XLIII. THE PECTIC SUBSTANCES OF PLANTS.

(Preliminary communication.)

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(Received November 14th, 1916.)

The substances known as the pectins, which are said to form the middle lamella of plant cells, offer many points of interest. It is supposed that they are readily attacked by various fungi which are the causes of plant diseases, and a knowledge of their chemistry is, therefore, of considerable importance to plant pathologists. They also, under certain conditions, which are not yet clearly understood, form gels, and it was mainly the interest in this particular property which first attracted the authors of this paper to their investigation. One of them (S. B. Schryver) had already commenced a systematic chemical research, when the other [Haynes 1914] published a paper dealing with the conditions under which pectic gels were formed, and they decided to continue in partnership their researches, which have, unfortunately, been repeatedly interrupted by other work.

Very little of the literature dealing with the subject of the pectins is of recent date, and the statements made by earlier authors are often confused and contradictory. It is not proposed to discuss the literature in any detail in this place, as it is dealt with in an adequate manner in various hand-books. (See more especially the works of Czapek [1913] and Tollens [1914].)

The materials employed by the authors for investigation comprised turnips, strawberries, rhubarb stem and apples, and the results so far obtained indicate that one single substance of acidic character is obtained from all sources; this has been designated *pectinogen*. On treatment with weak solutions of caustic alkalis at ordinary room temperature it readily undergoes conversion into a second substance to which the name *pectin* has been given from which gels can be readily obtained. It must be admitted that the

evidence as to the identity of the pectinogen from all the sources investigated cannot be considered as complete, but as relatively large amounts of material are now available for further work, it is hoped that subsequent researches which it is proposed to carry out in this laboratory, will lead to a definite conclusion as to this point. All the samples of pectinogen, however (which has not yet been isolated in a pure state) can be converted into pectins under the same conditions; the pectins from all sources, furthermore, give on combustion almost identical analysis numbers, which correspond very closely with a definite chemical formula containing seventeen carbon atoms (or multiples thereof); the furfural obtained by the distillation with hydrochloric acid of the pectinogen from two sources has also been estimated and the results indicate very definitely that each complex of seventeen carbon atoms contains one pentose group. The preliminary results point, therefore, to the conclusion that there is a single pectin which is a complex built up by the combination of a pentose group with another group containing twelve carbon atoms. There is no evidence that it is a carbohydrate, but it appears to be an acid *sui generis*. As several hundred grams have now been prepared, an opportunity exists for a more complete chemical research on the nature of this interesting substance.

PREPARATION OF PECTINOGEN.

The principle of the method employed is the same for all materials used, but it must be varied in details to suit their different textures. The expressed juices of the plant tissues appear to contain only small amounts of pectinogen, and only in the case of apples has any appreciable amount been obtained from such sources. It is generally extracted from the plant residues which remain after the expression of the juices by treatment with warm 0.5 % ammonium oxalate solution; from this extract after concentration to a small bulk it can be precipitated in a gelatinous form by the addition of acidified alcohol. By far the largest amount of pectinogen prepared in this laboratory has been obtained from turnips (or swedes) and these give the most satisfactory yield of all the materials hitherto investigated.

Preparation from turnips. The turnips are first coarsely disintegrated in a motor-driven mincing machine and the material thus obtained is placed in a cloth and pressed as rapidly and completely as possible in an ordinary tincture press; the juice collected, which contains no appreciable amount of pectinogen, is discarded. The residue is then mixed with acetone, a procedure which tends to stop fermentative action, and also facilitates the

subsequent drying processes. About $2\frac{1}{4}$ litres of acetone were mixed with the residue from about 40 lbs. of turnips (one Winchester quart to one bushel of vegetables). The moistened mass was then pressed again through the mincing-machine which was "set" to the small apertures so as to obtain the material in a finer state of aggregation. It was then wrapped in a cloth and pressed, first in a tincture press and then in a Buchner press at about 200 atmospheres pressure. The residue obtained in this way was then very rapidly dried (a process which takes a relatively short time) in a current of air at 37° , a "serum-drier" supplied by Hearsons having been employed for this operation. The powder obtained in this way was then finely ground by passage through a motor-driven grinding mill.

In one experiment 4730 g. of dry material were obtained from about 100 kilos of turnips.

The powder was then washed with cold water to free it from the last traces of soluble product. The amounts of water used for washing and of ammonium oxalate solution for the subsequent extraction of the pectinogen are so chosen that a paste is formed which can be enveloped in a cloth and pressed out afterwards in a tincture press. It is not always easy to do this, for the dried powder swells very considerably on the addition of water, and different powders absorb different amounts of the liquid. If the ratio of powder to water is too high, it is difficult to express the liquid in a press; if, on the other hand, too much water is added, a paste is obtained which is too thin to manipulate in the manner described. In this latter case large amounts of liquid must be used for washing and extraction and separated from the solid by decantation. By practice, however, such a contingency can be generally avoided.

Details of the experiment already mentioned will serve to illustrate the process. The 4730 g. of powder obtained from 100 kilos of turnips were washed twice with 24 litres of water, the washings being expressed each time with the tincture press. The moist residue was then divided into two approximately equal portions, each of which was extracted twice with hot 0.5 % ammonium oxalate solution. This solution was heated to $80-90^{\circ}$ in an enamelled bath, and the moist powder was then thrown in and well stirred with a wooden spoon; the paste thus obtained was wrapped quickly in a cloth and pressed whilst hot in a tincture press, a viscid extract being thereby obtained; the second extract of the first portion was used for the first extraction of the second portion; altogether, including the solution used for washing the cloths, 24 litres of extract were obtained. This ammonium

oxalate extract was then concentrated *in vacuo* (temperature of water bath 40–45°) to about 8 litres. Chloroform and toluene were added to the liquids as soon as they were obtained, to prevent growth of moulds and bacteria which readily attack pectinogen solutions, and small amounts of these liquids were also added from time to time to the extracts during the course of their concentration. To the concentrated extract of 8 litres, about 2–3 times the volume of 95 % alcohol was added, and the pectinogen then separated in the form of a very voluminous gelatinous precipitate. This was filtered off on to blotting paper contained in large funnels, and when it had drained sufficiently it was removed from the filters, wrapped in a cloth and pressed. The cake thus obtained was then redissolved in water, a process which requires several hours and occasional vigorous stirring. The turbid aqueous solution obtained in this way was then filtered through blotting paper pulp on a large Buchner funnel. It was necessary to change the pulp several times in the course of the filtration as it soon became clogged. All the pulp used was collected and heated with water in a beaker immersed in a water-bath and the washings thus obtained were filtered off on to a Buchner funnel, the residue being well pressed and subsequently washed on the funnel. These washings were added to the filtrate, and the total bulk in this particular experiment amounted to 12 litres. The opalescent fluid thus obtained still contained small quantities of suspended particles which could be separated from the fluid by centrifugalisation. (This operation was generally omitted at this stage when large amounts of pectinogen were being prepared owing to the great bulk of the fluid. In most of the work, the crude pectinogen was purified in small quantities as required; for this purpose it was dissolved in water to yield about a 3–4 % solution which was then centrifuged; the liquid was then poured off from the deposit and the purified pectinogen was precipitated from the cleared fluid by alcohol.) The fluid was then evaporated to about 5 litres, rendered distinctly acid to Congo red by dilute hydrochloric acid, and the pectinogen was precipitated by the addition of about twice the volume of 95 % alcohol. The gelatinous precipitate was then filtered, washed on the filter with 75 % then with 95 % alcohol, pressed, and finally allowed to stand for some time under absolute alcohol. It was then filtered off and air-dried, and was thus obtained in the form of a granular white powder. Yield from 100 kilos of turnips 355 g., which on heating in a steam-oven lost nearly 25 % of its weight.

Preparation from strawberries. About 150 lbs. of strawberries, after separation of the stalks, were mashed up with about 10 litres of acetone.

The mash was filtered on to cloths, and the residue thus obtained was allowed to stand under alcohol and then pressed, first in a tincture press, then in a Buchner press; it was then dried at 37° in a current of air. The dry powder weighed about 1·5 kilos. It was washed 5 times by decantation with large quantities of water and then extracted, first with 24 litres and afterwards with 8 litres of warm 0·5 % ammonium oxalate solution. The extract was evaporated to 6 litres before precipitation with alcohol. The purification of the precipitate was carried out by the method described in the case of pectinogen from turnips. 150 lbs. yielded about 95 g. of air-dried product.

Preparation from apples. The apples were minced and pressed in a tincture press, the juice was concentrated to a small bulk and alcohol was added. The gelatinous precipitate thus obtained was filtered off, pressed and extracted with warm ammonium oxalate solution. From this the pectinogen was obtained by the method already described. The greater part was, however, obtained from the residue of the apples remaining after expression of the juice, by the method employed in the extraction of pectinogen from turnips. The total yield from apples was very small.

Preparation from rhubarb stem. 16 kilos of rhubarb stem were beaten into fibre by a wooden mallet and the disintegrated mass was pressed in a tincture press. The residue was treated with 4·5 litres of acetone, and then pressed again, first in a tincture and afterwards in a Buchner press. The material thus obtained was dried in an air current at 37° and then ground. The method of preparation of pectinogen from the powder obtained in this way was similar to that employed in the case of turnips. Yield, about 25 g. of air-dried pectinogen, which was of brownish colour.

PROPERTIES OF PECTINOGEN.

Pectinogen is precipitated from aqueous solution by alcohol in the form of a very bulky gelatinous mass, which after treatment with absolute alcohol and drying in air, is converted into a granular powder. It is generally somewhat coloured, the tint depending on the source from which it has been prepared; the product obtained from turnips is nearly white. It dissolves in water somewhat slowly, as a considerable amount of imbibition of fluid must occur before dispersion takes place. It forms viscous, somewhat opalescent solutions which are distinctly acid to phenolphthalein.

0·3 g. of air-dried pectinogen from turnip required 13·3 cc. N/10 acid

for neutralisation. (The pectinogen was in this case dissolved in excess of alkali and the excess was titrated back.)

One mol of acid was therefore equivalent to 225 g. of air-dried pectinogen.

A solution of pectinogen made neutral to phenolphthalein by caustic alkalis yields no precipitate on the addition of either dilute acid or a dilute solution of a calcium salt, and in this respect differs from pectin into which it is so readily converted by the method described below.

No method has yet been discovered for preparing pectinogen in a pure state. The product examined was generally obtained from the crude pectinogen by dissolving it in water to form a solution of about 3 %, centrifuging, reprecipitating from this by alcohol, and washing with alcohol of graded strengths up to absolute, in which it was allowed to stand for some time. Specimens obtained in this way generally contained about 3 % of ash after drying *in vacuo* at 100° over phosphorus pentoxide. All specimens used for analysis both of pectinogen and pectin were dried in this manner. Two samples, one from turnips and the other from apples, yielded the following numbers on analysis:

0.2348 g. turnip pectinogen,	0.1129 g. H ₂ O,	0.3458 g. CO ₂ ,	0.0081 g. ash
0.1420 g. apple ,,	0.0729 ,,	0.2153 ,,	0.0042 ,,
	H	C	O
Turnip pectinogen	5.53	41.60	52.87
Apple ,,	5.88	42.61	51.51
			Ash
			3.33
			2.96

The H, C and O numbers are calculated for the ash-free product. They do not differ very markedly from those obtained for pectin, which, there is reason to believe, was obtained in a purer state.

THE CONVERSION OF PECTINOGEN INTO PECTIN.

As mentioned above, pectinogen rapidly undergoes a change when it is kept in alkaline solution at room temperature. A freshly prepared neutral solution gives a precipitate with neither acid nor a dilute solution of a calcium salt. If however it is made alkaline, a change is indicated by the fact that both the above-mentioned reagents will after a short time yield bulky gelatinous precipitates when added to the solution. A gelatinous precipitate is also formed when a pectinogen solution is allowed to stand in the presence of excess of lime-water. There is little doubt that pectinogen is rapidly converted by alkalis into another acid, pectin, which is insoluble in water and gives an insoluble calcium salt. The rate of change from pectinogen into pectin is very rapid and is nearly complete in a few minutes in the presence of normal sodium

hydroxide at room temperature. The pectin can then be precipitated as a gel on the addition of acids. It was generally prepared from a 2 % solution of pectinogen which was made distinctly alkaline and then allowed to stand over night at room temperature. From this solution the pectin was precipitated by the addition of an equal volume of concentrated hydrochloric acid, which was used rather than dilute acid as it dissolved out the greater part of the constituents of the ash (see analyses below). The gelatinous precipitate was filtered on to a Buchner funnel by a pump, washed with water till the washings were free from chlorine, and then with alcohol, and finally it was allowed to stand over night under absolute alcohol. It was dried *in vacuo* at 100° over phosphorus pentoxide before being submitted to analysis. It is soluble in alkali and is reprecipitated from the alkaline solution on addition of acids. The yield of pectin was generally about 60 % of the amount of pectinogen taken, but it was found that it is appreciably soluble in strong hydrochloric acid solution. Detailed research, which need not be described here, failed to reveal any other product than pectin, when pectinogen is acted upon by alkalis under the conditions described.

Pectinogen solutions also yield a gel (presumably also pectin) when allowed to stand with concentrated hydrochloric acid at room temperature. The rate of change is however much slower than that which takes place in alkaline solution.

Pectin is soluble in alkalis, and it was found that 0.3 g. of substance dried *in vacuo* at 100° over phosphorus pentoxide neutralised 15.6 cc. N/10 sodium hydroxide. It is not possible from this result, taken in conjunction with the numbers obtained from combustion, to draw any conclusions as to the basicity of pectin, owing to the probability of lactone formation. This point will form the subject of subsequent researches.

<i>Analyses.</i>			
<i>Turnip pectin :</i>			
*0.1857 g.	0.0812 g. H ₂ O,	0.2793 g. CO ₂ ,	0.0040 g. ash
0.2026 "	0.0948 "	0.3112 "	0.0005 "
<i>Strawberry pectin :</i>			
*0.1677 g.	0.0770 g. H ₂ O,	0.2545 g. CO ₂ ,	0.0022 g. ash
*0.1518 "	0.0693 "	0.2294 "	0.0020 "
0.1942 "	0.0890 "	0.2963 "	0.0004 "
0.1764 "	0.0807 "	0.2693 "	0.0004 "
<i>Rhubarb pectin :</i>			
0.1834 g.	0.0892 g. H ₂ O,	0.2813 g. CO ₂ ,	0.0010 g. ash
0.1842 "	—	0.2815 "	0.0009 "
0.1860 "	0.0864 "	0.2857 "	0.0010 "
<i>Apple pectin :</i>			
0.2078 g.	0.0966 g. H ₂ O,	0.3143 g. CO ₂ ,	0.0012 g. ash
0.1678 "	0.0726 "	0.2536 "	0.0009 "

Percentages.

	C	H	O	Ash
Turnip	41.92	4.97	53.11	2.15 *
	41.99	5.21	52.80	0.25
Strawberry	41.94	5.17	52.89	1.31 *
	41.95	5.14	52.91	1.32 *
	41.70	5.10	53.20	0.21
	41.73	5.10	53.17	0.22
Rhubarb	42.06	5.43	52.51	0.54
	41.88	—	—	0.49
	42.12	5.19	52.69	0.54
Apple	41.49	5.18	53.33	0.58
	41.44	4.83	53.73	0.54

The analyses marked with an asterisk were made from samples of pectin which were precipitated from alkaline solution by dilute acid; it will be noticed that in all these cases the ash content is high. The carbon and hydrogen numbers are all calculated on the ash-free product.

As the mean of the above numbers excluding those of the apple pectin, the percentages found are C = 41.93, H = 5.17, O = 52.90. These are in agreement with the formula $C_{17}H_{24}O_{16}$, which requires C = 42.15, H = 4.96, O = 52.89. The numbers for carbon obtained in the pectin sample from apple are a little too low (about 0.5 %), but the other numbers agree in a most satisfactory way with the suggested formula.

Pectin from all sources is soluble in alkali and if strong sodium hydroxide is added to such a solution, the sodium salt is precipitated in the form of a gel. The neutral solution of pectin gives a gelatinous precipitate with calcium chloride.

ESTIMATION OF THE PENTOSE GROUP IN PECTINOGEN.

Pectin itself is insoluble in acids and on heating with them readily undergoes charring. For this reason, the estimation of the pentose groups was carried out with the pectinogen, which is soluble in water, although no method has yet been discovered for purifying the crude product. The specimens employed were dried *in vacuo* at 100° over phosphorus pentoxide, after they had been precipitated from aqueous solution which had been centrifugalised. Both the turnip and rhubarb pectinogens contained 3.4 % of ash. They were heated with hydrochloric acid and the furfural which was evolved was estimated by the Tollens phloroglucinol method. The percentage is calculated on the weight of the ash-free product.

I.	0.3122 g. turnip pectinogen,	0.1114 g. phloroglucinide =	0.0605 g. furfural
II.	0.2935 " " "	0.1086 " "	= 0.0590 " "
III.	0.2784 rhubarb " "	0.0912 " "	= 0.0500 " "
Percentage of furfural from turnips = (I) 20.1 and (II) 20.8			
" " " rhubarb = (III) 18.6.			

A substance of the formula $C_{17}H_{24}O_{16}$ should yield 19.8 furfural, supposing it to contain one pentose group. As these estimations were carried out with crude pectinogens, the results are as near as could be expected and form strong evidence that each $C_{17}H_{24}O_{16}$ complex contains one pentose group.

SUMMARY.

(1) A method is described for the preparation of the pectic substances of plants, in which the essential operation is the extraction of the residue, obtained after the expression of the juice, with a warm 0.5 % ammonium oxalate solution.

(2) A substance of acidic character is thereby obtained, which is soluble in water and is designated *pectinogen*.

(3) This on keeping in alkaline solution at room temperature readily undergoes conversion into another substance, also of acidic character, which is precipitated from its alkaline solution by acids as a gel insoluble in water. To this substance the name *pectin* has been given. It differs from pectinogen in that it is insoluble in water, and that the solution of its sodium salt gives a gelatinous precipitate with calcium chloride. A solution of pectinogen also gives a gel when allowed to stand at room temperature with an excess of calcium hydroxide solution. In this case, presumably a calcium salt of pectin is obtained. [See however Haynes, 1914]

(4) Pectins from turnips, strawberries, rhubarb stem and apples have been prepared. The analyses agree well with the formula $C_{17}H_{24}O_{16}$. Only in the case of the apple pectin were numbers for the carbon obtained which were slightly too low.

(5) Pectinogen on distillation with hydrochloric acid yields furfural in such a quantity as to indicate that one pentose group is contained in each complex of seventeen carbon atoms.

REFERENCES.

- Czapek (1913), *Biochemie der Pflanzen*.
Haynes (1914), *Biochem. J.* 8, 553.
Tollens (1914), *Kurzes Handbuch der Kohlenhydrate*.