CII. THE PECTIC SUBSTANCES OF PLANTS. PART III.

THE NATURE OF PECTINOGEN AND ITS RELATION TO PECTIC ACID.

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IN previous communications [Schryver and Haynes, 1916; Clayson, Norris and Schryver, 1921] the preparation and properties of pectinogen and cytopectic acid were described, and the results obtained indicated that the latter was a definite chemical compound. The object of the present communication is to establish more definitely the nature of pectinogen as it occurs in the plant and on extraction in the laboratory, and its relation to cytopectic acid and the other constituents of the cell-walls of plants.

The pectic substances have been the subject of researches undertaken by a number of investigators, with the unfortunate result that some confusion in nomenclature has arisen. The term pectin has been used to describe every conceivable type of pectic substance. Pectinogen has also been widely used to describe a substance different from pectin and usually considered to be the precursor of pectin. As such it has also been called protopectin [Tutin, 1923, 2] and recently the old term pectose has been revived [Carré, 1925].

Now, there is very little doubt that the basal molecule of the pectic substances is that referred to above, cytopectic acid, and this name was introduced by us in an attempt to arrive at some standard nomenclature; there is also little doubt that this substance exists in a modified form in the cell-wall, and this precursor we called pectinogen. This distinction has been adopted in effect by recent workers [Nanji, Paton and Ling, 1925], who refer to pectic acid and its modification, pectinogen. We shall therefore adhere to this system in order to avoid further confusion.

Very little is known of the nature of pectinogen *in situ* and the contradictory and controversial statements that have been made arise in some measure from the lack of any standard of nomenclature, preparation and analysis.

Before dealing with pectinogen itself, it is necessary to consider briefly the nature of the basal molecule, pectic acid. A considerable advance in our knowledge of the constitution of this substance has been made recently. Ehrlich [1917] showed that the molecule contained galacturonic acid groups together with arabinose and galactose. These products of hydrolysis have been obtained in this laboratory, using a modification of a method due to Monier-Williams [1921]. The paper by Nanji, Paton and Ling contains a further important contribution to our knowledge of pectic acid. The carbon dioxide evolved on hydrolysis was quantitatively determined and from the results obtained it became apparent that the molecule must consist of four molecules of galacturonic acid with one molecule each of arabinose and galactose; they consider these molecules to be combined in a six-membered ring each side corresponding to the appropriate carbohydrate or acid molecule, the carboxyl groups of the acid being left free (see p. 685).

The empirical formula of this acid is $C_{35}H_{50}O_{33}$ and this agrees very well with the analytical results carried out both by them and by us on pectic acid; the formula tentatively suggested by Schryver and Haynes [1916], $C_{17}H_{24}O_{16}$, is a close approximation to the above. This was proposed as a formula for pectinogen, however, and in view of the fact that it has been used in connection with a method for estimating pectic acid, it is important that the corrected formula be speedily adopted.

Any attempt to correlate pectic acid and pectinogen should have reference to the above formula and it will be shown later that this formula helps to explain some of the problems which have arisen in connection with pectinogen. In the early paper of Schryver and Haynes [1916] it was suggested that the action of alkalis effected the change from pectinogen to pectic acid, and von Fellenberg [1918] showed that this change also involved the liberation of methyl alcohol. The first statement has been universally accepted, although perhaps on somewhat slender evidence, but the second has suffered some amplification and criticism. Tutin [1923, 1] states that he obtained from apple "pectin" both methyl alcohol and acetone in sufficient quantity to enable him to determine the physical constants and chemical nature of these substances. The presence of acetone in addition to methyl alcohol has been refuted by several workers and we, also, regard methyl alcohol as the sole volatile product of the reaction. This is founded on analytical methods it is true, but two methods of estimation were used, that of Zeisel and another due to Schryver and Wood [1920], the latter so delicate that the presence of acetone would be bound to affect the results to a considerable extent, whereas in fact they agreed very closely by the two methods. The previous history of Tutin's "pectin"—the apples were taken from a cider-press—would surely account for the presence of such volatile substances as impurities and not as definite groups in the molecule of the pectic substance.

Nanji, Paton and Ling [1925] agree with us in regarding the action as one of de-esterification and reiterate our statement that the analyses do not

warrant the assumption that any methylpentoses exist in the molecule. We, however, while regarding the main reaction as one of de-esterification, have found that there is also another factor to be considered. In addition to methyl alcohol, we have found another substance, which is non-volatile, in the alkaline extract, and this substance appears to belong to the class described as hemicelluloses. It appears to be associated with the main bulk of pectic substance, whether as a mixture or in loose combination is not as yet quite certain.

Some indications of the nature of pectinogen as it exists *in situ* were obtained and it seems probable that the formula for the basal molecule adapts itself very well to our results. Thus we regard pectinogen *in situ* as consisting of a methylated pectic acid in which three of the carboxyl groups are esterified, thus leaving one still free to account for the definitely acidic nature of pectinogen, in loose combination or admixture with a substance similar to the hemicelluloses.

The positions of the linkages in the molecule are as yet unknown but we have some evidence that they are not all of the same type. We consider that there is an intermediate product in the process of de-esterification containing only one methoxyl group, which seems to be more stable than the more completely methylated compound and consequently less susceptible to the action of weak alkalies.

We may now consider in more detail the basis of the above statements.

Preparation of pectinogen and its properties.

At the outset, the methods adopted for the extraction of pectinogen were similar to those used in the case of cytopectic acid, with the difference that no extraction with caustic soda took place before the pectic substance was obtained. The maximum yield was the prime consideration, and to this end the plant tissues were extracted to exhaustion and the fractions combined and precipitated as one. A number of extractives were used and after a time the large discrepancies in analytical results made the fact apparent that pectinogen was not of such constant composition as was cytopectic acid. It became obvious that the method of exhaustive extraction must be abandoned and that the pectinogen should be prepared under rigidly standardised conditions, having due regard to the factors involved. The most important of these factors were found to be:

- (i) Nature of source.
- (ii) Nature of extractive.
- (iii) Concentration of extractive.
- (iv) Time of extraction.
- (v) Temperature of extraction.

Each of these factors had a varying effect on the pectinogen in the following respects:

- (i) Yield.
- (ii) Purity. (Freedom or otherwise from adsorbed inorganic salts.)

(iii) Yield of furfural on analysis.

(iv) Yield of methyl alcohol on analysis.

Before proceeding to the examination of the effect of the above factors the experimental method of exhaustive extraction and the results obtained may be given.

The chief sources employed were turnips, onions and, to a lesser extent, pea-pods. The method of preparation of the cell-wall substance was that of Clayson, Norris and Schryver [1921]. The yield is small and, consequently, large quantities of raw material have been dealt with, amounting to about half a ton each of turnips and onions. Typical yields of cell-wall substance are, for turnip, 4 % and for onion, 2.25 % of the raw material.

In general, the air-dried cell-wall substance was treated with twenty times its weight of extracting solution and kept at about 90° in a water-bath for some time. Filtration followed and the residues were again extracted with an additional quantity of solution; this was repeated until the filtered extracts contained no pectinogen. Exhaustion was usually reached at the fifth extraction. The united extracts were then poured into two volumes of alcohol and after standing overnight the resulting gel was filtered through ordinary funnels. The gel is soluble in water and for this reason was difficult to precipitate in cases where the concentration of pectinogen in the extract was small, additional alcohol sometimes being required before the slimy mass was sufficiently coagulated to filter easily; in all cases the filtration was slow until the gel was placed in higher grades of alcohol. After allowing the product to stand in graded strengths of alcohol, the final drying was effected by absolute alcohol and ether, and air-drying for some days by means of a fan. Previous to analysis the pectinogen was dried over phosphorus pentoxide in a vacuum desiccator at room temperature. At this temperature constant weight was usually attained after a fortnight; this slow drying, however, was necessary, as the use of a vacuum dryer at 100° was found to be detrimental to the product, charring frequently resulting.

Pectinogen prepared by this method was found to be white and amorphous; it is brittle and impossible to powder by hand although susceptible to mechanical grinding. On standing with water, it swells considerably during the period of imbibition and finally goes into solution; the process is considerably accelerated by mechanical stirring. For general purposes a 1 % solution was found to be the maximum for easy working as the viscosity becomes high and solution slow and difficult.

Pectinogen contains methoxyl groups as shown by the action of hydriodic acid (method of Zeisel); on distillation with 12 % hydrochloric acid, furfural is obtained indicating the presence of pentose groups and/or sugar acids of the glycuronic acid type; when carefully oxidised with nitric acid mucic acid is obtained, indicating the presence of galactose or galactans.

Pectinogen appears to exist in the cell-wall in combination with calcium, and it has been suggested that magnesium may also be present in some

form. The evidence that calcium is in combination with the pectic complex is very strong, since it invariably appears that pectinogen is extracted by agents which throw down an insoluble calcium salt by double decomposition, leaving the pectinogen in solution. Ammonium oxalate and oxalic acid are familiar examples and even carbon dioxide is capable of liberating appreciable quantities of pectinogen. On the other hand, salts such as sodium chloride are unable to effect the change.

Nanji, Paton and Ling [1925] lay some stress on the presence of iron in the ash obtained from pectinogen. This has been noticed by Carré and Haynes and also by ourselves; no quantitative work has been carried out, however, and we do not consider that there is sufficient evidence at present to justify the assumption that iron is an essential constituent, although this is one of several interesting possibilities.

Note on the analytical methods employed. The principal determinations carried out on each sample of pectinogen were: ash content, yield of furfural, methoxyl content by two methods. As it is now firmly established that the basal molecule contains galacturonic acid, we have abandoned the practice of calculating furfural yields as pentose and give actual yields of furfural obtained by Tollens' method. Methoxyl groups were estimated by the well-known method of Zeisel and also by that of Schryver and Wood [1920] and are dealt with more fully later.

In some cases the yield of mucic acid was estimated, the results being calculated as galactose or galactan, and adsorbed salts were estimated where possible. Results quoted throughout are the averages of at least two determinations, concordant within the limits of experimental error, and are calculated on an ash- and salt-free basis.

Influence of source on pectinogen.

In the cases now to be considered, the method of extraction has been described and it is important to remember that the final product was the maximum amount of pectic substance that could be extracted, and that if the product is unstable under the conditions of extraction, variations in composition as shown by analysis are to be expected.

Yield of pectinogen from various sources.

Raw material	Weight of cell-wall substance taken in g.	Weight of pectinogen in g.	Yield %
Turnips	100	24	24
	200	34	17
	100	20	20
	250	54	21.6
Onions	25	4	16
	100	17	17
	150	22.5	15
	150	24	16
Pea-pods	100	8	8

In the above experiments, ammonium oxalate was used as the extractive

and the yields given are the total weights of pectinogen obtained after repeated extraction. It will be seen that turnips give the best yield although for some purposes to be detailed later onions are more suitable, in spite of the slightly smaller yield.

The effect of the source on pectinogen as shown by analytical results is indicated below. The extractives used are ammonium oxalate and 0.5 % oxalic acid, the cell-wall substance being exhaustively extracted at a temperature of 85–95°.

Source	Extractive	Ash %	Contaminating salts %	Furfural %	Methoxyl %
Turnips	Am. oxalate	3.12	15.7	20.32	7.1
	Oxalic acid	2.56	—	19.35	9.2
Onions	Am. oxalate	5.65	5.15	13.95	2.1
	Oxalic acid	2.44	—	9.06	2.2
Pea-pods	„	3.34	—	9.21	1.9

It will be seen from the above results that there is no uniformity in the product obtained, the furfural yields varying between 20 % and 9 % and the methoxyl content between 9.2 and 1.9 %. The only concordance to be observed is in the samples extracted from onions and pea-pods by oxalic acid, but these are in violent contrast with the others. The variability in composition of the product is thus clearly demonstrated and may be attributed to a variety of causes. The fact that each source used is at a different stage of growth may account for variations in the products to some extent, and it is certain that the action of the extractive agent is responsible for a change in the composition of the resulting product.

Another important point, bearing on the technique of extraction, arises from the foregoing results in that it will be noticed that two estimations of adsorbed salts are given. The retention of varying quantities of the substance used for extraction was to be expected, but was nevertheless a source of some trouble. The ordinary ash determination did not indicate the presence of ammonium oxalate, for instance, and it was necessary to estimate the nitrogen in the sample by the method of Kjeldahl in order to arrive at the correct value of the inorganic constituents. In some cases it was very difficult to estimate these contaminating salts as the gel itself prevented the use of the usual methods.

Apart from the difficulties of analysis, it was necessary to obtain the products in as pure a state as possible and methods of eliminating impurities were sought.

The method finally adopted was one of repeated solution in water and reprecipitation with alcohol. After three precipitations in this manner, over 90 % of the inorganic impurities were completely removed, and solutions were obtained from which it was difficult, but not impossible, to precipitate pectinogen by means of alcohol.

Dialysis followed by reprecipitation with alcohol was found to be unsatisfactory, since after dialysis precipitation with alcohol could only be

effected by adding either a salt, which reintroduced ash, or an acid, which affected the constitution of the gel.

Effect of extractive on pectinogen.

In order that the results under this heading should be more strictly comparable, one source only was used, all the cell-wall substance being derived from the same batch of turnips.

In each case the pectinogen was produced by exhaustive extraction with 0.5 % solutions, except in the last case quoted. In this the residues were placed in twenty volumes of water maintained at 90–95°, carbon dioxide being passed into the stirred mass for 6–8 hours. The analytical results are as follows:

Extractive	Yield %	Furfural %	Methoxyl %
Ammonium oxalate	20	20.32	7.1
Oxalic acid	20	19.35	9.2
Ammonium sulphate	10	23.16	7.4
Carbon dioxide	2.5	19.09	4.7

The furfural yield for the sample prepared with ammonium sulphate is quite abnormal and the chief effect of the extractive is observable in the yields, which show that ammonium oxalate and oxalic acid are the most effective.

Another result of some significance is noticeable in the case of the methoxyl content in the first two extractives above. It is known that the action of alkalis on pectinogen causes the elimination of methyl alcohol, and the lower methoxyl value in the first case above is due to the prolonged action of ammonium oxalate, which is slightly alkaline at the temperature and dilution of the extraction. One of the variants in the composition of pectinogen is thus seen to be a loosely combined methoxyl group or groups.

Other factors affecting the nature of pectinogen.

The effect of the temperature of extraction was found to be slight although the yields obtained at lower temperatures were much smaller. Cold reagents did not attack the cell-wall substance at all; after extraction of 100 g. of cell-wall substance for several hours at 36–37°, a small quantity of pectinogen was obtained which was just sufficient for analytical purposes.

In the table below, the source was onions and the extractive was 0.5 % oxalic acid:

Temperature of extraction	Ash %	Furfural %	Methoxyl %
37.5°	2.73	9.06	3.4
80.0°	2.44	9.06	2.2

The effect of temperature on the furfural yield was negligible but there is again a change in the methoxyl value under more drastic conditions of extraction, giving further evidence of the inherent instability of this group.

This fact was further exemplified in another experiment, in which an ammonium oxalate extract containing pectinogen was heated at 100° under

a reflux condenser for 8 hours before precipitation. Thus the pectinogen was in intimate contact with the extractive during this period. A comparison of analyses of a portion (a) freshly precipitated, and (b) after the above treatment, shows again the loss in methoxyl groups due to the prolonged action of the extractive:

Sample	Furfural %	Methoxyl %
(a)	20.32	7.1
(b)	20.64	5.0

The yield of mucic acid from pectinogen.

In view of the fact that the basal molecule of pectic acid and pectinogen contains one molecule of galactose and four molecules of galacturonic acid or its methylated analogue, we might expect that large yields of mucic acid would be obtained on oxidation with dilute nitric acid. This was found to be the case although the actual yields obtained were never those indicated by theory. The acid was estimated by the method of Tollens in which a weighed quantity of the product is gently evaporated with nitric acid of sp.g. 1.15 until the reaction is complete and a clear solution is obtained. The precipitate which forms on standing is re-dissolved in ammoniacal ammonium carbonate and slowly evaporated to dryness. The mucic acid is finally precipitated by addition of more nitric acid and weighed after filtration and drying in a Gooch crucible.

Nanji, Paton and Ling [1925], seem to have experienced some difficulty with this estimation, but we have obtained yields of mucic acid from pectinogens corresponding to 40–50 % galactose, and from pectic acid amounts equivalent to 50–60 %. We have found that the method required very careful working and that it was necessary to evaporate very slowly for 2 days or more in order to ensure maximum yields.

The yields obtained show considerable variations, due in part to the varying nature of the pectinogen and also, doubtless, to the estimation itself. They are considerably higher than those found by the above workers, but even now are not as high as theory requires. We have detected oxalic acid in appreciable quantity in the mother liquor after filtering off the mucic acid.

The table shows the yields of mucic acid calculated as galactose from pectinogen, the last three estimations referring to pectic acid.

Source	Method of extraction	Galactose %
Turnips	0.5 % oxalic acid at 90°	40.9
Onions	0.5 % oxalic acid at 90°	34.9, 37.0, 49.5
Onions	0.5 % ammonium oxalate at 90°	50.9
Onions	Pectic acid	50.1, 64.0
Turnips	Pectic acid	58.7

The above figures are the averages of several estimations, each one referring to a different sample.

In the experiments which follow, pectinogen was extracted under more standardised conditions and the effect of the concentration of the extractive and the time of extraction more fully examined.

Effect of concentration of extractive.

In this series of experiments turnip residues were used which, after the usual washing, pressing and drying, had been finely ground and passed through a fine sieve in order to ensure uniformity as far as possible. Ammonium oxalate was used as the extracting agent and in strengths of 1 %, 0.5 %, 0.25 %, 0.1 % and 0.05 %.

In each case, 20 g. of residues were weighed out and placed in a conical flask, 200 cc. of water were added, and, after about 2 hours, 200 cc. of ammonium oxalate solution. The mixture was heated in a water-bath under reflux for exactly 1 hour, then rapidly cooled, filtered through muslin and paper-pulp, and the pectinogen precipitated and dried as usual.

It was found that the best yield was obtained in the experiments where 0.5 % and 0.25 % oxalate had been used; nothing was gained by increasing the concentration to 1 %, and the extracts after treatment with 0.1 % and 0.05 % oxalate contained such small quantities of pectinogen that precipitation was impossible.

The variation in concentration of the extractive had little effect on the nature of the final product.

The following gives the results in tabular form:

Concentration of extractive %	Yield (approx.) %	Ash %	Furfural %
1.0	10	1.50	18.43
0.5	13	1.72	18.69
0.25	12	1.39	18.60
0.1	—	—	—
0.05	—	—	—

It was decided that for general purposes no advantage was to be gained by departing from the usual practice of using 0.5 % solutions.

Effect of time of heating on pectinogen.

In these small scale experiments a standard method was employed and the pectinogen produced was the result of a single extraction, no additional heating or washing taking place after the period of extraction decided on. The pectinogen was usually redissolved and reprecipitated to eliminate the greater part of the inorganic salts adsorbed in the first precipitate.

The procedure was as follows: 50 g. of air-dried residues were allowed to stand for 1 hour with 500 cc. of 0.5 % ammonium oxalate solution, after which a further 250 cc. were added and the mixture, in a flask under reflux, heated in a water-bath to 90–95° for a definite period. At the end of a stated time the mixture was rapidly filtered through muslin and paper-pulp in a Buchner funnel and flask. The extract was poured into two volumes of 95 % alcohol, filtered, washed, redissolved, and reprecipitated. After the usual treatment it was dried to constant weight in a vacuum desiccator over phosphorus pentoxide.

In a series of experiments under the conditions indicated, using turnips as the source of the pectinogen, the following results were obtained:

Time of heating in hours	Ash %	Furfural %	Methoxyl %
0.5	1.6	18.48	8.21
2.0	1.2	18.86	6.61
4.0	0.96	19.13	5.89
24.0	1.95	19.45	3.19

A short preliminary report to the Food Investigation Board for 1923, published in 1924, embodies some of these results in a modified form.

Interesting points arise from these results, and from them it is possible to form some idea of the nature of pectinogen as it exists in the cell-wall. By extrapolating the curves to zero time we have a hypothetical pectinogen containing 9.1 % methoxyl and yielding 18.27 % furfural. Now, a compound having the formula shown below, but in which three carboxyl groups of the basal molecule are methylated, should yield 8.94 % of methoxyl and 19.17 % furfural. The latter figure is only an approximation, being based on a yield of furfural from the pentose group equal to 90 % of the theoretical value and from the galacturonic acid groups of 31 %, these being generally accepted figures. We did not anticipate complete agreement with theory owing to the suspected presence of another substance in small quantity in association with the main product.

Another point arises in the consideration of these curves. It will be noticed that the results are almost constant at periods corresponding to 18 hours and longer. There is thus evidence that the product formed at this period is somewhat more stable under the slightly alkaline conditions of the reaction. The product obtained after extracting for 24 hours contains 3.19 % methoxyl and yields 19.45 % furfural. A substance having the formula shown herewith, in which the basal molecule contains only one methylated carboxyl group, gives theoretical yields of 3.05 % and 20.3 % of methoxyl and furfural respectively, calculated as above. There is thus the probability that this compound is the main product at this stage of the extraction, although again differences from theory may be expected due to the presence of another substance. It will be remembered that 4 % sodium hydroxide is generally used to effect the complete change to pectic acid, and it is probable that this

G = Galactose

A = Arabinose

Ga = Galacturonic acid

Ga' = Methylated galacturonic acid

compound is only completely de-esterified by the action of more strongly alkaline solutions than the hot ammonium oxalate; the positions of the various linkages in the molecule, at present unknown, may explain the comparative stability of this substance.

The methoxyl content of pectinogen.

It has been stated previously that methyl alcohol is one of the products of the action of alkalis on pectinogen. The preceding experiments show that the amount liberated varies according to the source of the pectinogen, the nature of the extractive, and the method and time of extraction.

In addition, the question has arisen as to whether the methyl alcohol thus obtained is the only product of the reaction. Tutin [1923, 1] states that acetone in addition to methyl alcohol was found in pectin from apples after use in a cider-press. In the case of pectinogen prepared by the usual methods we have been unable to find any evidence of the existence of acetone (see p. 677).

It is advisable to give the methods adopted in some detail in order that the evidence for the above statements may be more easily followed. The method of Zeisel was followed with but slight modifications now in common use. The products under examination were dried to constant weight over phosphorus pentoxide in a vacuum desiccator, more than a fortnight sometimes being required to effect this at room temperature. An accurately weighed sample in a capsule was placed in the Zeisel flask with 10 cc. of hydriodic acid of sp.g. 1.7. Carbon dioxide was passed into the reaction flask, which was connected to two flasks containing alcoholic silver nitrate solution; the reaction was carried out at 130–135°, methyl iodide being swept into the receiving flasks and silver iodide consequently precipitated. This was washed, dried and weighed in the usual manner.

The method proved of general utility in dealing with these products and gave results at least as accurate as those obtained in more orthodox organic chemical practice, as the following list shows. Duplicate results of estimations on different samples are shown:

(i) 3.77, 3.80.	(iii) 6.74, 6.80.
(ii) 3.18, 3.27.	(iv) 8.30, 8.37.

The method of Schryver and Wood [1920] is a micro-method of great delicacy, depending on the oxidation of methyl alcohol to formaldehyde by ammonium persulphate. The reaction is followed colorimetrically, by means of the well-known colour reaction of formaldehyde with phenylhydrazine and potassium ferricyanide. The great accuracy of the method is the chief recommendation for the process, which takes considerably longer to accomplish than the older method of Zeisel. In estimating the methyl alcohol liberated from pectinogen, a standard solution of the sample was prepared and accurate aliquot parts placed in conical flasks. An equal quantity of 8 % sodium hydroxide was added and the mixture allowed to stand with occasional shaking for 1 hour. Any gel that had formed was filtered off and a definite

quantity of the filtrate containing methyl alcohol was run into a distilling flask; the liquid was neutralised with glacial phosphoric acid and distilled into a receiver surrounded with a freezing mixture. An elaborate system of flasks acting as traps was also maintained at about -5° and distillation was carried out at such a rate that the drops froze in the first receiver as they came over. On melting, the distillate was made up to definite volume and estimated in the above manner.

It is obvious that the two methods are totally different in principle and procedure and it is of importance from the present point of view to note that the second method is very susceptible to the presence of ethyl alcohol and acetone, these two substances reacting under the conditions of experiment and thus causing big differences in the results. It would thus be legitimate to assume that if concordant results, within the limits of experimental error, are obtained by the two methods, then the only volatile product of the action of the alkali on pectinogen is methyl alcohol. The table gives a comparison of results by the two methods. They agree so well that the first method was adopted as the standard method, since it is quicker, the second being used in special cases.

Sample No.	Methoxyl %	
	Zeisel	Schryver and Wood
1	7.1	6.9
2	2.1	2.2
3	2.2	2.3
4	1.9	1.8
5	7.4	7.3
6	3.4	3.2
7	2.3	1.9
8	7.1	7.2

The conversion of pectinogen into pectic acid.

It has been stated and generally accepted that the main product of the action of alkalis on pectinogen is pectic acid. The object of the experiments now to be described was to determine the most suitable alkali for the purpose, and to discover whether this substance was the sole non-volatile product of the reaction. If there is another product of the reaction it must exist as a soluble substance in the alkaline extract after removal of the insoluble pectic acid. The choice of an alkali must therefore be governed by this consideration, and it would seem advisable to use one which can be easily eliminated from solution. It seemed feasible to employ lime or baryta water, thus effecting the alkaline change and the precipitation of a gel in one operation. It was not known, however, whether these alkalis were sufficiently strong to effect the change, and in order to show that the main insoluble product of the reaction was pectic acid the following procedure was adopted. A solution of pectinogen, about 1 % strength, was allowed to stand with an equal volume of saturated lime water in one case, and saturated baryta water in the other, for a period of 24 hours. The gel, which formed immediately, was thoroughly washed with water and then allowed to stand in moderately concentrated hydrochloric acid,

several changes being given. By this means the calcium or barium gel was converted to pectic acid and the inorganic constituents eliminated almost entirely. Further washing took place until the washings no longer reacted acid, and then the gel was dried in graded strengths of alcohol as usual. The products were vacuum-dried at room temperature to constant weight before analysis.

The products obtained all contained less than 1 % ash, showing that we were dealing with the acid and not the salt. The yield of furfural, calculated on an ash-free basis, on four products obtained by the action of lime water was: 21.42, 22.33, 22.39, 22.12; average 22.06 %. The product of the action of baryta gave as an average of two determinations, 21.94 %. These figures are within very close limits of those usually obtained for pectic acid, and we may reasonably conclude that these alkalies are effective.

Owing to the ease with which these bases can be removed from solution they were of great use in the examination for soluble products of the reaction on a larger scale. Evidence of the existence of such products was obtained in a somewhat unusual manner.

Anomalies in the methyl alcohol content of pectinogen.

The series of experiments undertaken at this point was designed to determine the rate at which the methyl alcohol was liberated from pectinogen solutions by the action of lime-water.

The pectinogen used in this series was prepared by heating the residues with 0.5 % ammonium oxalate solution for 24 hours, and gave the following analyses:

Ash: 1.95 %; Furfural: 19.45 %; Methyl alcohol: 3.29 % (Zeisel).

A standard solution of pectinogen (about 0.6 %) was prepared and 50 cc. placed in each of five conical flasks; 50 cc. of saturated lime-water were then added and the flasks, loosely stoppered, allowed to stand in the ice-chest for 3, 5, 24 and 72 hours. At the end of the allotted time, the mixture was centrifuged, the calcium gel being thrown down, and 25 cc. of the supernatant liquid were made up accurately to 100 cc. The methyl alcohol in this solution which contained the equivalent of 0.053 g. pectinogen per 100 cc. was determined by Schryver and Wood's method without distillation. The following are the results obtained:

Sample No.	Period of reaction hrs.	Critical concentration of persulphate %	CH ₃ OH %	Pectinogen %	CH ₃ OH 100 g. pectinogen	Difference
1	1.3	0.143	0.0084	0.053	11.56	8.27
2	3.3	0.153	0.0090	—	12.39	9.10
3	5.3	0.174	0.0102	—	14.04	10.75
4	24.0	0.266	0.0157	—	21.48	18.19
5	72.0	0.255	0.0151	—	18.50	15.11

The figures in thick type in the final column indicate the difference between the observed apparent methyl alcohol content and the value obtained by the method of Zeisel. The results indicate (i) that about 24 hours is the optimum time for the reaction; (ii) that there must be some substance other than

methyl alcohol present in the alkaline extract. This substance must be capable of reducing the ammonium persulphate used in the above method, thus giving a falsely high apparent methyl alcohol content. As will be shown later, large scale experiments resulted in the isolation of this substance. Thus we have as products of the action of alkalis on pectinogen: pectic acid, methyl alcohol and a new substance, shown later to have many properties in common with hemicelluloses previously described.

Isolation of a hemicellulose from pectinogen.

1. *Preparation of pectinogen.* 400 g. of the dried cell-wall substance of turnips were allowed to stand with 4000 cc. of water for about 2 hours, when all the water had been taken up. 4000 cc. of 1 % ammonium oxalate solution were then added with constant stirring. The mixture was placed in large flasks and the temperature maintained at 90–95° for 24 hours by means of a constant-level water-bath.

On cooling and filtering the extract amounted to about 6 litres and the pectinogen was precipitated by pouring this into about 10 litres of 95 % alcohol. The precipitate was very gelatinous and filtered slowly, and when most of the alcoholic liquid had passed the filter, the gel was redissolved in water and again precipitated. The pectinogen was finally dried by standing in graded strengths of alcohol and ether, and then in a current of air from a fan. The air-dry weight was 68 g., representing 17 % of the residues.

2. *Separation of the hemicellulose.* 65 g. of the air-dried pectinogen were dissolved in 6500 cc. of distilled water, and an equal quantity of saturated lime-water added with stirring. The mixture was allowed to stand for 24 hours, and then the gel was filtered off and washed thoroughly. The filtrate and washings were saturated with carbon dioxide to remove excess of lime, and again filtered. The final volume of the liquid, after concentration *in vacuo*, was about 100 cc. and this was filtered into 300 cc. of alcohol. A light flocculent precipitate came down gradually and settled on shaking. This was dried in a vacuum desiccator over phosphorus pentoxide.

The substance was very similar to the cytopentans and hemicelluloses described elsewhere. It was a white powder, soluble in water, and gave strong reduction with Fehling's solution after hydrolysis with hydrochloric acid. The presence of pentose or other furfural-yielding groups was indicated by a strong positive phloroglucinol reaction.

The entire process was repeated with a different batch of turnips and the yield of hemicellulose in each case was between 3 and 4 % of the original pectinogen.

The following analytical results were obtained:

	Ash %	Furfural %
1st product	17.16	12.04
2nd product	23.29	12.77
	Average	12.40

The high ash content of these samples is inherent in the method of preparation, as the calcium carbonate formed in the cold lime-water extract is very finely divided and difficult to separate completely.

A portion of the substance was dialysed for some days in presence of very dilute acid, reprecipitated and dried. This gave the following:

Ash: 5.1 %; Furfural yield: 12.71 %.

An important point arises from the foregoing with regard to the procedure adopted. The time of heating—24 hours—was decided on because the composition of the pectinogen appears to be more stabilised after heating for that period. We may expect the maximum amount of chemical action after such a period, and, therefore, an increased yield of any substances associated with pectinogen. There is only one element of uncertainty and that is in respect of the solubility of the hemicellulose. The extracting solution is sufficiently alkaline to liberate the methyl alcohol in the course of the extraction; is it sufficiently alkaline to liberate the hemicellulose, and is there likely to be any of this substance lost in the alcoholic filtrate after precipitation of the pectinogen?

This point was settled by the following experiments: 120 g. of cell-wall substance from turnips were heated for 24 hours with 2400 cc. of 0.5 % ammonium oxalate solution at 85–95°. The extract was filtered through a Buchner funnel and the pectinogen precipitated with 2–3 volumes of 95 % alcohol. The product was redissolved and reprecipitated, and finally dried in graded strengths of alcohol. The combined alcoholic filtrates were evaporated to small bulk under diminished pressure and lime-water added to precipitate the ammonium oxalate and any pectinogen still in solution. Air was passed through the solution while hot, to free it from ammonia, and further evaporation took place after careful filtering through fine filters to remove calcium oxalate. The concentrated liquid gave no reduction with Fehling's solution before or after hydrolysis with dilute hydrochloric acid, and the addition of alcohol and ether failed to produce any precipitate. From this we concluded that the hemicellulose was not split off under the action of the extractive, but that a stronger alkaline action was necessary.

In view of the success attained by following out the indications of the small-scale experiments (p. 688) on turnip pectinogen, it was decided to adopt the same large-scale procedure in the case of pectinogen derived from onions. The objects were to determine whether a hemicellulose was usually associated with pectinogen, and if so, whether the characteristics of such a substance were the same from different sources.

Using onions as the source, the yield was somewhat larger than that obtained from turnips. The product was prepared in an exactly similar manner to that already described. The following are average results from two samples of different origin:

	Ash %	Furfural %
1st product	12.03	9.64
2nd product	10.76	8.62
Average		9.13

It will thus be observed that although substances of the same general type were obtained in each case, they did not appear to be identical, as shown by furfural yields. It will be remembered that an earlier paper [Clayson, Norris and Schryver, 1921] described the cytopentans, substances similar to the hemicelluloses, and found that no agreement in analyses was to be observed in these substances prepared from a variety of sources. It is probable that they are mixtures, or substances varying with the metabolic processes in the plant. Our present knowledge does not enable us to form any rigid theories as to their nature.

Reverting to our suggested formula for the chief constituent of pectinogen, it will be seen that the slight departure from theoretical analytical results can be explained by the presence of this small quantity of furfural-yielding substance. There is therefore every reason to believe that pectinogen consists of a methylated pectic acid, in which the methoxyl groups are readily eliminated together with a small quantity of another furfural-yielding substance, similar in general character to the hemicelluloses. The conversion of pectinogen to pectic acid is not one solely of de-esterification, but involves in addition the separation of this secondary substance.

Additional note on the hemicelluloses.

Clayson, Norris and Schryver have previously shown [1921] that by direct treatment of the cell-wall substance with 4 % sodium hydroxide products similar to the hemicelluloses of Schulze were obtained. In this paper we have described the preparation of substances of the same general type by the action of alkalis on pectinogen, with which they appear to be associated. In addition, we have found that, after extracting pectinogen by the usual methods, sodium hydroxide is still capable of liberating hemicelluloses from the cell-wall substance. It will be convenient to represent these results by the following scheme:

We thus have three types of hemicellulose, and the object of further researches will be to determine the relation, if any, existing between them and the other constituents of the cell-wall.

Hemicelluloses of the type *C* were prepared by treating the cell-wall substance, from which all pectinogen had been extracted and washed out,

with 4 % sodium hydroxide. The alkaline extract was neutralised with dilute acetic acid and the product precipitated with 95 % alcohol. Solution and reprecipitation followed, after which drying was carried out in the usual manner.

The table shows the furfural yields of hemicelluloses of the three types. The products were obtained as far as possible from the same batch of turnips or onions; results in which an asterisk is shown are either abstracted from a paper by Clayson, Norris and Schryver [1921] or are from different batches of raw material.

Source	A		B		C	
	Ash %	Furfural %	Ash %	Furfural %	Ash %	Furfural %
Turnips	5.9*	41.11*	17.16 23.29	12.04 12.77	8.18 9.4	18.39 19.31
Onions	3.8 2.5*	10.89 20.32*	12.03 10.76	9.64 8.62	6.1 4.3	9.80 9.28

The relationship between the various types as shown by these analyses is very slight, and it would be inadvisable to attempt any generalisation. It is hoped to conduct further enquiries into the nature of these substances at a later date.

The hemicelluloses are not simple products of the condensation of pentose and hexose groups, but contain in addition sugar acids of the galacturonic acid type as indicated by the evolution of carbon dioxide on hydrolysis in accordance with the method advocated by Nanji, Paton and Ling [1925].

SUMMARY.

1. Recent investigations on pectic substances are summarised, and indicate that pectic acid is a definite chemical substance and is, in fact, the basal molecule of this class of substance. The conception of Nanji, Paton and Ling, whereby the molecule may be regarded as a ring with six sides, these corresponding to one molecule of galactose, one molecule of arabinose, and four of galacturonic acid, the carboxyl groups of the last being free, may be accepted as a convenient working hypothesis.

2. Pectinogen occurs in the cell-wall as a methylated pectic acid in loose combination with metallic ions such as calcium. It is probable that three of the free carboxyl groups in the parent acid are methylated, leaving the remaining one to fulfil its acidic function. There are indications that an intermediate compound, containing only one methoxyl group, may be obtained, and that it is more stable than the trimethylated compound.

3. Pectinogen is obtained in the laboratory by extracting the cell-wall substance with warm 0.5 % solutions of such substances as ammonium oxalate or oxalic acid. Its composition, especially with regard to methoxyl content, varies considerably with certain factors, of which the period of extraction is the most important. The shorter the period of extraction, the more nearly does the composition of the resulting pectinogen correspond to

that of pectinogen as it occurs in the cell-wall, although the yield is not theoretical.

4. Alkalies, such as lime-water, effect the conversion of pectinogen to pectic acid, which is precipitated. The action is not one solely of de-esterification, since, in addition to methyl alcohol, a substance similar to the hemicelluloses is found in the alkaline filtrate. This hemicellulose may be an unstable or variable constituent, since its composition as shown by furfural yield, does not appear to be constant.

5. Substances of the general type described as hemicelluloses may also be obtained by direct extraction of the cell-wall substance with caustic soda or by similar treatment after extraction of pectinogen. It is difficult at present to draw any conclusions with regard to the relation of these substances to the pectic complex, as they appear to be mixtures, variable in composition, such variations being very probably due to the processes of metabolism in the plant.

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