XXVI. THE NATURE OF THE PECTIC SUBSTANCES OF FLAX.

A PRELIMINARY INVESTIGATION.

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As the result of recent researches our knowledge of the pectic substances of plants has become more systematic, and it is proposed in the present paper to adhere to the nomenclature adopted by the American Pectin Symposium of 1925. Thus we are now acquainted with protopectin, the naturally occurring pectic substance; pectin, the substance extracted from the plant by chemical or other means with the minimum of chemical degradation; and pectic acid, a derived product.

For the purposes of the present communication it is necessary to refer briefly to some recent investigations, commencing with the isolation of pectic acid [Clayson, Norris and Schryver, 1921]. It was shown that pectic acid could be prepared from a large variety of plant sources, that it appeared to be of constant, or very nearly constant, composition without reference to the particular source, and was, in fact, a definite chemical entity. Important researches, notably by Ehrlich [1917, 1924], culminated in the suggestion of a ring formula for pectic acid by Nanji, Paton and Ling [1925]. According to this suggestion, pectic acid is the basal substance to which the other pectic substances may be referred, and the molecule is a closed six-membered ring, whose members represent one molecule of anhydro-arabinose, one molecule of anhydro-galactose and four molecules of galacturonic anhydride, the four carboxyl groups of the last being free. Pectin, or the "pectinogen" of Schryver and Haynes [1916] and Norris and Schryver [1925], contains, in addition, methyl alcohol to a varying extent, in ester combination with the free carboxyl groups of the original pectic acid.

No serious objections to this suggested formula have been advanced since its publication, nor has the uniform nature of pectic acid derived from different sources been disputed. Only in one case has there been any indication of a departure from this generalisation, that of the pectic substance of flax. Ehrlich and Schubert [1926] suggested the presence of xylose in addition to the usual constituents of the molecule, but this has not been confirmed. In a recent paper on the subject, Henderson [1928] casts some doubt on the validity of the ring formula in the case of flax pectin, and rejects it in favour of a simpler galactose-tetragalacturonic acid formula:

arabinose is regarded as an adventitious adsorbed impurity.

The present investigation was undertaken to test the correctness of Henderson's criticisms. His method of work was not repeated exactly, as it was considered advisable at the present stage to prepare the pectic substances by the general methods which have been adopted in previous communications [Clayson, Norris and Schryver, 1921; Norris and Schryver, 1925]. Thus there is nothing novel in the methods adopted, which consisted of a first treatment of unretted flax with hot water until colourless washings were obtained. The flax was then divided into two portions, each representing 200 g. of the original flax. One portion was treated directly with 0.5 % ammonium oxalate solution at 95–98° for 2 hours; the extract was then filtered off and precipitated with 2 volumes of 95 % alcohol. The gel was purified by re-solution in distilled water and reprecipitation in alcohol some five or six times, when a colourless product was finally obtained. This is the soluble pectin, the "pectinogen" of Schryver and the "ammonium oxalate pectin" of Henderson.

The other portion of flax was treated first with cold 4 % sodium hydroxide, which was subsequently completely washed from the fibre. The latter was then treated as usual with hot 0.5 % ammonium oxalate solution, and a gel was precipitated from this extract by concentrated hydrochloric acid. The gel in this case was not free from colour, but after repeated solution in the absolute minimum of dilute ammonia, and reprecipitation in acid alcohol, most of the colour was removed and a good sample of pectic acid obtained.

The ash content, furfural and carbon dioxide produced on hydrolysis with 12 % hydrochloric acid were determined for each product, and in the case of the soluble pectin, methoxyl groups were also estimated, the results being shown below.

	Pectic acid	Pectin
	%	%
Ash	0.99	5.3
Furfural	20.16 (average of 3 det.)	20.9 (average of 3 det.)
Carbon dioxide	17.80 (average of 3 det.)	Not det. owing to lack of material
Methoxyl	—	9.65 (average of 4 det.)

DISCUSSION OF RESULTS.

The results summarised above are quite in accordance with expectations based on the ring formula of Nanji, Paton and Ling. Considered side by side with analyses of pectic acid and pectin from widely different sources, they present no unusual features. This is clearly indicated by reference to Table I (Nos. 17 and 18).

Yield of carbon dioxide on hydrolysis.

Henderson bases his suggested formula on this value, which he finds to be an increasing one with increasing apparent purity of the sample. Thus, on repeated re-solution in caustic soda, followed by reprecipitation with acid alcohol, a product is finally obtained which yields 20.9 % of carbon dioxide. Although most of the experimental errors in the determination of carbon

dioxide are likely to lead to high results, this progressive increase is difficult to understand, especially in view of the fact that recent work by Candlin and Schryver [1928] would seem to indicate that a loss of radicles which yield carbon dioxide takes place on prolonged treatment of pectin with caustic soda.

Table I. *Analyses of pectic substances.*

Nature of product	Source	Reference	Furfural %	CO ₂ %
1. Pure pectic acid	Pea-pod	Clayson, Norris and Schryver [1921]	20.7	—
2. "	Turnip	" "	21.6	—
3. "	Onion	" "	20.7	—
4. "	Orange	" "	21.5	—
5. "	Cabbage	" "	20.3	—
6. "	Apple	" "	21.7	—
7. Crude pectin	"	Nanji, Paton and Ling [1925]	—	13.79
8. Purified pectin	"	" "	—	18.56
9. Pectin A	Beet	" "	—	18.08
10. " B	"	" "	—	19.81
11. Pectic acid	Orange	" "	—	18.09
12. "	Onion	" "	—	18.00
13. Calcium pectate (calc. on ash-free basis)	"	" "	—	17.64
14. Pectic acid	Orange juice	Norris [1926]	20.4	—
15. Pectin	"	"	19.2	—
Average of observed figures			20.75	17.71
Calculated figures from ring formula			20.85	17.64
Calculated from Henderson formula			14.03	20.70
16. Pectic acid	Flax	Henderson [1928]	—	20.90
17. "	"	Present communication	20.16	17.80
18. Pectin	"	"	20.90	—

In discussing the results of Nanji, Paton and Ling, Henderson suggests that the yields of carbon dioxide obtained by them approach the higher figure, 20.7 %, required by his formula, and that with further purification of the products this figure might have been reached. In point of actual fact, the reverse is true—with increasing purity of product, their results more nearly approach the lower figure, 17.6 %, required by the ring formula. These results are shown in the table (Nos. 7–13); calcium pectate (No. 13) can be prepared in a high state of purity, and, with a carbon dioxide yield of 17.64 % (on ash-free basis), gives the exact figure required by the ring formula. Of the other products, the pectic acids, Nos. 11 and 12, were very carefully prepared and purified by the writer and give results of 18.09 and 18.00, again very nearly approaching the theoretical figure required by the ring formula. Pectic acid obtained from flax in the present instance (No. 17) shows a result, 17.8 %, much more closely in agreement with that required by the ring formula than with the formula of Henderson.

Yield of furfural on hydrolysis.

The case for the formula suggested by Henderson is not strengthened by his omission to quote the yields of furfural obtained from his products. The average yield of furfural from pectin is about 20.75 % (see Table I); the theoretical yield on the basis of the ring formula is 20.85 %, and in the present instance the pectic acid and pectin from flax give 20.16 and 20.9 % respectively.

These figures are in good agreement, therefore, with those usually obtained, and here it may be remarked that the calculated figures are only approximate, although the errors involved in the calculation are of the same order as the experimental ones.

If the suggestion of Henderson, that arabinose is an adsorbed and consequently variable impurity, is correct, then no constant value for furfural could be expected. Further, a substance of the type suggested by him—galactose-tetragalacturonic acid—would yield only approximately 14 % of furfural. It is extremely unlikely that so low a figure has ever been quoted in the literature in the case of a pure pectic substance, and it is quite outside the range indicated in the table. The apparent constancy of the furfural yield—*circa* 20 %—militates against the suggestion that arabinose is a variable constituent of pectin.

It may be remarked in passing that the methoxyl content, 9.65 %, is also quite a normal figure for pectin produced by 2 hours' extraction with hot 0.5 % ammonium oxalate.

It is possible that the abnormal results obtained by Henderson may be due to an impurity, in spite of protracted attempts at purification. Thus it is highly probable that pectin is frequently contaminated with substances of the type known as hemicelluloses [Norris and Schryver, 1925]; further, O'Dwyer [1928] has indicated that this type of substance contains methoxyl in a form of combination which renders it highly resistant to the action of alkali. The observation by Henderson, that even after purification his product still contained a small quantity of residual methoxyl, is in harmony with the suggestion that hemicellulose might be an impurity.

In conclusion it cannot be said that a valid argument has been advanced by Henderson, and there seems to be no adequate reason for rejecting the ring formula in the case of the pectic substances of flax.

The results obtained by Henderson with reference to the progressive hydrolytic breakdown of pectin are of considerable interest and will form the subject of a further communication.

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REFERENCES.

- Candlin and Schryver (1928). *Proc. Roy. Soc. Lond. B.* **103**, 365.
Clayson, Norris and Schryver (1921). *Biochem. J.* **15**, 643.
Ehrlich (1917). *Chem. Ztg.* **41**, 197.
— (1924). *Deutsche Zuckerindustrie*, **49**, 1046.
— and Schubert (1926). *Biochem. Z.* **169**, 13.
Henderson (1928). *J. Chem. Soc.* 2117.
Nanji, Paton and Ling (1925). *J. Soc. Chem. Ind.* **44**, 253 T.
Norris (1926). *Biochem. J.* **20**, 993.
— and Schryver (1925). *Biochem. J.* **19**, 676.
O'Dwyer (1928). *Biochem. J.* **22**, 381.
Schryver and Haynes (1916). *Biochem. J.* **10**, 539.