CXXI. THE PECTIC SUBSTANCES OF PLANTS.

PART IV. THE PECTIC SUBSTANCES IN THE JUICE OF ORANGES.

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(Received August 5th, 1926.)

OUR knowledge of the chemistry of the pectic substances of plants has made considerable strides in the last decade. The important work of Ehrlich [1917, 1924] on the hydrolysis of pectic substances indicated that the constituents of the molecule were arabinose, galactose and galacturonic acid. In these laboratories [Clayson, Norris and Schryver, 1921] a pure pectic substance was prepared, shown to have a definite chemical identity, and designated pectic acid. This substance was further examined by Nanji, Paton and Ling [1925] who found that the chemical properties of this substance were fully explained by a formula involving a six-membered ring, the sides of which represented arabinose, galactose and four molecules of galacturonic acid, the carboxyl groups of the latter being free. This substance may be regarded as the basal molecule to which members of the class of pectic substances may be referred. This formula received striking confirmation in the result of investigations on pectinogen, the precursor of pectic acid [Norris and Schryver, 1925]. It was found that the analyses of pectinogen corresponded with methylated derivatives of the basal molecule, and it was deduced that the pectic substance in the cell wall consisted chiefly of the trimethyl derivative of pectic acid, one carboxyl group being left intact; this is probably the point of attachment of the cellulose or hemicellulose with which pectinogen is associated.

The object of the present communication is to indicate the relationship between the pectic substance of the cell wall and that which is found in the juice of such a fruit as the orange.

It is generally held that the pectic substances of the juice are derived from the cell wall in the first instance, although the mechanism of the process is not as yet completely understood. It seems probable, however, that the acid in the juice is responsible for the scission of the pectic complex from the other constituents of the cell wall with which it is associated.

The reaction whereby the pectic substance is brought into solution is considerably less drastic than the chemical means employed to extract the product from the cell wall, and thus we should expect to find that this soluble pectic substance is highly methylated. The results of the present investigation

show that this is the case; the soluble product was precipitated with alcohol, could be redissolved in water and reprecipitated, and on analysis yielded both furfural and methoxyl corresponding almost exactly with those calculated for trimethylpectic acid.

Two points of interest are to be observed in connection with the preparation of this product.

Firstly, it is necessary to ensure that the juices come into intimate contact with the cell wall substance. If the juice is very carefully expressed in order to prevent the cells from being ruptured to any great extent, the amount of pectic substance present is small. If, however, the fruit is minced and filtered, the amount present is considerably increased, thus indicating that both cell wall and juice are intimately concerned in the production of the pectic substance.

Secondly, if after mincing in order to ensure the intimate association of cell wall and juice, the juice is allowed to stand for twenty-four hours or longer, a smooth jelly is formed, and this differs markedly from the soluble gel. The change is generally attributed to the action of enzymes and this view is supported by the observation that, if the juice is heated to 90–100° for an hour or two immediately after extraction, the formation of the smooth jelly is completely inhibited.

These two considerations, therefore, have some bearing on the method to be adopted in the preparation of the soluble gel. The oranges chosen were placed in boiling water for 2 hours; the peel was subsequently removed and the whole fruit minced. In this way enzyme action was stopped, and the intimate association of cell wall and juice and the maximum yield of soluble gel ensured.

The next step in the investigation was an attempt to identify the smooth jelly which is formed when the fruit juice is allowed to stand. Its properties were in direct contrast to those of the soluble gel. It was insoluble in water and contained no methoxyl groups. It could be dissolved in dilute ammonia and from this solution it was reprecipitated as a clear white gel by addition of excess of hydrochloric acid. These properties are characteristic of pectic acid and the yield of furfural obtained further supported this view.

Although the gels obtained were bulky, the actual weights finally obtained were very small; just sufficient for the analyses was obtained and further purification was therefore impossible. The ash content was not high, but a small quantity of nitrogen due to protein was present, and corrections were made in the analyses accordingly.

Attention was called in Part II of this series [Clayson, Norris and Schryver, 1921] to the fact that the analytical figures for pectic acid from oranges differed very slightly from those obtained for pectic acid from a variety of different sources. The same was true to a lesser extent in the case of apples. It is significant that these are the only cases in which any considerable quantity of pectic substance is to be found in the juice. Although at the present stage it

is not possible to explain this in detail, there seems little doubt that the activity of the acid and enzyme in the juice effects changes which might account for the slight differences in analysis cited above.

EXPERIMENTAL.

Preparation of the soluble gel.

About 30 Denia oranges, which were just ripe, were used for the preparations.

In order to stop the action of enzymes at the outset, the oranges were placed in boiling water and maintained at a temperature of 95–100° for 2 hours. The peel was then removed and the oranges were passed through a fine mechanical mincer. The moist mass was then filtered through muslin: this was somewhat slow, but by stirring the mass most of the liquid came through. The muslin bag was then placed in cloth and subjected to pressure. The extracts contained small quantities of cell wall material and were filtered on ordinary funnels; it was necessary to change the filter papers at intervals owing to clogging. Finally, however, the liquid was sufficiently free from solid particles to enable the first precipitation to be carried out. Accordingly it was thrown into two volumes of 95 % alcohol and the resulting gel allowed to stand overnight. The gel was filtered off with some difficulty through ordinary filters. At this stage the alcoholic filtrate was bright yellow and the gel itself was by no means colourless. The gel was redissolved in the minimum of distilled water, the solution filtered through paper pulp and again thrown into two volumes of alcohol. The gel had now lost most of its original colour, but further solution and precipitation were deemed necessary in spite of the fact that loss was involved.

After the second precipitation an almost colourless gel was obtained and this was then dried in graded strengths of alcohol for some days. After washing with ether and drying in a current of air, the product was placed in a vacuum desiccator over phosphorus pentoxide until it became constant in weight.

The analyses were carried out by the same methods as have been adopted in previous communications; furfural and methoxyl groups were estimated by the methods of Tollens and Zeisel respectively. The figures quoted were calculated on an ash-free basis. The calculated figure is based on accepted yields of furfural from the groups concerned [cf. Norris and Schryver, 1925], and shows the yields of furfural and methoxyl which may be obtained from trimethylpectic acid.

The results are indicated in the table below:

	Ash	Furfural	Methoxyl
	%	%	%
Found (i)	6.56	18.61	9.27
(ii)	—	19.12	8.44
(iii)	—	19.91	9.06
Average	—	19.21	8.92
Calculated		19.76	8.94

These results, therefore, correspond very closely with the figures calculated for trimethylpectic acid, and it is probable that the soluble gel consists almost entirely of this product. It resembles, in almost every respect, the pectinogen obtained by chemical means and described in Part III. It will be remembered that a hemicellulose was invariably found in association with pectinogen: owing to the small quantity of gel available in the present instance, it was impossible to effect a separation, and the question of the presence or otherwise of a hemicellulose in the soluble gel obtained from the juice must be held over until larger supplies have been prepared and examined.

Preparation of the insoluble gel.

The oranges were peeled and minced as already described. The minced mass was allowed to stand for 2 or 3 hours and then filtered through muslin. Filtration was slow, the juice in this instance being in contact with the cell wall for some hours. When filtration was complete, the cell wall substance was washed with a small quantity of distilled water and the filtered washings added to the main bulk of extract. Toluene and chloroform were added and the mixture allowed to stand for 3 days. At the end of this period, a considerable bulk of a brightly coloured jelly was formed and the residual liquid was poured off. The jelly was dissolved in ammonia, the mixture stirred mechanically for some hours and then filtered through paper pulp. A slight excess of concentrated hydrochloric acid was added, whereupon a flocculent gel was thrown down; the gel was thoroughly washed with many changes of distilled water and the process of solution and precipitation repeated. After another washing, the gel was almost colourless and was then dried as usual in graded strengths of alcohol, finally attaining constant weight after drying for a fortnight in a vacuum desiccator containing phosphorus pentoxide.

The results obtained on analysis are shown below:

	Ash %	Furfural %
Found (i)	1.01	20.18
(ii)	—	20.66
(iii)	—	20.13
Average	—	20.32
Calculated	—	20.59

These results correspond very closely with those calculated for pectic acid; the properties of the gel are also similar and we may conclude that this product is identical with that obtained by chemical means from the cell wall.

It would seem, therefore, that the action of the enzyme on the pectic substance in the juice follows much the same course as was shown by the action of lime water on the soluble gel extracted from the cell wall (Part III). That is to say, the methylated pectic acid is gradually de-methylated, pectic acid being the final product. In the case of the pectinogen from the cell wall, however, it was shown that lime water effected, in addition, the scission of

a hemicellulose in association with the pectic substance. In the case of the product obtained from the juice, this has not yet been demonstrated and is left for future investigation when a larger supply of material is available.

SUMMARY.

1. The pectic substances which occur naturally in the juice of fruit are essentially the same as those extracted from the cell wall by chemical means, as described in the earlier papers of this series.

2. They are derived from the cell wall by the action of the acids and enzymes in the juice; this view is supported by the observation that the quantity of pectic substance in the juice is considerably increased if the fruit is macerated before expression of the juice, thus allowing the juice to come into intimate contact with the cell wall.

3. The pectic substance in the juice corresponds with a trimethylated derivative of pectic acid similar to the pectinogen described in a former paper. This on standing is gradually converted into an insoluble gel unless the fruit is boiled before maceration and expression of the juice. The action is evidently due to the enzymes present in the juice. The insoluble gel has been identified as pectic acid.

4. Owing to the small quantity of material available, it has not yet been possible to determine whether a hemicellulose was present in association with the soluble gel. This was always found to be the case with preparations of pectinogen prepared from the cell wall by chemical means.

REFERENCES.

- Clayson, Norris and Schryver (1921). *Biochem. J.* **15**, 643.
Ehrlich (1917). *Chem. Ztg.* **41**, 197.
— (1924). *Deutsch. Zuckerind.* **49**, 36, 1046.
Nanji, Paton and Ling (1925). *J. Soc. Chem. Ind.* **44**, 253 T.
Norris and Schryver (1925). *Biochem. J.* **19**, 676.