CCXXXVIII. THE PECTIC SUBSTANCES OF PLANTS

V. FURTHER OBSERVATIONS ON THE NATURE OF PECTIN AND PECTIC ACID

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IN the course of investigations, to be published elsewhere, on the pectin and pectic acid of the flower of the hop, it was established that these conformed in general physical and chemical properties to the products obtained from more usual sources, such as the citrus fruits, turnip, beet etc. In order to demonstrate this similarity, a number of different methods of preparation were adopted and a number of different sources of pectic substance utilized, with the result that some comparison of the products obtained by different methods was possible. In addition some attempt has been made to explain the mechanism of the various methods of extraction and, since it has become obvious that theories of the constitution of pectic substances require some revision, suggestions on this point have been made.

EXPERIMENTAL

Pretreatment of material

Orange peel. After drying and mincing, the peel, weighing 660 g., from some 60 ripe oranges, was placed in a percolator [Liston & Dehn, 1933] and extracted for 24 hr. with 95 % alcohol and with further fresh alcohol until the extracts were colourless. The peel was then extracted with water and again dried.

Apple. 2250 g. of apples were peeled, cored and minced and then treated exactly as described above for orange peel. The air-dried pomace weighed 50 g. In addition a large sample of apple pomace remaining after cider manufacture was available and was utilized in some preparations of pectin.

Beet. Beet shavings, obtained after use in a beet-sugar factory, were dried and used direct in the preparations.

Hops. 10 kg. of fresh hop flowers were air-dried at about 50° for 4–5 days and then ground to a suitable fineness in a Christie and Norris laboratory mill. 1·8 kg. of the ground hops were placed at the earliest moment in 10 l. of 95 % alcohol to arrest enzyme action. The mass was poured through a muslin bag which was placed in an extractor of a semi-continuous type, similar to that described by Morris [1933] and extracted with warm 95 % alcohol for 10–12 hr. each day for 9 days. The alcohol was then expressed in a hand press and the hops allowed to stand in 13 l. of methyl alcohol in which they were extracted for 8 days. The methyl alcohol was likewise expressed and the hops then placed in 5 l. round-bottomed flasks, covered with ether and extracted under reflux for 3 hr. After repetition of the ether extraction the hops were finally dried in air and were ready for further treatment. Prior to extraction of pectin the dried hops were extracted exhaustively with water at room temperature, at 45–50° and at 95°. Kilned hops were also used and treated similarly throughout.

Preparation of pectin

(1) *Aqueous extraction under pressure.* The dried material was stirred into ten times its weight of water in a beaker of suitable size and the mixture heated in the autoclave at 110° for 1 hr. After expression of the liquid the process was repeated and the combined extracts poured into 84 % alcohol containing 5 ml. of concentrated hydrochloric acid per litre, so that the final concentration of alcohol was not less than 70 % [Morris, 1933; Ehrlich, 1932]. The precipitated pectin was redissolved in distilled water, filtered and precipitated three times, the final filtration being conducted through washed kieselguhr between layers of filter-paper pulp. The product was then dried. In this manner samples of pectin were obtained from orange peel, apple pomace, fresh hops and kilned hops (samples A-E).

(2) *Extraction with ammonium oxalate.* Four extractions with 0.5 % ammonium oxalate solution at 80–85° for 1 hr. each produced a large volume of extract which was concentrated under diminished pressure to about 3 l., from which the pectin was precipitated with acid alcohol. The precipitate was redissolved in the minimum of water, treated with chloroform and dialysed for 2 weeks. This protracted dialysis was necessary, despite frequent changes of distilled water without the membrane, owing to the accumulation of ammonium oxalate in concentration. At the conclusion of dialysis, the pectin was reprecipitated twice and finally dried in the usual manner [Norris & Schryver, 1925]. This method was used for preparations from orange peel, fresh and kilned hops (samples F, G, H).

(3) *Extraction by the method of Ehrlich [1932].* Beet shavings and kilned hops were used in the preparations by this method. Two portions of 500 g. each of the dried material were placed in enamel vessels with 10 l. of water and maintained at 50–55° for 1 hr. The extract was filtered off through muslin and then at the press. Extraction at the same temperature was repeated until the extracts no longer gave a positive α -naphthol reaction. The heating was then continued with fresh additions of water at 75° and the extracts again removed. Finally, the material was boiled for 1 hr. with three successive additions of water and the united extracts filtered.

In the case of one batch, the extracts were concentrated at as low a temperature as possible until the volume was reduced to 5 l. This concentrated extract was then precipitated as already described and the product purified and dried as usual (samples J₁, K₁).

The extracts from the second batch were also concentrated, but in this case the process was allowed to go to completion, giving a yellow-brown dry residue. This was boiled under reflux with successive additions of fresh 75 % alcohol until the alcoholic extracts no longer gave a positive orcinol reaction. The residue insoluble in 75 % alcohol became lighter in colour; it was dissolved in 1 l. of water, filtered through kieselguhr and precipitated by pouring into 2 l. of 95 % alcohol containing 50 ml. of concentrated hydrochloric acid. Purification and drying then proceeded as usual (samples J₂, K₂).

Analytical methods

Ash: by heating to a white ash of constant weight at dull red heat.

Uronic anhydride: by the apparatus of Dickson *et al.* [1930] modified by Norris & Resch [1935].

Furfuraldehydphloroglucide: by the method of Kröber [1901] using a modified apparatus. Anhydroarabinose, *A* is obtained from the equation

$$A = 0.9942P - 1.9081C + 0.01313,$$

where *P* = phloroglucide and *C* = carbon dioxide from uronic anhydride. Anhydrogalactose is obtained by difference [Angell *et al.* 1936].

Methoxyl: by the method of Zeisel [1886] as modified by Campbell [1932].

Analysis of pectin preparations

The results of analysis of the samples prepared as indicated are shown in Table I.

Table I. *On an ash-free basis*

Source and sample		Ash %	Methoxyl %	Uronic anhydride %	Non-urone		
					Total %	Anhydro- arabinose %	Anhydro- galactose %
Autoclave treatment							
Orange peel	A	0.38	9.6	71.1	19.3	11.7	7.6
Apple	B	0.70	11.4	70.2	18.4	14.0	4.4
Apple pomace	C	3.98	10.0	76.2	13.8	10.4	3.4
Fresh hops	D	2.84	7.8	71.2	21.0	8.3	12.7
Kilned hops	E	10.37	5.9	60.8	33.3	9.4	23.9
Ammonium oxalate extraction							
Orange peel	F	0.21	9.4	73.3	17.3	16.5	0.8
Fresh hops	G	0.98	3.7	77.6	18.7	11.1	7.6
Kilned hops	H	1.36	4.0	86.1	9.9	1.2	8.7
Ehrlich method: alcoholic precipitation							
Beet	J ₁	0.50	8.3	71.7	20.0	9.8	10.2
Kilned hops	K ₁	8.82	5.6	50.0	44.4*	12.5	31.9*
Ehrlich method: complete evaporation							
Beet	J ₂	1.44	8.3	73.3	18.4	13.4	5.0
Kilned hops	K ₂	1.57	4.9	63.2	31.9*	4.4	27.5*

* These samples were found to contain appreciable amounts—up to 7%—of proteins; hence the anhydrogalactose and total non-urone figures carry a larger error than usual.

Presence of araban in pectin preparations

Indirect evidence of the presence of araban, soluble in 70–75% alcohol, in several of the preparations was obtained as follows. The sample was boiled under reflux with approximately 70% alcohol for several hours, at the end of which the pectin was separated at the centrifuge. This procedure was carried out ten times with fresh alcohol for each extraction. The alcoholic residue on concentration gave positive results to tests for the presence of arabinose, and galactose and/or galacturonic acid. The residual pectin was dried and analysed as usual, and in one case the uronic anhydride and anhydroarabinose contents had changed from 60.8 and 9.4% respectively in the original pectin to 70.2 and 3.6% in the alcohol-treated product. In another case the change was from 73.3 and 13.4% to 80.4 and 4.9% uronic anhydride and anhydroarabinose respectively.

The complete experimental results for pectin are discussed later.

Preparation of pectic acids

(1) *By the method of Clayson et al.* [1921]. The cell-wall material, pretreated as described, was mixed with 4% sodium hydroxide which was subsequently completely removed by washing and pressing. The material was then extracted

twice with hot 0.5% ammonium oxalate. From the combined extracts the pectic acid was precipitated by the addition of concentrated hydrochloric acid. Purification of the gel was effected by washing in 40–50% alcohol until the wash liquid was free from chlorides. The gel was then redissolved in the minimum of dilute ammonia and reprecipitated with hydrochloric acid; the re-solution and re-precipitation were carried out five times with thorough washing before drying as usual. In this way pectic acids were prepared from turnip, marrow, lemon albedo, beet shavings and kilned hops.

(2) *By alkali hydrolysis of prepared pectins.* For this preparation orange peel pectin (A), apple pectin (B), kilned hop pectin (K₁) and a commercial citrus pectin were used. The pectins were dissolved in distilled water to a concentration of 1% and then made alkaline by addition of sodium hydroxide to a concentration of 0.2%. After standing, the pectic acid was precipitated by adding the minimum quantity of hydrochloric acid necessary to effect complete precipitation. The mixture was allowed to stand overnight, filtered and washed with quantities of water until washing became difficult by reason of the tendency of the gel to disperse. Re-solution of the gel in ammonia and re-precipitation in hydrochloric acid were repeatedly carried out as described in method (1) above until the purified gel reached the drying stage.

Analysis of pectic acids

By the methods previously mentioned the composition of the samples of pectic acid obtained was determined as in Table II. Full discussion of these

Table II. *On an ash-free basis*

Source and sample		Ash %	Uronic anhydride %	Non-urone		
				Total %	Anhydro- arabinose %	Anhydro- galactose %
(i) From tissue by NaOH and oxalate extraction						
Turnip	L	0.54	75.6	24.4	12.5	11.9
Marrow	M	2.11	86.8	13.2	5.0	8.2
Lemon peel	N	0.28	74.0	26.0	15.9	10.1
Beet	P	0.38	64.0	36.0	22.6	13.4
Kilned hops	Q	2.00	84.4	15.6	5.0	10.6
(ii) By alkali hydrolysis of prepared pectin						
Orange peel (A)	R	1.15	89.6	10.4	N.d.	N.d.
Apple (B)	S	0.00	93.2	6.8	N.d.	N.d.
Citrus (commercial)	T	1.44	92.4	7.6	3.0	4.6
Kilned hops	U	0.79	87.2	12.8	0.2	12.6

results is included at a later stage; for the purposes of the experimental section it must be pointed out that the varying composition of pectic acids by method (i) above was quite unexpected, since Clayson *et al.* [1921] had apparently established the constancy of composition of pectic acid. Since two reagents—sodium hydroxide and ammonium oxalate—are used in the preparation, it was decided to examine the effect of varying times of standing with the reagents on the composition of the pectic acids produced. The experiments which follow were carried out with hops, but since the pectic substances of hops conform to type as shown by the preceding results and by Fink *et al.* [1935; 1936], the results may be presumed to be of general application.

Effect of duration of alkali treatment. Six batches of the same bulk samples of pretreated cell-wall material, each weighing 50 g. were used. To each batch 500 ml. of water were added and the mixture allowed to stand for 19 hr. With

thorough stirring, 500 ml. of 8 % NaOH were then added to each and the alkali subsequently removed at the end of the following periods: 30 min., 6, 12, 25, 98 and 193 hr. The removal of NaOH was accomplished by pouring the mixture on to muslin on a Büchner funnel, the liquid being removed by suction as far as possible. The funnel was filled at least twice with distilled water and drained thoroughly each time; 300 ml. of *N* acetic acid together with 400 ml. of water were then added, the acid being more than sufficient to neutralize the remaining alkali. The "period of standing" with alkali was measured from the time of addition of alkali to that of the dilute acid. The mass was then washed with large quantities of distilled water until there was little or no reaction to litmus paper. Finally each batch was separately treated with 500 ml. of 1 % ammonium oxalate at 85–90° for 1½ hr., after which the filtered extracts were precipitated, the pectic acid being purified and dried as usual. The effect on the composition of the pectic acids of varying times of alkali treatment is shown in Table III.

Table III. *On an ash-free basis*

Time of alkali treatment hr.	Ash %	Uronic anhydride %	Anhydro-arabinose %	Anhydro-galactose %
0.5	1.47	86.4	5.3	8.3
6	0.95	88.5	4.0	8.1
12	1.19	90.6	3.1	6.3
25	0.86	91.0	N.d.	N.d.
98	1.06	90.5	N.d.	N.d.
193	0.58	88.5	3.8	7.7

The effect of increasing the period of alkali treatment is evidently at first to increase the uronic anhydride content of the products, this reaching a maximum which remains approximately constant for 12 hr. treatment or longer, but falls off again for prolonged treatment of over a week or more. Clearly, however, these analyses bear little relation to those of Table II (i) and the length of time of standing with alkali is not a factor in the lowering of uronic anhydride content of the pectic acids.

Effect of duration of oxalate treatment. Fresh samples of cell-wall material were treated for a standard period with alkali and then exhaustively extracted with hot ammonium oxalate, the combined extracts being precipitated and the pectic acids dried and analysed as usual. It at once became evident that prolonged treatment with ammonium oxalate is the cause of low uronic anhydride content, a typical hop pectic acid obtained in this manner having the composition: uronic anhydride 70.0 %; anhydroarabinose 4.7 %; anhydrogalactose 25.3 %; ash 1.23 %.

This is closely similar to the composition to be anticipated from the work of Clayson *et al.* [1921] and of Nanji *et al.* [1925] but it now appears that such composition is only attained after prolonged treatment with ammonium oxalate and that short and incomplete extraction gives products of similar physical properties but of differing constitution.

DISCUSSION

It is still not possible to define within narrow constitutional limits exactly what is meant by pectin. It becomes increasingly obvious that pectin is a complex mixture of substances having largely similar properties. There are abrupt differences in composition of pectins derived by different methods from similar sources. It is probable that the usual sample of pectin consists of a central nucleus of galacturonic acid units to which arabinose and galactose units may

be chemically attached. In accordance with modern views on polysaccharide structure, it is probable that the members of this central nucleus are united in chain formation; but in addition to this there must be varying amounts of araban and galactan or arabogalactan which are held to the main nucleus by physical forces. The amounts of added araban and galactan appear to vary with the source and method of extraction of the pectin. Although Ehrlich [1935] has made the most important and sustained contributions to the chemistry of pectin, his suggested structures involving units of four uronic acid residues in ring formation cannot be accepted in the light of modern research on carbohydrate structure and the subtle differences in anhydride and lactone structure claimed for certain of his preparations appear to rest on the somewhat inadequate foundation of titration experiments.

The mechanism of extraction is correspondingly far from clear. It would appear that all methods of extraction give products which contain added araban or galactan, although the higher uronic anhydride content of ammonium oxalate-extracted pectins would appear to show that this method yields pectins in which there is less araban in physical combination. If, in fact, there is a chemically combined uronide nucleus then it will be seen that the action of extractants may alter the composition of the prepared pectin in at least two ways: (a) the extractant acting on the cell-wall substances dissolves out the central pectin nucleus together with varying quantities of araban or galactan, the resulting pectin being of lower uronic anhydride content owing to "dilution" with non-uronic constituents; (b) the extractant may react not only on the cell-wall material but on the extracted pectin, removing non-uronic constituents with the result that the final product contains a higher uronic anhydride content and a correspondingly lower non-uronic content. It may be tentatively suggested that aqueous extraction acts in the first manner, whilst ammonium oxalate is an extractant of the second type. It should be pointed out, however, that where the material has already been treated by autoclave, subsequent ammonium oxalate extraction yields a pectin very heavily "diluted" with non-uronic constituents.

The application of the methods of Ehrlich to beet shavings gives pectin of similar composition to that by other methods, but does not seem well suited to the case of hops. The hop pectins K_1 and K_2 show a very heavy dilution of the main uronide complex with araban and galactan.

The effect of the long period of boiling involved in the Ehrlich method is doubtless to produce a number of more or less complex changes in the products finally obtained. Nevertheless, the method cannot be said to be exhaustive, as there still remains an appreciable amount of pectin which is only extracted by ammonium oxalate. Thus with the hops remaining after preparing samples K_1 and K_2 , further treatment with ammonium oxalate resulted in a product having the usual physical properties of pectin and the following composition: uronic anhydride 55.6%; anhydroarabinose 14.9%; anhydrogalactose 27.1%; methoxyl 2.5%.

On consideration of the present results for pectic acids, it is evident that views which have been fairly generally held for some time must now be modified. The pectic acid of Clayson *et al.* is shown to be not a chemical entity of definite constitution, but a mixture which under certain conditions of preparation approaches constant composition. With this conception must likewise be abandoned the well-known 6-ring formula of Nanji *et al.* [1925] which in any case became untenable when it was realized that the formula was calculated by means of incorrect factors [Norris & Resch, 1935].

In the preparation of pectic acid from the cell-wall material it has been shown that the effect of the alkali is to increase the uronic anhydride content of the resultant pectic acid. In addition, of course, it profoundly affects the substances of the cell wall, releasing hemicelluloses and doubtless breaking or modifying other carbohydrate linkages. The ammonium oxalate subsequently attacks cell-wall substances which have already been somewhat disrupted and it is not surprising to find that the more prolonged the action of the oxalate the higher the non-urone constituents of the final pectic acid, which then has the approximately constant composition assigned to it by Clayson *et al.* That the attack of the oxalate is directed to the cell-wall substances rather than the pectin itself would seem to be indicated by arrival at this "resting stage" where the products average about 70% uronic anhydride. If the pectin itself were being degraded there seems no reason why the action should stop at this point.

The effect of alkali in eliminating non-urone constituents as above is seen again in the composition of pectic acids derived by alkaline treatment of prepared pectins. In every case the uronic anhydride content of the original pectin (samples A, B and K₁, Table I) has risen considerably in the resulting pectic acid (samples R, S and U, Table II).

The pectic substances as normally isolated appear then to consist of complex mixtures in which, so far, it has proved difficult to distinguish the essential pectin structure from the incidental adjuncts and artefacts. Whilst the ultimate solution of pectin structure probably lies in the hands of the organic chemist, this primary difficulty must still be faced.

SUMMARY

1. Pectins prepared by different methods from different sources show the same general characters but differ in detail of constitution.
2. Pectic acid is not a chemical entity; its composition depends on the period of treatment of the cell-wall material with alkali and with ammonium oxalate. Prolonged treatment with the latter produces a pectic acid which is probably a mixture of constant composition.
3. The pectic substances of the hop flower conform to type.
4. Suggestions are made with respect to the chemical constitution of the pectic complex and the mechanism of methods of extraction.

REFERENCES

- Angell, Norris & Resch (1936). *Biochem. J.* **30**, 2146.
Campbell (1932). *J. Soc. chem. Ind. Lond.* **51**, 590.
Clayson, Norris & Schryver (1921). *Biochem. J.* **15**, 643.
Dickson, Otterson & Link (1930). *J. Amer. chem. Soc.* **52**, 775.
Ehrlich (1932). *Ber. dtsch. chem. Ges.* **65**, 352.
— (1935). *Festschr. Tech. Hochschule. (Breslau.)*
Fink & Hartmann (1935). *Wschr. Brau.* **52**, 221.
— & Just (1935). *Wschr. Brau.* **52**, 341, 349, 362.
— (1936). *Wschr. Brau.* **53**, 33.
Kröber (1901). *J. Landw.* **48**, 357.
Liston & Dehn (1933). *Industr. Engng Chem.* **5**, 306.
Morris (1933). *Principles of Fruit Preservation.* (London.)
Nanji, Paton & Ling (1925). *J. Soc. chem. Ind. Lond.* **44**, 253 T.
Norris & Resch (1935). *Biochem. J.* **29**, 1590.
— & Schryver (1925). *Biochem. J.* **19**, 676.
Zeisel (1886). *Mh. chem.* **6**, 989.