XXXIII. THE DECARBOXYLATION OF PECTIN.

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EARLY work on the occurrence of pectins and hemicelluloses showed that these substances were normally to be found in association in the cell-wall, the relative amount of each being dependent on the condition of the tissue, *i.e.* whether lignified or non-lignified [Clayson, Norris, and Schryver, 1921]. Subsequent work [Candlin and Schryver, 1928] indicated the existence of an intimate relation between the two classes of substances, since pectin was shown to undergo decarboxylation under the influence of alkali, yielding *inter alia* a substance of the hemicellulose type. The yield of hemicellulose obtained by this treatment was invariably low, amounting only to 12–20 % of the pectin taken; further, these workers were unable to determine the nature of the products which represented the major part of the pectin. This might have been due to the fact that, as is well known, the sugars readily undergo profound changes in the presence of alkali, giving a variety of indefinite degradation products. It would follow therefore that if, as would seem possible from its accepted constitution, the pectin molecule yields sugars on decarboxylation, these latter would be destroyed immediately by the alkali present.

Since, however, the decarboxylation of pectin is a reaction likely to throw some light upon the question of the relationship between certain of the cell-wall substances, it is desirable to establish the nature of the substances other than hemicelluloses produced thereby. With this object it is necessary to employ a decarboxylating reagent which will not tend to destroy these other products, and it has been found possible to use hot water for this purpose. By this means, pectin has been shown to yield an insoluble substance, of the hemicellulose type, and one or more soluble substances, probably sugars.

The insoluble product finally obtained appears to have undergone much more complete decarboxylation than the end-product (hemicellulose) described by Candlin and Schryver [1928]. From this insoluble product, a substance of the hemicellulose type has been separated, containing 6.2 % of uronic anhydride, and yielding a mixture of sugars on hydrolysis.

The investigation of the soluble products is not complete, but it is thought that the observation that pectin can be decarboxylated by means of hot water is of sufficient interest to warrant the publication of a brief account of the work at the present stage.

EXPERIMENTAL.

Methods of decarboxylation. For the earlier experiments, sodium pectate, prepared by the method given by Candlin and Schryver, was used. The reactions were carried out in silica vessels, as it was thought that the use of hot water under pressure might result in the liberation of free alkali from glass vessels.

About 0.4 g. sodium pectate was placed in a silica boiling-tube, with 8 cc. water, and the mixture was heated in an autoclave for 6 hours at approximately 125° (20 lb. per sq. in.). At the end of this period, the solution of pectin had assumed a faintly orange colour. The "uronic anhydride" content of the whole of the product was determined by the method of Nanji, Paton and Ling [1925]. It was found that little decarboxylation had taken place, the uronic anhydride content having fallen only by 3 % (calculated on the original uronic anhydride content of the sodium pectate).

It was thought, however, that the elimination of carbon dioxide from pectates might be a balanced reaction, in which case removal of carbon dioxide from the system would alter the equilibrium and induce further decarboxylation. With this point in view, the experimental conditions were modified as follows.

A nickel crucible containing approximately 2 g. potassium hydroxide was placed in the autoclave, the top of the crucible being well above the level of the water, but below the level of the mouth of the tube containing the sodium pectate. By the use of this device, any carbon dioxide evolved during the reaction was removed immediately.

A sample of sodium pectate heated thus for 5 hours at 153° (60 lb. per sq. in.) gave a product whose uronic anhydride content was 58 % less than that of the original sodium pectate, indicating that by this modification of the experimental conditions decarboxylation could readily be achieved. It was found that raising the temperature or prolonging the time of heating resulted in increased decarboxylation, but that at temperatures above 153° charring was liable to occur.

The possibility existed, however, that hydrolysis of the sodium pectate might occur during this process, giving rise to free alkali, which would produce secondary changes in the products of decarboxylation. For this reason it was decided to abandon the use of sodium pectate, and to use citrus pectin itself. This substance is considered by most workers to be entirely organic.

On heating citrus pectin (0.2 g. in 10 cc. water) at 153° for 1½ hours, a fall in the uronic anhydride content of 35 % was observed. The resultant products consisted of a dark brown solid, together with an orange coloured liquid.

The insoluble products. The brown solid, which on drying was light grey in colour, was further examined.

2 g. of citrus pectin dissolved in 100 cc. water were heated in the autoclave at 153° (60 lb. per sq. in.), in the presence of potassium hydroxide, as described

above. Experiments were made to determine the effect of the time of heating on the yield of the brown product. In each case, at the end of the period of heating the reaction mixture was filtered while hot through a Munroe crucible, and the solid product was washed thoroughly with boiling water, and dried at 100°. The uronic anhydride content of the product obtained in each case was determined (Table I).

Table I.

Time of heating (hrs.)	Wt. of insoluble product		Uronic anhydride content (%)
	A† (g.)	B† (g.)	
(0	—	—	69.4*)
2	0.086	0.086	58.2
4	0.128	0.137	32.1
6	0.156	0.147	20.8; 21.2
16	0.218	0.204	12.7; 12.0
48	0.346	0.352	3.6; 3.6
96	0.403	0.413	— —

* Original citrus pectin.

† Duplicate experiments.

From these figures it seems that the final product given by treatment with hot water has a very low uronic anhydride content, and that the amount of insoluble product reaches a limit on prolonged treatment. The maximum yield of the product amounted to about 20 % of the pectin taken, a figure comparable with that obtained by Candlin and Schryver for the yield of hemicellulose given by alkali decarboxylation.

For further investigation of the insoluble product, a rather larger sample was prepared by heating citrus pectin with 50 times its weight of water at 153° for 48 hours; 2 g. of product were obtained in all. This was extracted with 300 cc. of 4 % sodium hydroxide, the insoluble residue being removed. To the extract glacial acetic acid was added until the liquid was very faintly acid, after which the solution was treated with an equal volume of 95 % alcohol. A brownish coloured precipitate was produced. This settled after standing for some hours and was filtered off, washed thoroughly with 95 % alcohol, and dried. The substance thus obtained was in the form of a brown powder. A yield of 1.14 g. was obtained from 2 g. of the original insoluble product. Its uronic anhydride content was found to be 6.2 %. This is considerably lower than the uronic anhydride content of the hemicellulose described by Candlin and Schryver. The general behaviour of the substance, however, indicated that it belonged to the hemicellulose class. It yielded a mixture of sugars on hydrolysis with dilute sulphuric acid.

The soluble products. As stated above, an orange coloured liquid was obtained together with the insoluble brown product on heating pectin with water for some hours at 153°. This liquid became slightly opalescent on cooling; it was not possible to obtain a perfectly clear solution by filtration. On treatment with 4 volumes of alcohol the solution became quite clear, there being no precipitate of a gel of pectin; it was concluded therefore that the original pectin had been destroyed completely on heating. The addition of acid to the solution gave no gel of pectic acid.

The solution was neutral to litmus, and did not give the naphthoresorcinol test for sugar acids. It possessed strong reducing properties, and appeared to contain sugars. It seems certain that the use of hot water as a decarboxylating agent avoids that destruction of the soluble products which renders the use of alkali objectionable.

SUMMARY.

1. It has been found possible to produce decarboxylation of pectin by means of hot water under pressure, provided that the carbon dioxide evolved during the reaction is removed from the system.

2. The products obtained by such decarboxylation consist of an insoluble substance, probably a hemicellulose (mixed with a small quantity of undetermined matter), and one or more soluble products, possibly sugars. The hemicellulose-like substance has a uronic anhydride content of 6.2 %.

The work described in this paper was undertaken at the suggestion of the late Prof. S. B. Schryver, under whose direction the early experiments were carried out. The author wishes to place on record his indebtedness to Prof. Schryver for advice and encouragement.

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