LXXIV. STUDIES ON PECTIN.

V. THE HYDROLYSIS OF PECTIN.

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(Received May 1st, 1930.)

INTRODUCTION.

MANY workers have shown that pectin exists normally as the methyl ester of the tetrabasic pectic acid. The carboxyl groups may be, and in fact are usually, only partially esterified. The first stage in hydrolysis is the process of de-esterification which involves no change in the six-membered ring. This may be brought about in the cold by alkali as dilute as $N/20$ or by warming in weak acid solution. Heating with stronger acid or alkali results in the destruction of a portion or all of the pectin. In other words, there is a rupture of the pectic ring. A knowledge of this process may be of value in three directions. In the first place, it is of importance to know the degree of stability of the pectin molecule and the critical concentrations of various reagents above which the ring is attacked. Such conditions can then be avoided in the preparation and handling of pectic substances. In the second place, information on the rate and route of hydrolysis may throw some light on the arrangement of the units and nature of the linkages in the ring, about which so little is as yet known. Nanji, Paton and Ling [1925] suggest that the linkages are "other than 1 : 6." While it is certain that group 6 does not participate, since the carboxyl groups of the galacturonic acid units are free, it is equally certain that group 1 is involved for pectin is a non-reducing body. The amylenoxide ring theory for sugars would suggest a 1 : 4 linkage, but this has yet to be proved. Further, it must not even be assumed that the linkage between each unit is of necessity of the same type. If suitable methods of attack could be devised, any difference in type or strength of linkage would become evident from the rate of hydrolysis and nature of the products formed. In the third place, a full knowledge of the degradation products given on hydrolysis might throw much light on the down-grade processes in the metabolism of the pectic substances. There is no definite information as to the nature of the intermediate and ultimate degradation products of pectin in the plant, the metabolic picture being thus incomplete. Pectin is not a final stable substance, as is cellulose or lignin, but more labile, since it disappears during the process of lignification.

O'Dwyer [1926] found that in unligified tissues containing pectin the hemicellulose content is low, but in lignified tissues it is considerably higher and pectin is absent or practically so. She suggested [1928], therefore, that a stage in the process of lignification is the conversion of pectin to hemicellulose. Candlin and Schryver [1928] grouped both these substances as "polyuronides," and investigated the production of hemicellulose-like bodies by the alkaline hydrolysis of pectin. They claim that the conversion is a process of decarboxylation of the uronic acid groups brought about by the action of the alkali. They quote figures for the percentage loss of uronic anhydride content of pectin treated with various concentrations of alkali under different conditions, and hold the loss of uronic acid to be an index of decomposition. However, the extent of decarboxylation of the uronic acids could only be regarded as any measure of the degree of hydrolysis of the pectin, if the uronic acid groups were the most sensitive in the molecule and were instantaneously destroyed by the alkali as the ring was ruptured. This did not seem to be in agreement with the known properties of these acids and accordingly in the work to be described the degree of hydrolysis was determined by the only valid method possible, namely by the yield of calcium pectate. For comparison, the loss of uronic acid anhydride was also estimated as in Candlin and Schryver's work. Since it was hoped at the same time to obtain information as to the relative sensitivities of the various units to alkaline treatment, and the route of the degradation, the furfuraldehyde yield of the hydrolysis liquid was also determined.

METHODS.

The methods employed were as follows.

(A) *Calcium pectate yield*; Nanji and Norman's [1928] modification of the Carré and Haynes [1922] method.

(B) *Total furfuraldehyde yield*; distillation with 12 % HCl, and precipitation of the furfuraldehyde as the phloroglucide, which is subsequently extracted with hot alcohol to remove impurities.

(C) *Total carbon dioxide yield*; Nanji, Paton and Ling's [1925] modification of the Lefèvre and Tollens [1907] method for uronic acids.

The pectin which was used throughout this work was a commercial sample prepared from Citrus fruits by the method of Wilson [1925].

The analytical figures obtained for this pectin calculated on 1 g. calcium pectate were:

furfuraldehyde yield (B)	17.72 %
carbon dioxide yield (C)	18.29 %
uronic anhydride content	73.16 %

It is seen that these figures do not precisely correspond with the theoretical. Calcium pectate should contain 69.7 % galacturonic acid anhydride, and yield approximately 19.5 % furfuraldehyde. However, commercial preparations,

and, indeed, most general preparations show a divergence from these figures. The difference is accounted for in this case by a content of anhydroarabinose rather below theoretical, *i.e.* 10.33 % instead of 14.25 %, and of galacturonic acid rather higher. It is not proposed to discuss this discrepancy here except to note that the Citrus-pectin employed by Candlin and Schryver in their work gave figures differing even more widely from the theoretical. Its content of uronic acid was 88.6 % and total furfuraldehyde yield 23.1 %. Their figures are not, however, calculated on calcium pectate obtained but on the crude preparation.

Calculation of results.

Knowing the composition of the original pectin solution, it was possible from the determinations mentioned above to calculate the pentose and uronic acid content of any degradation product present in the hydrolysis liquid. The calculations involved are given below.

$$(D) \text{ Furfuraldehyde due to pectin, } A \times \frac{17.7}{100}.$$

$$(E) \text{ Uronic acids in pectin, } A \times \frac{73.16}{100}.$$

$$(F) \text{ Non-pectin uronic acid, } 4C-E.$$

$$(G) \text{ Furfuraldehyde due to non-pectin uronic acid, } F \times \frac{16.66}{100}.$$

$$(H) \text{ Furfuraldehyde due to pentose not in pectin (B-D)-G.}$$

Whereas the calcium pectate yield (A) will be the true measure of degree of hydrolysis of the pectin, the non-pectin uronic acid (F) and furfuraldehyde due to pentose groups not in pectin (H) will show the resistance or otherwise of the uronic and pentose groups to the hydrolysing agent. If either is very low or nil it will indicate that that constituent is destroyed as rapidly as it is liberated from the pectin and is, in all probability, the point at which the pectin ring is susceptible to attack.

EXPERIMENTAL.

Alkaline hydrolysis.

The work of Candlin and Schryver [1928] indicates that considerable decarboxylation of pectin is effected by boiling with alkali of concentration *N*/8. They record 33 % decarboxylation in 30 minutes and 80 % decarboxylation in 4 hours. This strength of alkali was therefore employed in the first experiments. The various hydrolyses were carried out independently for ease and rapidity of operation.

50 cc. of a 0.4 % solution of Citrus-pectin were taken in a 250 cc. round-bottomed flask, and to it were added 5 cc. *N*/10 sodium hydroxide, which was the quantity necessary to convert the pectin to sodium pectate. The solution was brought to the boiling point and 20 cc. of 1.88 % NaOH were added. This brought the volume to 75 cc. and the final concentration of alkali to 0.5 %.

The liquid was boiled under a reflux for the requisite time, rapidly cooled, and exactly neutralised with *N* HCl. In certain cases in which the hydrolysis was nearly complete double quantities had to be taken.

For the furfuraldehyde estimation and uronic acid determination the acid concentration was brought up to 12.5 % by the addition of 42 cc. concentrated HCl. In the former case the distillation was then carried out in the usual way, while in the latter a stream of CO₂-free air was passed through the cold solution for half an hour to remove any carbon dioxide in solution.

Table I. *Alkaline hydrolysis of pectin.*

Expressed on 1 g. calcium pectate.						
Time	Calcium pectate yield	Loss of calcium pectate %	Total furfurald. yield	Loss of furfurald. yield %	CO ₂ yield	Loss of CO ₂ yield %
Hydrolysed with 0.5 % NaOH.						
Blank	1.0	—	0.177	—	0.183	—
5 min.	0.550	45.0	0.118	33.2	0.124	32.5
10 "	0.256	74.4	0.052	70.8	0.098	46.7
20 "	0.184	81.6	0.051	71.1	0.081	55.7
30 "	0.122	87.8	0.043	75.9	0.073	60.1
1 hour	0.057	94.3	0.038	78.9	0.061	66.9
3 hours	0.054	94.6	0.030	83.0	0.052	71.4
Hydrolysed with 0.2 % NaOH.						
Blank	1.0	—	0.177	—	0.183	—
10 min.	0.305	69.5	0.103	41.8	0.142	22.2
20 "	0.235	76.5	0.089	49.4	0.136	25.9
30 "	0.207	79.3	0.069	60.9	0.132	28.1
1 hour	0.202	79.8	0.059	66.7	0.113	37.9
3 hours	0.156	84.4	0.057	68.0	0.064	65.2

Since hydrolysis with 0.5 % alkali appeared to be very rapid and extensive a similar series was carried out with 0.2 % NaOH. The results obtained in both series are quoted in Table I.

All the figures are the means of two or more closely agreeing determinations.

It was clear from the above figures that the hydrolysis of pectin or, rather, the rupture of the pectin ring is more rapid than the destruction of furfuraldehyde-yielding groups and than apparent decarboxylation, and does not seem to be conditioned by either of these. It is necessary to deduce the liberation of carbohydrate units either free or in simple combination in the hydrolysis liquid. Accordingly it was of importance to determine the behaviour of sugars in the presence of alkalis under similar conditions. The sugars tested were those present in pectin, namely, arabinose and galactose. In addition, lactose was similarly treated since it contains galactose in a combined form with glucose, the last mentioned being also treated independently. 0.2 g. of sugar in 25 cc. was boiled with 5 cc. of alkali of the necessary strength to give a 0.5 % or 0.2 % solution. The solution was exactly neutralised and the reducing power measured by the iodimetric method [Baker and Hulton, 1920]. The results are expressed as percentage loss of reducing power as measured by iodine absorption (Table II).

Table II. *Treatment of sugars with alkali.*

Sugar	Conc. of NaOH %	Percentage loss of reducing power					
		2 min.	4 min.	10 min.	20 min.	30 min.	1 hour
Arabinose	0.5	44.5	49.7	51.8	—	52.8	—
"	0.2	38.2	43.5	49.7	—	53.9	—
Galactose	0.5	41.0	41.9	45.0	—	52.8	—
"	0.2	27.5	32.6	40.5	—	46.1	—
Glucose	0.5	31.7	34.3	40.4	—	44.5	—
Lactose	0.2	+62.4	+37.3	+21.4	+13.9	+9.1	+7.2

Very rapid destruction of the sugars therefore takes place in the first few minutes of treatment with dilute alkali, and the reducing power observed at 10 minutes, which thereafter remains practically constant, was thought to be due not to sugars but to iodine absorption by the degradation products formed. That this is so was shown by treating the above sugars with 0.2 % alkali for 10 minutes, cooling and neutralising with acetic acid. Phenylhydrazine was then added under the usual conditions for preparation of an osazone, but none was obtained in the cases of the monosaccharides tested or even in the case of lactose.

It seemed therefore that units with free aldehydic groups would be destroyed within a few minutes of liberation, and that any uronic acid and pentose split off from pectin by rupture of the ring and found in the hydrolysis liquid must be linked with the aldehydic group shielded. In order that the quantity of such degradation products might be determined the methods of calculation previously described were applied to the figures quoted in Table I, and are given in Table III.

Table III. *Alkaline hydrolysis of pectin.*

Expressed on 1 g. calcium pectate.

Time	Calcium pectate yield (A)	Furfurald. due to pectin (D)	Non-pectin furfurald. (B-D)	Apparent total uronic content (4 C)	Pectin uronic content (E)	Apparent non-pectin uronic content (F)	Furfurald. from non-pectin uronic acid (G)	(H)
Hydrolysed with 0.5 % NaOH.								
Blank	1.0	0.177	—	—	0.732	—	—	—
5 min.	0.550	0.098	0.020	0.494	0.402	0.092	0.015	0.005
10 "	0.256	0.045	0.007	0.390	0.187	0.203	0.034	-0.027
20 "	0.184	0.033	0.018	0.324	0.134	0.190	0.032	-0.014
30 "	0.122	0.222	0.021	0.292	0.089	0.203	0.034	-0.013
1 hour	0.057	0.010	0.028	0.242	0.041	0.201	0.033	-0.005
3 hours	0.054	0.009	0.021	0.210	0.039	0.171	0.028	-0.007
Hydrolysed with 0.2 % NaOH.								
Blank	1.0	0.177	—	—	0.732	—	—	—
10 min.	0.305	0.054	0.049	0.569	0.223	0.346	0.058	-0.009
20 "	0.235	0.042	0.048	0.542	0.172	0.370	0.062	-0.014
30 "	0.207	0.037	0.032	0.526	0.151	0.375	0.062	-0.030
1 hour	0.202	0.036	0.023	0.452	0.148	0.304	0.051	-0.028
3 hours	0.156	0.028	0.029	0.254	0.114	0.140	0.023	0.006

This method of calculation, though based on justifiable theoretical knowledge, when applied to the figures in question showed certain marked discrepancies. In the tables above, the final column, headed (H), should in theory

give the furfuraldehyde due to pentose units not in pectin. It was found, however, that a negative result was obtained in practically every case. This could be due to one of two reasons. Either the total furfuraldehyde yields as obtained were uniformly low, or else the CO_2 yields were too high. It did not seem likely that any substance present in the hydrolysis liquid could lower the production of furfuraldehyde from the pentose units present on distillation with hydrochloric acid. It did, however, appear possible that the action of the alkali might produce substances, apart from uronic acids, which would give CO_2 on boiling with hydrochloric acid, although this reaction has been presumed to be specific.

The action of dilute alkalis on the polysaccharides is a field of carbohydrate chemistry as yet unexplored. The work of Lobry de Bruyn and van Ekenstein [1895, 1896, 1897] and of Nef [1907, 1910, 1914] and his co-workers on the effect of alkali on the monosaccharides has shown the very complicated nature of the reactions which take place. More recently Lloyd Evans [1929] has re-investigated these problems and extended the work to certain disaccharides. The results of all these workers have thrown much light on the constitutional relationships between the sugars. In practically all cases, however, they found it necessary to employ moderate temperatures so that the course of the reactions might be followed, and intermediary and transitory substances identified and estimated. However, differences in behaviour and quantities of end-products which were observed between individual sugars of the same series at a given alkalinity at lower temperatures were found to disappear as the temperature was raised. Evans [1929] states that at 75° the various hexose sugars yield practically identical amounts of oxidation products since they form a common 3:4-dienol. It is not unreasonable to assume that the reaction at 100° proceeds similarly to that described as taking place at 75° , but at a much increased rate. The hexoses are presumed to give in the first place an equilibrated mixture of three different dienolic forms, the 1:2, the 2:3, and the 3:4 isomers. Rupture then takes place at the double bonds of the several dienols and various degradation products are formed. In the cases just considered the 1:2-dienol gives an active pentose and hydroxymethylene, the 2:3-dienol, a tetrose and glycollaldehyde, and the 3:4-dienol, two molecules of glyceraldehyde. The pentoses and tetroses similarly give dienolic forms which on rupture yield the same products. Subsequent oxidation results in the production of CO_2 from hydroxymethylene, and in part from glycollaldehyde and glyceraldehyde. Glycollaldehyde at the same time gives oxalic acid, or may give some glycollic acid. Glyceraldehyde is converted into pyruvic aldehyde which in turn gives lactic acid or may give acetic and formic acids.

The extent to which this process is modified by linkage of the sugar is, as yet, not fully determined. The only linked hexose product which has been investigated under alkali treatment is maltose. Evans and Benoy [1930] have shown that alkaline solutions of this sugar contain an equilibrated system

similar to that observed with the hexose sugars, the components of the system being the 4-glucosido-glucose dienols and their fission products. In the case of the treatment of pectin with alkali the processes of hydrolysis and dienolic fission must go on simultaneously, and upon the precise point or points of rupture of the ring will depend the course of the subsequent fission. In addition to the products given above, derivable from the hexose and pentose units, the presence of galacturonic acid renders other products likely. These it is proposed to investigate later. A 3 : 4 dienol of galacturonic acid would give one molecule of glyceraldehyde and an aldehyde acid, giving in turn probably pyruvic, glyoxylic or oxalic acid.

The possible ultimate fission products of pectin on alkaline treatment are therefore, formic, acetic, oxalic, lactic, glycollic, glyceric, glyoxylic and pyruvic acids. It seemed not unlikely that one or more of these might yield CO_2 on treatment with 12 % HCl, and its presence in the hydrolysis liquid be responsible for the unaccountably high figures for CO_2 yield obtained in the experimental work just described. Samples of each were therefore boiled with HCl under the usual conditions, and the CO_2 evolved, if any, estimated (Table IV).

Table IV. CO_2 evolution from certain acids on treatment with 12 % HCl.

Acid	CO_2 evolved %	Acid	CO_2 evolved %
Formic acid	Trace	Glycollic acid	Nil
Acetic acid	Trace	Glyoxylic acid	Nil
Oxalic acid	0.42	Glyceric acid	Nil
Lactic acid	0.08	Pyruvic acid	5.4

It will be seen that among these only pyruvic acid was found to yield any quantity of CO_2 . It was not, however, found possible to characterise this acid in the hydrolysis liquid, owing to the presence of other decomposition products, but theoretical considerations and this indirect evidence point to its formation in this process. Oxalic acid is known to be formed in some quantity and undoubtedly must contribute to the yield of CO_2 .

For comparison a hexose and a disaccharide were treated with 0.2 % alkali for various periods and after neutralisation 12 % HCl was added and the CO_2 obtained on distillation measured (Table V).

Table V. *Hydrolysis of sugars with 0.2 % alkali.*

Sugar	Time of heating min.	CO_2 yield %
Glucose	2	0.98
"	5	0.82
"	15	0.70
"	30	0.73
Lactose	30	1.12

In the method of calculation described earlier, CO_2 from the dienolic fission products would be calculated as uronic acid anhydride thus giving for this, as was suspected, a high and unreliable figure. Since it is not possible

satisfactorily to remove these fission products from the hydrolysis liquid, it is patent that this method of calculation is inapplicable for the purpose of throwing light on the composition of the higher degradation products of pectin.

Certain other methods of hydrolysis were tested, since it was supposed that these disadvantages might be confined to alkaline treatment. Acid hydrolysis seems to have received even less attention than hydrolysis by alkali. Ehrlich [1917] states that mild acid hydrolysis by boiling for several hours with 1 % oxalic acid yields an araban and a monobasic acid, galactose-galacturonic acid, $C_{12}H_{20}O_{12}$. Complete acid hydrolysis by boiling for 2-3 hours with 1 % HCl is stated to go further and to give free galactose and galacturonic acid. Hydrolyses were therefore carried out employing these two reagents under precisely similar conditions as described for the alkaline treatments. Since it was necessary to remove the oxalic acid before the uronic acid determinations were made, a slight excess of calcium chloride was added and the precipitated calcium oxalate filtered off. The washing of the alcohol precipitate in the pectin estimation with acid alcohol had to be carried out with great care till the oxalate test was negative. The results obtained, together with the calculations therefrom, are given in Table VI.

Table VI. *Hydrolysis of pectin.*

Expressed in g. per 1 g. calcium pectate.

Time hours	Calcium pectate yield (A)	Total furfurald. yield (B)	CO ₂ yield (C)	Furfurald. due to pectin (D)	Non-pectin furfurald. (B-D)	Apparent total uronic content (4 C)	Pectin uronic content (E)	Apparent non-pectin uronic content (F)	Furfurald. from non-pectin uronic acid (G)	(H)
Hydrolysed with 1 % HCl.										
Blank	1.0	0.177	0.183	0.177	—	—	0.732	—	—	—
1	0.549	0.255	0.210	0.097	0.158	0.840	0.401	0.439	0.073	0.085
3	0.155	0.242	0.208	0.207	0.215	0.832	0.113	0.719	0.120	0.095
5	Nil	0.231	0.128	—	0.231	0.512	—	0.512	0.085	0.146
Hydrolysed with 1 % oxalic acid.										
Blank	1.0	0.177	0.183	0.177	—	—	0.732	—	—	—
4	0.635	0.206	0.191	0.112	0.094	0.764	0.465	0.299	0.050	0.044
8	0.325	0.282	0.190	0.058	0.224	0.760	0.238	0.522	0.087	0.137
12	Nil	0.210	0.190	—	0.210	0.760	—	0.760	0.126	0.084

An examination of these results showed two features of interest. In the first place there was observed in both cases a definite and considerable increase in total furfuraldehyde yield which can be accounted for with little difficulty. The action of the dilute acid on the galacturonic acid is in part at least a simple decarboxylation. From a unit which yields 16.66 % of its weight of furfuraldehyde is given a pentose unit which yields nearly 60 % of furfuraldehyde. The observed increase of furfuraldehyde yield is therefore due to the formation of pentose groups by simple decarboxylation of the uronic acids. In the second place an increase in carbon dioxide yield was observed in both

cases despite the fact that uronic acids have undergone decarboxylation to pentoses. It is seen, therefore, that the theoretical method of calculation employed is again invalid, and it is necessary to postulate in these cases also the production of non-uronic degradation products yielding CO_2 on treatment with 12 % HCl . It is not possible to speculate on the nature of these degradation products since so little is known as to the course of mild acid scission of polysaccharides.

A final hydrolysis was carried out by heating a pectin solution alone in an autoclave at 1 atmosphere for 1 hour. Considerable acidity is developed, and the final p_{H} was approximately 2.0. It is this development of acidity which prevents the setting of agar media containing pectin. The results are given in Table VII. Once again an increase in total furfuraldehyde yield was observed which is only in part balanced by the loss of uronic acid, necessitating again the postulation of the production of CO_2 -yielding degradation products.

Table VII. *Hydrolysis of pectin solution in autoclave at 1 atmos.*
(Final $p_{\text{H}} = 2.0$.)

Expressed in g. per 1 g. calcium pectate.

Time	Calcium pectate yield (A)	Total furald. yield (B)	CO_2 yield (C)	Fur- furald. due to pectin (D)	Non- pectin fur- furald. (B-D)	Ap- parent total uronic content (4 C)	Pectin uronic content (E)	Ap- parent non- pectin uronic content (F)	Fur- furald. from non- pectin uronic acid (G)	(H)
Blank	1.0	0.177	0.183	0.177	—	—	0.732	—	—	—
1 hour	0.904	0.219	0.172	0.160	0.059	0.688	0.687	0.001	—	0.059

Preparation of hydrolysis product.

Candlin and Schryver [1928] prepared two polyuronide products by the alkaline hydrolysis of pectin. These products both had a low uronic anhydride content, which was not surprising, since the hydrolysis was continued for 4 hours, a period which, in view of the figures given in Table I, would seem to be rather too long. Accordingly a hydrolysis was carried out for a shorter period. 20 g. of Citrus-pectin were dissolved in 1000 cc. water and NaOH added to make a concentration of 0.5 %. The whole was boiled for 1 hour under a reflux and exactly neutralised with hydrochloric acid. The solution was concentrated under reduced pressure to 200 cc. and made acid with hydrochloric acid. The precipitate which formed was filtered off, and will be referred to as the residue. The filtrate was poured into two volumes of 95 % alcohol and, after standing overnight, filtered. The precipitate was redissolved in dilute acid and reprecipitated with alcohol, and finally dried in graded strengths of alcohol. The residue was treated similarly. The yield of product was about 4 g. and of residue rather less.

The furfuraldehyde and CO_2 yields were determined on both the residue and the product.

	Residue %	Product %
Ash	6.67	16.47
CO ₂ yield (ash-free)	18.27	14.31
Uronic anhydride (ash-free)	73.08	57.24
Furfurald. yield (ash-free)	17.24	14.68

It is seen that the composition of the residue closely approximates to that of the original pectin, and it is therefore unchanged pectic acid.

From the figures given above it is possible to calculate the composition of the hydrolysis product. The composition of the products obtained by Candlin and Schryver [1928] has similarly been calculated from their results, and is given for comparison in Table VIII.

Table VIII. *Composition of alkaline hydrolysis products of pectin (%)*.

	(Norman and Martin) Citrus-pectin	(Candlin and Schryver) Citrus-pectin	(Candlin and Schryver) Onion-pectin
Uronic anhydride	57.24	37.25	21.55
Total furfurald.	14.68	17.40	8.43
Furfurald. due to uronic anhydride	9.53	6.21	3.59
Furfurald. from pentose	5.15	11.19	4.84
Anhydropentose	9.64	20.84	9.06
Anhydrohexose	33.12	41.91	69.39

Like the products obtained by Candlin and Schryver [1928] the hydrolysis product in this case was non-reducing, but hydrolysable by N H₂SO₄ into reducing substances. Arabinose was characterised by the diphenylosazone and galactose by the production of mucic acid.

GENERAL DISCUSSION.

It is not possible from the results presented above to come to any conclusions as to the arrangement of the units in the pectin molecule, or as to the type of linkage involved. Nor is one able to point to any single linkage or unit as being peculiarly susceptible to attack by a particular hydrolysing agent, and being therefore the point at which rupture of the ring takes place. A theoretically sound method of calculation for determining the composition of the polyuronide derivatives from pectin breaks down both in the case of alkaline and acid hydrolysis, owing to the production of simpler degradation products which interfere with the determination of the uronic carboxyl groups.

The susceptibility of pectin at 100° to alkali as dilute as $N/20$ is particularly noteworthy since it indicates that the carbohydrate linkages are of a type apart from those found in some other polysaccharides, such as cellulose and starch. Since the production of simple degradation products, yielding CO₂ on treatment with 12 % HCl, can be accounted for by a process of dienolic fission following upon the rupture of these linkages, it seems likely that hydrolysis follows this course. It is clear that the CO₂ yield cannot be taken as a measure of decarboxylation and still less of hydrolysis of pectin as was done by Candlin and Schryver [1928]. They quote the percentage loss of uronic

anhydride calculated from CO_2 yield from pectin treated with different concentrations of alkali for various times and at several temperatures. The error in their figures due to the presence of dienolic fission products will vary with the conditions and will be considerable, as is seen in the work described above. Where it is possible to compare the apparent decarboxylation produced by the different concentrations of alkali employed by them, it appears that the lower concentrations are more effective, and in fact these workers comment on this. It seems likely that with the higher concentrations of alkali the enolic fission products are more completely degraded than with the lower, and so do not give CO_2 or as much CO_2 on treatment with HCl .

The principles underlying the mild acid hydrolysis are even more obscure, and, apart from the observation that pentose units are formed by simple decarboxylation of uronic groups, it is difficult to make further assertions. That there is simultaneously the production of non-uronic units giving CO_2 on treatment with HCl is clear from the results, but there is no evidence as to the nature of these.

It seems that valid information as to the nature of the polyuronide degradation products can only be obtained by the tedious process of actual isolation at various stages. Even then it is probable that the products isolated may be mixtures of derivatives with similar properties. That polyuronides similar in properties to the natural hemicelluloses may be obtained by the alkaline hydrolysis of pectin, as claimed by Candlin and Schryver [1928], is certain, but the process, as is shown above, is something more than simple decarboxylation, and, furthermore, can hardly be imagined as taking place in the plant. Norman [1929] has suggested that the production of hemicelluloses and of pectin in the plant is due to the mild oxidation of hexosan units. Recently Norman and Norris [1930] have shown that decarboxylation may be effected by a process of mild oxidation, thus lending some support to this theory. It is possible that enolic fission may play a part in the metabolism of these substances. Nef [1914] stated that dienols did not exist in any solution containing less than $N/30$ alkali, but this has recently been disproved by Evans *et al.* [1928] who show that limited dienol formation takes place in solutions of p_{H} less than 7.0. By employing copper acetate as a mild oxidant they were able to isolate some of the characteristic products of dienolic fission. It is conceivable therefore that these two methods of mild oxidation may be jointly responsible for the degradation in the plant of pectin to hemicelluloses. Oxidation of linked units may take place as in the hydrogen peroxide work of Norman and Norris [1930] and of unlinked units by limited oxidative dienolic fission owing to the p_{H} of the cell sap.

SUMMARY.

1. Hydrolyses of pectin have been carried out at 100° with 0.5 % and 0.2 % alkali. The yields of calcium pectate, furfuraldehyde, and CO₂ were estimated at intervals.

2. A rapid hydrolysis was observed in both cases; the rupture of the pectin ring proceeding faster than the destruction of furfuraldehyde-yielding groups and apparent decarboxylation.

3. When a calculation was made to determine the nature of the immediate degradation products of the pectin, certain discrepancies became apparent. The CO₂ yield was higher than could be accounted for from the uronic acids present.

4. On theoretical grounds the presence of certain dienolic fission products of sugars was suspected, and the CO₂ yields of these were tested. Pyruvic acid was found to yield a considerable amount of CO₂, and oxalic and lactic acids a little. There is therefore indirect evidence for their presence in the alkaline hydrolysis liquid.

5. In the course of mild acid hydrolyses an increase in furfuraldehyde yield was observed, due to simple decarboxylation of uronic acids with the production of pentose. Despite this decarboxylation, an increase of CO₂ yield was noted, owing to the formation of non-uronic degradation products of unknown composition, yielding CO₂.

6. A polyuronide derivative of pectin containing 57 % uronic anhydride was prepared by alkaline hydrolysis of pectin, and its composition was determined.

The work described in this paper was carried out at the Rothamsted Experimental Station by the courtesy of the Director, Sir John Russell.

One of us (A.G.N.) is indebted to the Department of Scientific and Industrial Research for a Senior Research Award.

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