CCLXXVIII.—*The Pectin and Hemicelluloses of the Flax Plant.*

By Stanley Thomas Henderson.

On the constitutive problem of the plant pectins the most important papers are those of von Fellenberg (*Biochem. Z.*, 1918, **85**, 118), Ehrlich (*ibid.*, 1926, **168**, 263; **169**, 13), and Nanji, Paton, and Ling (*J. Soc. Chem. Ind.*, 1925, **44**, 253T). Von Fellenberg and Ehrlich extracted their material from plants by water boiling under pressure, whereby products were obtained differing considerably from the "ammonium oxalate pectin" described in the present work.

1. *The Ammonium Oxalate Pectin.*—Flax fibre separated mechanically from the unretted straw was extracted with hot dilute (0·5%) ammonium oxalate solution, and the pectin worked up in the usual way, the characteristic methoxyl groups being removed by alkaline hydrolysis. There remained a substance which from analysis and properties was galactosetetragalacturonic acid, $(C_6H_{10}O_5,4C_6H_8O_6 - H_2O)_n$. This has roughly the same ultimate composition as the hypothetical galacto-arabino-tetragalacturonic acid, $C_{35}H_{50}O_{33}$, considered by Nanji, Paton, and Ling to represent the demethylated form of the pectin complex. But the calculated amounts of carbon dioxide liberated from the "-uronic anhydride" groups by boiling hydrochloric acid (*d* 1·06) are, respectively, 20·7 and 17·6% for these two molecules. Some of Nanji, Paton, and Ling's estimations did, in fact, approach the higher figure, and in the present work it was observed that the yield of carbon dioxide rose steadily to 20·9% with repeated purification of the pectin. Nanji, Paton, and Ling evidently did not complete the separation of the pectin gel from adsorbed carbohydrate produced, presumably, by hydrolysis of the original pectin complex.

It appeared impossible by precipitation methods to render the pectin free from inorganic matter and a small residual methoxyl content. A copper salt was prepared in an attempt to obtain a more nearly homogeneous product. Determinations of "-uronic anhydride" on the copper salt were, however, very variable, but the quantities of carbon dioxide evolved in the reaction were large enough to rule out the possibility of any non-carboxylated residue in the molecule. This therefore appeared to be

$$3(C_6H_7O_6)_2Cu,2C_6H_8O_6,4H_2O.$$

Hydrolysis of the ammonium oxalate pectin with boiling dilute sulphuric acid gave an almost insoluble polygalacturonic acid, $(C_6H_8O_6)_n$ or $(4C_6H_8O_6,H_2O)_n$. From this reaction were also obtained a small amount of a soluble acid, giving a soluble barium salt, and a syrup containing arabinose, galactose, and a little fermentable hexose of unknown origin. The arabinose in the syrup might arise from adsorbed pentose (see above), or from partial decarboxylation of galacturonic residues.

A better yield of the polygalacturonic acid resulted from hydrolysis with 1% hydrochloric acid. A highly purified specimen (A7) agreed with the formula $(C_6H_8O_6)_n$. Besides this main substance the same barium salt was obtained as in the sulphuric acid hydrolysis.

Ehrlich described two "digalacturonic acids" derived by hydrolysis of the "pectic acid" of sugar beet, namely, insoluble $2C_6H_8O_6,H_2O$ (*a*) and soluble $2C_6H_8O_6$ (*b*). From his flax "pectic acid," only the soluble *b*-acid could be produced. In the present work the main yield closely resembled his *a*-acid in properties. Differences in molecular weight or configuration may be involved. Exception may reasonably be taken to some of Ehrlich's results, for in determining the equivalent of "digalacturonic acid *b*" from flax pectin he recorded titrations differing from the calculated value by 22, 26, and 31% for three separate preparations, although the ash content of the material, to which these discrepancies were attributed, was less than 1% in the single instance where it was determined.*

For simplicity it has been assumed in the above discussion that the carboxylated groups in pectin and its hydrolysis product are entirely galacturonic acid and not glycuronic residues. This has been indirectly demonstrated by the workers already mentioned, although direct quantitative proof is lacking.

2. *Hemicellulose extracted by Water under Pressure.*—When flax fibre or straw, after a preliminary leaching with hot water, was subjected to the action of water under pressure at temperatures between 100° and 145°, various soluble substances were extracted

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* In a recent descriptive paper (*Z. angew. Chem.*, 1927, **40**, 1305) Ehrlich has discarded these "digalacturonic acids" in favour of his earlier views on "tetragalacturonic acids."

in the form of calcium-magnesium salts. This was Ehrlich's method of preparing " calcium magnesium pectate " from whole flax straw, but the chemical and genetic relations between the non-gelatinous, easily soluble " pectic acid " so derived and the ammonium oxalate pectin are obscure. It appears desirable, if only for convenience of description, to restrict the term " pectin " to gel-forming substances of high carboxyl content such as the " ammonium oxalate pectin."

Even when purified from adherent tissues, waxes, pectin, and free hemicelluloses, flax fibre still contained carboxylated hemicellulose (CO_2, 5·2%) soluble in water under pressure (Cashmore, J., 1927, 718). The distinction between pectin and non-pectic hemicellulose was evident in this case. The salts and acids resulting from pressure extraction will therefore be referred to as carboxylated hemicellulose, or non-pectic hemicellulose.

On the question of the possible derivation of non-pectic hemicellulose by degradation of pectin, no definite evidence can be given, except that the autoclave treatment for removal of hemicellulose left behind no pectin soluble in ammonium oxalate. On the other hand, purified ammonium oxalate pectin heated with water under similar conditions was converted into a syrup with no recognisable constituents except -uronic residues.

In the present work, two preparations of hemicellulose were made from unretted flax fibre, and one from unretted flax straw. The first two agreed fairly well with the empirical formula $C_6H_{10}O_5$, and yielded 5·6 and 4·3%, respectively, of " -uronic " CO_2.

The extract from whole straw was first purified and analysed as a calcium magnesium salt with 9·7% of -uronic carbon dioxide. Conversion into the free acid lowered this value to 6·04%. Once again, however, the acid corresponded to the formula $C_6H_{10}O_5$. Part of the material obtained by repeated extraction of the straw at higher temperatures gave only 2·74% of carbon dioxide. Ehrlich's so-called " pectic acid " from flax had a higher oxygen content and 13·0 and 13·9% of -uronic carbon dioxide. These varied figures suggest that mixtures of hemicelluloses were produced by pressure extraction. The calcium magnesium salt from straw was a soluble, amorphous powder agreeing closely with the formula $(C_6H_7O_6)_4CaMg,(C_6H_{10}O_5)_6,H_2O$.

3. *Hexopentosan from Flax Straw.*—The yield of calcium magnesium salt was less than 2% on the weight of straw. A more abundant product (5%) was a solid hexopentosan free from -uronic residues. Pentose estimations indicated the presence of equivalent amounts of hexosan and pentosan, and hydrolysis gave galactose, arabinose, and xylose, also a small amount of fermentable hexose.

Note on the Estimation of Pectin.

Since hemicelluloses without the gel-forming characteristics of pectin may contain -uronic groups, it is clearly inadmissible to estimate pectin in flax straw or fibre, or in other tissues containing cellulose, by determining the carbon dioxide evolved on boiling with hydrochloric acid (*d* 1·06). For example, ripe unretted flax fibre separated mechanically from the straw contained 7·0 to 7·8% of -uronic anhydride in several samples, whereas the pectin found by the precipitation method (*Biochem. J.*, 1922, **16**, 60) was only 4·5 to 5%. This fibre, when all adherent tissues had been removed by repeated treatment with hot soap solution, still contained 1·6% of -uronic anhydride in the cleanest sample examined. After ammonium oxalate extraction this value fell to 1·3% (compare Cashmore, *loc. cit.*). A further source of error is the carbon dioxide derived from oxycelluloses under the conditions of estimation.

In Carré's method of estimation (*Ann. Bot.*, 1925, **39**, 811) the pectin is extracted by boiling *M*/75-hydrochloric acid, and precipitated as a calcium salt. This process applied to clean flax fibre lowered the -uronic anhydride content from 1·9% to 0·3%, but the calcium salt amounted to 0·03% only. Further extraction with boiling *M*/100-sodium hydroxide to remove " middle lamella pectic substance " left the -uronic anhydride content unchanged at 0·3%.

EXPERIMENTAL.

1. *Ammonium Oxalate Pectin.*—The straw used was " J. W. S." flax pulled when ripe; the fibre was removed by breaking, scutching, and hackling without retting. Tannins and other soluble substances were removed by one or two extractions with water at 80—90° for a few hours.

One extraction lasting about 7 hours with ammonium oxalate solution (0·5%) at 80—90° was sufficient to remove most of the pectin. 100 G. of fibre, or tow from the hackling, required about 1 l. of liquid. After extraction the filtered liquid was precipitated by an excess of alcohol to give a pale yellow gel. The solid was finally squeezed nearly " dry " in a linen cloth, redissolved in water to not more than 0·5% concentration, reprecipitated by alcohol with a little hydrochloric acid, and so on. Hydrolysis of methoxyl groups was effected by making the aqueous solution of pectin alkaline (*N*/40 in NaOH) and leaving it for about 18 hours at room temperature before adding acid and alcohol. Finally, the gel was macerated with 90% alcohol, then with alcohol and ether, and dried to constant weight in a vacuum over phosphoric oxide. The dried product was pale cream in colour, granular and hard to powder, and very slowly soluble in cold water. Excess of acid precipitated it from aqueous solutions, and salts of the alkaline earths at once produced colourless gels. Tollens's naphtharesorcinol reagent gave a violet ethereal extract indicating the presence of galacturonic (or glycuronic) groups. Oxidation with nitric acid (*d* 1·15) on the water-bath gave a small yield of mucic acid presumably derived from the galactose residue, for experiments on polygalacturonic acid failed to produce mucic acid except by the slow action of concentrated nitric acid.

Two samples were analysed: (*a*) six times precipitated, and twice hydrolysed in *N*/40-sodium hydroxide: ash, 1·5%; (*b*) ten times precipitated, and five times hydrolysed in *N*/40-sodium hydroxide: ash, 0·9%. Found, on ash-free basis: (*a*) C, 41·8, 42·2, 41·8; H, 5·0, 4·6, 5·0; CO_2 from -uronic anhydride, 19·7, 20·3, 19·7. (*b*) C, 41·4; H, 5·1; CO_2, 20·9; OMe, 0·77; equiv. by back titration with phenolphthalein as indicator, 228·6. $C_6H_{10}O_5,(C_6H_8O_6)_4 - H_2O = C_{30}H_{40}O_{28}$ requires C, 42·4; H, 4·8; CO_2, 20·7%; equiv., 212·1; one methoxyl group per molecule requires OMe, 3·6%. $C_{30}H_{42}O_{29}$ requires C, 41·6; H, 4·9; CO_2, 20·3%; equiv., 216·6. A thrice precipitated sample, not hydrolysed, gave OMe, 2·2; CO_2, 19·3%.

2. *Copper Salt.*—Pectin purified by several precipitations and hydrolyses was dissolved in water at 70—80° to give a solution of about 0·3% concentration, to which was added one-tenth of its volume of *N*-acetic acid, followed by twice this amount of *M*-copper sulphate solution. The mixture was stirred and heated at 80° for an hour, and the gelatinous copper salt was separated, washed free from soluble copper compounds with large quantities of very dilute, hot acetic acid and then with hot water, alcohol, and ether in succession, and dried to constant weight in a vacuum over phosphoric oxide. It formed hard, granular masses of a bright green colour. Not more than about 4 g. could be conveniently prepared in one operation [Found: C, 34·3; H, 4·2; Cu, 11·3 (mean of seven combustions on three different preparations). $(C_6H_7O_6)_8H_2Cu_3,4H_2O$ requires C, 34·6; H, 4·0; Cu, 11·45; CO_2, 21·1%.

$$C_6H_{10}O_5,(C_6H_7O_6)_4Cu_2,3H_2O$$

or $C_{30}H_{36}O_{28}Cu_2,4H_2O$ requires C, 34·5; H, 4·25; Cu, 12·2; CO_2, 16·9%].

The -uronic anhydride estimation gave values for CO_2 varying from 18·0 to 19·8%, and concordant results could not be obtained for any single sample. Taking the elementary analyses and the *maximum* value found for CO_2, it is possible to deduce the formula $C_{32}H_{46}O_{35}Cu_2$, containing *five* -uronic groups. The number of carbon atoms could hardly be explained by the presence of methoxyl groups, for a determination by Zeisel's method yielded no trace of silver iodide.

3. *Hydrolysis of Pectin by Sulphuric Acid.*—Dry pectin (16 g.) was softened by heating with water and then made up to 900 c.c. of liquid containing 2% of sulphuric acid. After 15 hours' refluxing, the residual gelatinous precipitate (A1) was separated and the hot liquor was treated with excess of barium carbonate and norit, filtered, and concentrated under reduced pressure to a syrup. This syrup was thoroughly extracted with boiling 90% alcohol. Part was insoluble (B, about 0·5 g.) and the extracts were again concentrated to a syrup (C), which was dried (1·7 g.) over sulphuric acid in a vacuum. A1 was purified by three precipitations from dilute soda or ammonia solution by dilute hydrochloric acid with norit treatment. The solid was washed with dilute hydrochloric acid, water, alcohol, and ether, and dried over phosphoric oxide in a vacuum, yielding 1·3 g. of polygalacturonic acid as a colourless powder (see below). Shorter periods of hydrolysis produced greater yields of polygalacturonic acid. Pectin (5 g.) boiled for 2 hours with 500 c.c. of 2% acid gave 0·88 g. (A2), and 10 g. in 600 c.c. of 3% acid gave 2·75 g. in 5 hours (A3).

B, a barium salt, was three times precipitated from aqueous solution by alcohol with a little acetic acid to cause flocculation (Found : "-uronic" CO_2, 12·14%. Compare barium salt from hydrolysis with hydrochloric acid).

C contained arabinose (identified by the diphenylhydrazone, m. p. 196°), and a larger amount of galactose (α-methylphenylhydrazone, m. p. 182°). Melting points were always checked by the "mixed melting point" with an authentic specimen. No xylose could be detected in C by Bertrand's cadmium xylonate test, no ketose by Pinoff's test, and no methyl pentose by Rosenthaler's test.

The syrup, tested in dilute solution for (i) total reducing sugars by Pavy's method and (ii) hexose fermentable by yeast at 35° in Lohnstein's apparatus, showed the presence of 7·4% of fermentable hexose on the total reducing sugars.

4. *Hydrolysis of Pectin by Hydrochloric Acid.*—Dry pectin (10 g.) was dissolved in about 400 c.c. of hot water containing 30 c.c. of *N*-sodium hydroxide to assist solution. Sufficient concentrated hydrochloric acid having been added to make the solution of 1% concentration after neutralisation of the soda, the whole was heated on the water-bath and the gel was separated and purified as before. The yields of dried acid from 10 g. of pectin were : A4, 8 hours' heating, 3·5 g. ; A5, 1½ hrs., 2·75 g. ; A6, 1 hr., 3·85 g. Part of the solid prepared by hydrolysis with hydrochloric acid was heated for

a further 2 hours in fresh 2% hydrochloric acid, reprecipitated three times, and dried (A7).

Polygalacturonic acid. The samples so prepared were almost colourless, amorphous powders, slightly soluble in hot water but insoluble in cold water and in the usual organic solvents, soluble in caustic alkali or hot ammonium oxalate solution. The substance reduced Fehling's solution on warming and gave a strong positive naphtharesorcinol reaction. Heated with concentrated nitric acid on the water-bath, it gave a small amount of mucic acid, m. p. 211°; this product was not obtained by treatment with dilute nitric acid, or with bromine and 5% hydrobromic acid in a sealed tube at 100°.

The sodium salt, a stiff, colourless gel, or granular powder when dried, was prepared by adding the acid to less than an equivalent amount of *N*/10-soda, filtering the solution, and precipitating the salt three times from an aqueous solution by alcohol.

A sample of A4, carefully washed after five precipitations and dissolved in an excess of *N*/10-sodium hydroxide (0·2343 g. in 20 c.c.), gave $[\alpha]_{\mathrm{Hg\ yellow}}^{15^\circ}$ + 334° and $[\alpha]_{\mathrm{Hg\ green}}^{15^\circ}$ + 376°.* The highest value recorded by Ehrlich is $[\alpha]_{\mathrm{D}}^{20^\circ}$ + 285° (*Z. angew. Chem.*, 1927, **40**, 1305). Found, on ash-free basis:

Sample.	% C.	% H.	%-uronic CO_2.	Equiv.
A4	38·7	4·9	22·9	187
$2C_6H_8O_6,H_2O$ requires ...	38·9	4·9	23·8	185
A2	39·7	4·8	23·7	—
A5 and A6 (mean)	40·0	4·9	24·2, 22·5	194
$4C_6H_8O_6,H_2O$ requires ...	39·9	4·7	24·4	180·5
A1 and A3 (mean).........	40·8	4·6	25·0, 24·5	—
A7 (mean of three)	40·7	4·3	24·8	185
$C_6H_8O_6$ requires	40·9	4·6	25·0	176

The molecular weight of the sodium salt in 2·5% aqueous solution was 292, but the ice separating seemed to be contaminated with gelatinous matter.

In the A5 experiment, a barium salt was isolated as a colourless powder from the mother-liquor by a method similar to that used before [Found: Ba, 19·2; "-uronic" CO_2, 12·04. $C_6H_{12}O_6,(C_6H_9O_7)_2Ba$ requires Ba, 19·5; CO_2, 12·5%].

5. *Extractions by Water under Pressure.*—(1) Unretted flax fibre was repeatedly extracted with water at 50—60° till the washings were colourless. The hemicellulose required was then removed by 6 hours' heating with water (1 l. to 100 g. of fibre) in an autoclave at 30—35 lb. per sq. in. excess pressure, and worked up as described by Cashmore. The substance, dried in a vacuum over phosphoric oxide, was brown, granular, and easily soluble in water to a mobile solution. Yield, about 1·5%. It reduced Fehling's solution slowly

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* The author is indebted to Dr. F. G. Mann, of the Cambridge University Laboratories, for these determinations.

on boiling, was stained brown by iodine, and gave a positive naphtharesorcinol reaction (Found, on ash-free basis : C, 44·8, 44·6 ; H, 6·3, 6·1 ; " -uronic " CO_2, 5·57 ; ash, 4·0. $C_6H_{10}O_5$ requires C, 44·4 ; H, 6·2%).

Another preparation was finally precipitated from water by strongly acid (HCl) alcohol. The ash content fell to 0·6% (Found : C, 45·0 ; H, 6·3 ; " -uronic " CO_2, 4·32 ; OMe, 1·24 ; equiv., corrected for methoxyl, 1290).

(2) 450 G. of broken, unscutched straw were used in an experiment similar to Ehrlich's. Soluble substances were removed by extraction with water at 50°. There followed five extractions for hemicellulose, first for 2 hours in 5 l. of water at 30—35 lb. per sq. in. excess pressure, then similarly at higher pressures up to 50 lb. per sq. in. From the straw, now considerably darkened, no pectin could be extracted by ammonium oxalate, although cold *N*-caustic soda removed " free hemicelluloses " precipitable by excess of acetic acid.

The aqueous extracts were concentrated to small bulk on the water-bath, and the yellow, flocculent " calcium magnesium pectate " precipitated by alcohol. That from the first pressure extract (P_1) was worked up separately from the less pure material (P_2) arising from later extracts. The aqueous-alcoholic filtrates from P_1 and P_2 were evaporated under reduced pressure, and fractional precipitation then separated a gummy substance, soluble in water but insoluble in alcohol, from the main yield, which was soluble in both solvents. The aqueous-alcoholic solution of the latter was concentrated and freed from a resinous substance soluble in alcohol but insoluble in water. The hexopentosan present in the aqueous solution was finally obtained as a brown, brittle solid (23 g.) by concentration to a syrup and drying in a vacuum over sulphuric acid.

Calcium magnesium salt from whole straw. This, after treatment with norit and precipitation by alcohol, formed a pale yellow or brown powder easily soluble in water or dilute acid. That described above as P_1 (yield, 4·5 g.) gave, after four precipitations, C, 41·1, 41·1 ; H, 5·4, 5·0 ; CO_2, 9·7 ; ash (CaO, MgO), 5·46, 6·13%. The weights of ash, assumed to be CaO and MgO in equivalent quantities, gave (Ca + Mg), 3·64, 4·08%. $(C_6H_7O_6)_4(Ca + Mg),6C_6H_{10}O_5,H_2O$ requires C, 41·0 ; H, 5·2 ; CO_2, 10·0 ; (Ca + Mg), 3·67%.

The fraction P_2 (yield, 3·7 g.) was converted into the soluble acid by three precipitations from dilute hydrochloric acid (Found : CO_2, 2·74%). The main yield P_1 was similarly treated (Found : C, 44·4 ; H, 6·4 ; CO_2, 6·04. $C_6H_{10}O_5$ requires C, 44·4 ; H, 6·2%).

Hexopentosan. Oxidation with nitric acid (*d* 1·15) yielded mucic acid, denoting the presence of galactose residues. Bial's orcinol reaction for pentoses gave a positive result, but the tests for methyl pentose and -uronic groups failed (Found, by the Krüger–Tollens–Kröber method: pentosan, calc. as a mixture of arabinose and xylose, from Kröber's tables, 44·0, 40·9. $C_5H_8O_4,C_6H_{10}O_5$ requires pentosan, 44·9%).

The dried solid (5 g.) was hydrolysed by boiling for 4 hours with 250 c.c. of 1% sulphuric acid. Excess of barium carbonate and charcoal were added to the hot liquid, which was filtered, concentrated to a dark brown syrup under reduced pressure, and dried in a vacuum over sulphuric acid. Arabinose was identified in it by the diphenylhydrazone, xylose with difficulty by Bertrand's reaction. Oxidation with nitric acid gave mucic acid as for the unhydrolysed substance. No methyl pentose, ketose, or -uronic groups were distinguished. By the method previously outlined, it was determined that the syrup contained 4·5% of yeast-fermentable hexose calculated on the total reducing sugars.

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