid, and phenol, as determined by Pringsheim, Aronowsky, and Lassmann (*Ber.*, 1921, **54**, 1281; 1922, **55**, 1409). Bergmann and Knehe (*Annalen*, 1926, **449**, 302; *Ber.*, 1926, **59**, 2079) find only two anhydrofructose units in acetyl inulin prepared in the presence of pyridine. Again, Hess and Stahn (*Annalen*, 1927, **455**, 104) find that triacetyl inulin as prepared by Bergmann and Knehe has in air-free acetic acid a (cryoscopically determined) molecular weight which varies with time from that corresponding with the presence of one anhydrofructose unit up to infinity. Later work of Pringsheim and Fellner (*Annalen*, 1928, **462**, 231) indicates that the molecular weight of " inulin," regenerated from inulin acetate heated at various temperatures in presence of tetrahydronaphthalene, is variable according to the temperature of the heating, two, three, or four anhydrofructose units being present in the molecule. Direct determinations of the molecular weight of inulin in water are stated by these authors to agree in indicating that seven anhydrofructose units are present in the molecule.

It is clear that derivatives of inulin (such as the acetyl and methyl compounds) can throw no light on the complexity of inulin itself until it is demonstrated that these derivatives are simply related to the carbohydrate; and, although it has been alleged that inulin may be regenerated from the triacetyl compound, no definite proof of this statement has yet been given. Indeed, it has by no means been shown that inulin consists of uniform molecules.

Assuming that inulin consists of anhydrofructose units, these may be united either by residual affinities, in which case the structural units are retained as such in the complex, or by ordinary valencies, when the fructofuranose units are linked in chain formation. If the latter be true, as seems probable at present (compare Haworth and Learner, this vol., p. 619), the chain of γ-fructose (fructofuranose) units in the inulin molecule may be either open or closed to a ring. If it be an open chain, aqueous solutions of inulin should show slight reducing properties, due to the terminal reducing groups of the chains; if the ends of the chains be joined to give a ring, reduction may not be made manifest.

It has generally been held that pure inulin has no reducing action on hot Fehling's solution, but we have not yet succeeded in obtaining a sample of inulin which did not show slight and progressive reducing action on the boiling reagent. Such action may be due initially to the presence of open-chain molecules augmented subsequently by hydrolysis, or it may be due to the hydrolysis alone.

The inulin of commerce, derived from dahlia tubers, contains a considerable amount of ash consisting of mineral bases and salts, part of which is soluble in water after ignition, yielding an alkaline solution. The main bulk of this mineral matter may be separated from the inulin by the following simple process: The inulin is dissolved rapidly in hot water, and the solution boiled for a few minutes with a little active charcoal which has been carefully freed from soluble salts; it is then filtered, and the process is repeated unless the filtrate is colourless and water-clear. The bulk of the inulin is then frozen out, redissolved, boiled momentarily with a little more charcoal, again frozen out, then redissolved and precipitated by a large excess of alcohol, washed with alcohol, and dried. The white powder obtained has no reducing action on

Fehling's solution during 24 hours in the cold, but shows slight reduction on boiling or even on keeping at 50°. It contains about 1 part in 1000 parts of ash, as determined by heating in a platinum boat with or without sulphuric acid (*e.g.*, 14·139 mg. gave 0·014 mg. of ash). The ash appears to consist mainly of silica. The water-content was determined by heating during 2¾ hours at 75° to 110° in a stream of dry air at low pressure; the loss of water was 10% of the original weight of the sample (*e.g.*, 5·571 mg. lost 0·553 mg. of water). The resulting powder, which was extremely hygroscopic, gave on analysis: C, 44·3; H, 6·5 [$(C_6H_{10}O_5)_x$ requires C, 44·4; H, 6·2%].

The determination of the molecular weight, or average molecular weight, of "ash-free" inulin in boiling water was now attempted. The initial assumption was necessarily made that inulin is unaltered in molecular structure by solution in boiling water. That an alteration, at least in physical structure, does occur, however, is evident from the abrupt change in the solubility of inulin in water between 80° and 100°: 100 c.c. of solution at 14° contain 0·02 g. of inulin; at 80°, 4·0 g.; and at 100°, no less than 36·5 g. This view receives some support, also, from our own observation that the position of the decomposition range on the temperature scale varies markedly with the amount of water retained by different samples of inulin; *e.g.*, the decomposition range is situated above 200° for specimens of "glassy" inulin rich in water, but is much lower for drier powdery samples. Inulin, as would be expected, never shows a true melting point. The molecular state of inulin in boiling water would nevertheless be of interest, and it is unfortunate that another complication enters into the determination. The apparent molecular weight of inulin in boiling water shows continuous diminution over a considerable period of time, and we have found that the reducing power of the solution rises as the molecular weight decreases, and that the action is not completely, if at all, reversed in the cold. These facts point distinctly towards the occurrence of hydrolysis.

The hydrolysis probably depends upon the presence of minute traces of acid electrolytes, since pellets made from the same bulk sample of inulin on different occasions did not always show the same initial molecular weights nor the same rate of hydrolysis, and pellets which had been handled with the fingers seemed to hydrolyse relatively rapidly. Contamination by the surface of the pellet-press was difficult to avoid and may partly account for the discrepancies. The following are typical examples of the results, *W* indicating the weight of water, *w* that of the water-free inulin, and the times being reckoned from that of the addition of the inulin (the pellets were seen to dissolve completely in from 1 to 2 minutes):

I.			II.			III.		
W, 14·7 g.; w, 0·8748 g.			W, 14·95 g.; w, 1·0701 g.			W, 14·35 g.; w, 1·2542 g.		
Mins.	Rise of b. p.	M.	Mins.	Rise of b. p.	M.	Mins.	Rise of b. p.	M.
3	0·008°	3870	5	0·012°	3100	4	0·011°	4130
7	0·011	2810	8	0·013	2860	7	0·014	3250
10	0·017	1820	23	0·020	1860	13	0·017	2670
17	0·024	1290	48	0·025	1490	28	0·022	2070
						39	0·025	1820

The times of observation were extended, in some cases, beyond those shown, and it was found that the molecular weight continued to fall, even below 900. It is evident that no exact significance attaches to the figures, nevertheless they appear to us to indicate that the initial molecular weight of inulin in boiling water is not less than 3200 or 3600; *i.e.*, the inulin molecule contains not fewer than 20 or 24 anhydrofructose units. Initial experiments showed that a perfectly steady indication of the boiling point of the water could be obtained by placing clean fragments of garnets, mixed with pieces of platinum foil, in the boiling-bulb (provided with a thick piece of platinum wire sealed into the lower extremity), so that the height of the column of garnets, which terminated just below the bulb of the thermometer, bore a certain ratio to that of the column of water above it. Four minutes were allowed for the re-attainment of the true boiling point after the addition of the pellets, this time having been determined roughly by other measurements with the apparatus.

It will be clear that the molecular weight of inulin may be much higher than that indicated by these experiments, both because the initial changes of temperature are necessarily very small and because the initial effect of traces of electrolytes may be relatively very great. We hope to investigate this aspect of the matter in further experiments.

It seems clear that the results of Pringsheim and his co-workers (*loc. cit.*) can have little significance, unless he has succeeded in obtaining a specimen of inulin that is stable to boiling water. In any case, his procedure for the cryoscopic determination of the molecular weight (namely, to make up a solution of inulin in boiling water and then to cool it and determine the freezing point) is inadmissible, and the agreement of his cryoscopic and ebullioscopic results is possibly fortuitous.

It has frequently been stated that inulin shows, on hydrolysis by mineral acids, no intermediate stage between itself and fructose. Certain observations which we have made during the past two years indicate that this view is erroneous; and although our experiments have not resulted in the isolation of demonstrably homogeneous intermediates, it seems desirable at this stage briefly to indicate their nature.

When a stream of carbon dioxide is passed during about 2 days through an aqueous solution of inulin, the solution being occasionally warmed to redissolve any inulin which has separated, a considerable proportion of the inulin becomes changed to a white powder having a lower range of decomposition on heating than inulin and shows a tendency to effervesce at higher temperatures. The powder differs from inulin, also, in that it is readily soluble in cold water to a water-clear solution which does not foam when shaken. The product, the specific rotation of which in water is rather higher than that shown by inulin, has a greater reducing action towards hot Fehling's solution.

The same effect can be produced by treating an aqueous solution of inulin in an autoclave with carbon dioxide at a pressure of about 230 lb./sq. in. during about 24 hours. Unchanged inulin is filtered off, and the clear solution is precipitated by the addition of an excess of acetone, a product similar to the foregoing being obtained.

The transformation may also be brought about in the dry way at the ordinary temperature. For instance, when inulin powder is kept in a desiccator in which is present a little vaporous ethyl chloroformate, the inulin is gradually changed into a product similar to that already described. The change may take many months; its speed seems to depend upon the proportion of water present in the inulin, very dry inulin requiring a longer period. This behaviour indicates that the change is hydrolytic, and that it is produced by traces of hydrochloric acid generated by the decomposition of the chloroformate in the presence of water.

A sample of inulin powder which has become " infected " in this manner suffers progressive degradation even when the chloroformate vapour is removed; and, if the infection be acute or if the water content of the original inulin be high, the powder may finally change to a yellow syrup which contains much fructose. Inulin may be " infected " without the agency of chlorinated substances; *e.g.*, inulin which has been recovered by precipitation with acetone from aqueous solutions treated with carbon dioxide undergoes the change when kept in a desiccator over phosphoric oxide or calcium chloride. In such cases the change is slow and the final product is a flaky powder apparently free from ordinary fructose.

The change may in most cases be arrested by repeatedly dissolving the sample in water and reprecipitating it by means of acetone. In some cases, however, even this treatment does not prevent the occurrence of slow subsequent change.

Naturally, the method of isolation by precipitation leads to fractionation of the product, the more soluble products of lower molecular weight remaining in solution. Our attention has been mainly directed towards the water-soluble product (precipitable by acetone) which can usually be obtained in high yield.

We have not succeeded in showing that this product is molecularly homogeneous, but we believe that it approximates to this condition and that it is an open-chain compound, of high molecular weight, containing one or two free reducing groups in the molecule. The following is a description of the product, which may be regarded as analogous to a starch dextrin.

Inulin "lævulin." The substance obtained in the foregoing manner was carefully separated from unchanged inulin and from substances of lower molecular weight by repeated solution in cold water, filtration, and reprecipitation with acetone. The fine white powder so obtained (7 g.) dissolved to a slightly opalescent solution in 75 c.c. of water at 19° ; the solution was passed through a stream-line filter, which was rinsed with a further 125 c.c. of water. The clear, colourless solution, neutral to litmus and quite free from halogen and methoxyl, was now treated slowly with 300 c.c. of acetone. After 1½ hours, the white, granular precipitate (5 g.) was collected, washed with 100 c.c. of acetone, and kept over phosphoric oxide. The substance melted to a white, opaque paste at about 142°, and the paste became a white froth near 185°. It was dehydrated in a vacuum during 11 hours at 80°, then during 24 hours at 95°, and finally at 120° during 6 hours, the loss of water amounting to about 7·5% (Found : C, 43·8 ; H, 6·5%). The product contained 1% of ash, which was allowed for in the analysis. A sample dried at 90° in a vacuum had $[\alpha]_{5461}^{21^\circ}$ $-45·8°$ in water ($c = 4·778$) and showed no mutarotation after a month. Other samples showed approximately the same rotation.

Cryoscopic determinations of the molecular weight in water gave results varying between 900 and 1100 for the undried material, and between 1200 and 1600 for the material dried at 90° in a vacuum. Ebullioscopic determinations gave results varying between 1400 and 1800.

It is doubtful whether much significance can be attached to these figures ; but it seems possible that the effect of dehydration or of hot water tends to change the product back in the direction of inulin by the relinking of chains opened in the former degradation process.

Another sample of this product, prepared in a similar way, was ash-free, lost 7% of water when dried in a vacuum at 80° during 8 hours, melted to a white paste at about 145° and frothed at a higher temperature (Found : C, 43·5 ; H, 6·5%). Many other analyses were carried out on similar products dried under various conditions, the average of these results showing C, 43·3; H, 6·5 (octasaccharide requires C, 43·8; H, 6·2%; *M*, 1314. Hexasaccharide requires C, 43·6; H, 6·3%; *M*, 990). The substance reduces dilute Fehling's solution at 50°, the reduction commencing after a few minutes and being considerable in 10 minutes, whereas inulin under these conditions showed only a trace of reducing action after ½ hour.

Experience has shown that it is important to remove the ash as completely as possible from inulin before investigating the properties of this substance, a consideration which we did not at first closely regard. We intend to repeat our experiments with ash-free inulin if this is attainable. It is possible that inulin is protected from the hydrolysing action of boiling water by the presence of weakly alkaline mineral matter contained among the "ash," and that the removal of ash, either by purification or by neutralisation with traces of acid, permits the onset and continuation of the hydrolysing process which we have described; but it is not yet certain that the hydrolysis of inulin by boiling water is a process identical with the catalytic degradation taking place in the dry way.

By allowing the transformation of inulin to proceed to different lengths, products of lower decomposition range have been isolated. These are considered to be mixtures of compounds containing shorter chains. The best-defined was a *substance* obtained, by the action of carbon dioxide on aqueous inulin, as a white powder which melted to an opaque paste at 85—86° and effervesced at 120—125°. This was readily soluble in cold water and had $[\alpha]_{5461}^{29^\circ}$ −45·6° in water ($c = 2·28$), the negative rotation slightly increasing with time. A further specimen, of initial m. p. 92—95°, gave C, 42·0; H, 6·8%; *M* (cryoscopic), 520 in water. The substance may be an impure tetra- or tri-saccharide in a partly hydrated condition. It reduced warm Fehling's solution, and also cold aqueous potassium permanganate on standing.

Summary.

It is considered probable that inulin consists of chains of fructofuranose or γ-fructose rings, the molecule containing at least 20 or 24 such rings.

Degradation, by means of carbon dioxide or a trace of hydrogen chloride, consists in the hydrolytic scission of the chains at junctions between the rings, polysaccharides with shorter chains being produced. Dehydration may in certain circumstances tend to regenerate the longer-chain polysaccharides or inulin itself. Complete scission probably leads to the production of γ-fructose, which is

then irreversibly changed to ordinary fructose. The migration of inulin in plant stems may possibly occur through the formation of water-soluble polysaccharides which are subsequently dehydrated to inulin in the tubers.

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