XCII. STUDIES ON PECTIN. PART III.

THE DEGREE OF ESTERIFICATION OF PECTIN IN THE JUICE OF THE LEMON.

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DURING the past few years interest in the chemistry of the pectic substances has revived, and considerable advances have been made in our knowledge. The recent work of Nanji, Paton and Ling [1925] has thrown much light on the constitution of the basal unit; and the conclusions of these workers have been confirmed by Norris and Schryver [1925]. On the other hand very little exact knowledge exists as to the precise nature of these substances *in situ*. Examination after extraction is apt to give results which are misleading, since several changes are known to occur during this process. For example, it is quite certain that de-esterification must occur during the usual processes of extraction to a quite considerable extent. Consequently the methoxyl content of the pectin preparation cannot be taken as that of the pectin as it existed in the plant before extraction.

It is now generally accepted that the pectic substances occur in the plant in at least two forms, one soluble in water, and the other insoluble. Tutin [1923] did not agree with this statement, arguing that the retention of pectin in a form apparently insoluble in water was due solely to mechanical difficulties of extraction. His objections, however, were fully met by the work of Carré [1925], who produced good evidence for the existence of a pectic substance insoluble in water, called by her "pectose," and supposed to be in combination with cellulose. This substance, which will form the subject of a further communication, corresponds to the "protopectin" of other workers. The soluble modification has been supposed by most workers to arise from the insoluble form, although the mechanism of the process is not understood. Further it is clear that the soluble pectin does form a part of the cell wall, not only in young tissue, but in mature plants; in other words it is by no means all of the total soluble pectin which is in solution in the cell sap. Consequently an examination of the condition of the soluble pectin in the cell fluid provides a reliable reflection of the state of the same substance *in situ* in the cell wall.

Von Fellenberg [1918] pointed out that methyl alcohol is a scission product of pectin, and assigned to pectin a formula which contained eight methoxyl groups. He even suggested that pectin might be estimated in plant materials

by the yield of methyl alcohol. The formula given by Nanji, Paton and Ling [1925] includes four galacturonic units, each of which may be present as the methyl ester. Norris and Schryver [1925] conclude that in the case of the soluble pectic substance of the plant, three of the four carboxyl groups are esterified, whilst one is still free to react with a metallic ion in normal salt formation. This conclusion is based on the fact that these workers never found more than 9.2 % methoxyl in any of the many samples of pectin prepared by them in various ways. The usual figure obtained by them was much below 9.2 %. The theoretical percentage for the tetra-methyl ester is 11.76, and that for the tri-methyl ester, 8.94.

More recently Norris [1926] has studied the pectic substances in the juice of the orange, and proved them to be essentially the same as those extracted from the cell wall by purely chemical means. He states that his product from the juice corresponds almost exactly with a tri-methoxylated derivative of pectic acid, having an average methoxyl content of 8.92 %. However, the method of preparation used by Norris is not above criticism, since it probably involves the loss of a portion of the methoxyl content. The oranges which he employed were placed in boiling water for 2 hours to inactivate the enzymes. The hydrogen ion concentration of the juice of oranges is of the order of $p_H = 3.5$, and reference to the tables given by Nanji and Norman [1926], and further unpublished work, indicates that heating for 2 hours at this hydrogen ion concentration results in the splitting off of at least 10 % of the total methoxyl content. The effect of natural buffering may be to reduce this somewhat, but nevertheless it is a factor which must not be overlooked.

EXPERIMENTAL.

An investigation was carried out on lemon juice with the same object in view, namely the determination of the degree of esterification of the pectin present in solution in the juice.

About 50 lemons, nearly ripe, were employed in this preparation. The lemons were peeled, cut into two portions and the juice was squeezed out immediately by hand. It was at once filtered through muslin into excess of 95 % alcohol. This precludes any possibility of the intervention of enzyme action, although as a method it is not economical. Ordinary pressing, however, was avoided, as it was not considered desirable for the juice to come in contact with any metallic surface. The precipitated gel, yellowish in colour, was allowed to stand overnight, filtered on fluted filter-papers, and well washed with cold 95 % alcohol. It was then redissolved in cold water, filtered through paper pulp, and reprecipitated by pouring into alcohol as before. This time a greyish-white precipitate resulted. This process of solution and precipitation was repeated five times. The gel was then again dissolved in water, rather more in quantity than before, and the solution filtered through fluted filter-papers before being poured into alcohol. Though very slow, this final

filtration through paper is necessary to ensure that no fibres from the paper pulp are present in the finished product. The gel obtained was then dried with repeated changes of absolute alcohol for over a week, and placed in a vacuum desiccator containing phosphorus pentoxide till of constant weight. The yield from the juice of 50 lemons was rather less than 2 g.

It is to be noted that throughout the preparation the temperature was kept as low as possible, so that de-esterification would not occur. The methoxyl yield was determined by the method described by Nanji and Norman [1926].

The results obtained were as follows:

Ash (i) 11.90 %, (ii) 11.86 %, Mean 11.88 %.

Apparent *methoxyl* yield (on ash-free basis):

(i) 8.64 %, (ii) 8.51 %, Mean 8.57 %.

These results, then, would appear to confirm the conclusions of Norris and Schryver [1925], and of Norris [1926], that the tri-esterified form is the normal state of soluble pectin in the plant. It is possible, however, that this is not the case, since there is no criterion of purity of the pectin preparations. Pectin on precipitation with alcohol carries down with it other substances, which are extremely difficult to remove later even by repeated solution and reprecipitation. This is reflected in the high ash content. Further it is more than possible that there may be present with pectin in the juice some other organic substance, insoluble in alcohol and soluble in water, which will not be removed by the method of purification adopted by Norris [1926], and also used in this work. Accordingly a determination of pectin present in the sample was carried out by the calcium pectate method as modified by Nanji and Norman [1928], and a surprising result was obtained. The calcium pectate yield is the only analytical figure for pectin which is characteristic, and further it has been accepted by many workers as quantitative [cf. Nanji, Paton and Ling, 1925].

The mean yield of calcium pectate from the apparently pure product obtained by the method given above, was only 67.2 %, which on the ash-free product corresponds to 76.3 %. Calculating on this basis then the true methoxyl content of pectin present is (i) 11.56 %, (ii) 11.40 %, giving a mean figure of 11.48 %, the calculated figure for tetra-methoxyl pectic acid being 11.76 %. If this correction for calcium pectate yield is justifiable, and it seems that it is so, it is clear that the soluble pectin as it exists in the juice and in the cell wall is practically completely esterified. It is not claimed that it is uniformly in the tetra-methoxy condition; the acids in the juice would probably prevent this, but the evidence points to a further degree of esterification than that asserted by other workers.

If this correction for calcium pectate yield is applied, the question naturally arises as to the nature of the substance or substances also present with pectin in such preparations to an extent of over 20 %. It was thought at first that a portion might be protein in nature, but the preparation was found to be

free from nitrogen. The furfural figures as recorded by Norris and Schryver [1925], and Norris [1926] for their preparations agree fairly closely with the theoretical figures assuming only pure pectin to be present. The capacity for yielding furfural is not, of course, limited to the pectic substances, so it is clear that the unknown substance must yield approximately the same amount of furfural as pectin, that is, about 20 %, and possess similar solubilities. Norris and Schryver [1925] isolated a hemicellulose associated with pectin in turnips. This, however, was only found to be present to the extent of 3 to 4 %; and further, its yield of furfural was low, being only of the order of 12 %. The substance "hemicellulose C" also described by these workers gives a furfural yield approaching that apparently indicated in this case, but the vague character of these substances renders it impossible to carry the parallel further.

It seems likely that this associated material consists of pectin degradation products, the six-membered pectin ring having been broken, producing compounds of a conjugated galacturonic type retaining in all probability one pentose group. In view of this observation, work is being carried out on the breakdown of pectin beyond the pectic acid stage, and will be described in a further communication.

SUMMARY.

1. Special precautions are taken to obtain a preparation of the pectin from its solution in lemon juice, without the possibility of the occurrence of partial de-esterification.
2. Such a preparation gives an apparent methoxyl content corresponding to a tri-methoxylated derivative of pectic acid.
3. If a correction for the calcium pectate yield of the preparation is employed—the only criterion of purity known—then the corrected figures for methoxyl approach the theoretical value for tetra-methylpectic acid.
4. It is claimed that no good evidence exists for stating that the soluble pectic substance exists uniformly in the tri-methoxylated condition; and that it must be regarded as being almost completely esterified.
5. Some other material, probably pectin degradation products, giving a furfural yield similar to that of pectin, is associated with pectin in such preparations.

In conclusion it is a pleasure to express my thanks to Prof. A. R. Ling for his advice and encouragement.

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