CVI.—*The Constituents of the Cell-wall of the Flax Fibre.*

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In the past, investigations of the chemical constituents of the flax fibre dealt nearly always with materials, such as commercial raw fibre and yarn, in which non-fibrous tissues adhered to the fibre proper. This complex assemblage of tissues, comprising fragments of epidermis, cortex, soft-bast, and usually wood, contains a large variety of substances, and although it is customary to refer to the flax fibre as a pectocellulose, there is little definite knowledge of the distribution of the pectic constituents among the various tissues present in the raw fibre. Moreover, the loss in weight suffered by linen materials in the preliminary treatments with boiling alkalis is generally attributed, on very insecure grounds, to the removal of pectic constituents. This assumption is based

mainly on the evidence of Kolb (*Bull. Soc. Ind. Mulhouse*, June, 1868. See also Cross and Bevan's "Cellulose," 1918, p. 218), who states that pectic acid is precipitated when the spent alkaline lyes obtained in the kier-boiling of linen are acidified.

During the past few years our knowledge of the chemistry of pectin has been considerably enlarged (see, for example, Carre, *Ann. Bot.*, 1925, 811, where a summary of the literature dealing with the properties and constitution of pectin is given) and better methods have been devised for the extraction of pectin from plant tissues and for its estimation, either after extraction or *in situ*. Honneyman (*J. Text. Inst.*, 1925, **16**, 370T) has recently carried out estimations of pectin in commercial flax fibre, using the method of Ling, Nanji, and Paton (*J. Soc. Chem. Ind.*, 1925, **44**, 253T), and has shown that the pectic content of the retted fibre is very low (some 4—6%), whereas by the action of boiling dilute alkalis the fibre loses some 25% in weight. Similar results have been obtained in unpublished work carried out in these laboratories, and it has been shown that the fibres, freed from extraneous tissues by treatment with a hot soap solution as described later, contain less than 1% of pectin.

Reference may be made at this point to a paper by Schmidt, Haag, and Sperling (*Ber.*, 1925, **58**, 1394) on the so-called incrusting substances of flax. The authors do not state definitely the nature of the material used, but presumably it was the retted and scutched fibre of commerce. By successive treatments of the material with solutions of chlorine dioxide and sodium sulphite, a separation of the "incrusting" substances was effected, leaving the "skeletal" substances. In this way, the following figures were obtained :

Skeletal substances	49·9% (wt. of ash deducted).
Incrusting substances	38·9% (wt. of ash deducted).
Moisture content	7·7%.

The skeletal substances so obtained contained 3·8% of a hemicellulose soluble in cold 5% sodium hydroxide solution, and when this was removed the residual "cellulose" still contained 5% of "pentosan." The cellulose content of the fibre thus indicated is only about 40%, and it is clear that the "incrusting substances" of these authors include the bulk of the non-cellulosic constituents of the fibre wall with which we are concerned in this communication. There is, however, no evidence as to which of the constituents examined by them came from the fibre-strands and which from the adherent tissues.

In a paper entitled "The Incrustations of Flax," Ehrlich and Schmidt (*Biochem. Z.*, 1926, **169**, 13) describe the substances obtained by the extraction of flax straw with water at temperatures

varying from 50° to 130°, but in this case also no evidence was given to show from which tissues the substances examined were derived.

The fibre employed in the present investigation was obtained from unretted flax straw of good quality, pulled at about the customary stage of ripeness. This was broken and scutched without retting, and after hand-combing to remove the bulk of the adherent shives, was extracted with a hot soap solution. In this way, the remaining adherent tissues were loosened from the fibre-strands, and, as microscopic examination showed, could be completely removed by careful washing. The adherent tissues could not be readily removed from retted fibre by this treatment. It was found that the unretted fibre-strands cleaned by the soap treatment lost practically nothing in weight when placed for several days in a retting tank containing actively retting flax straw. It may be concluded, therefore, that the fibre-strands from unretted straw, purified as described, contained no appreciable amount of substances which are normally removed in retting. In order to remove small quantities of waxy and resinous constituents, the fibre-strands were next extracted with a mixture of ethyl alcohol and benzene. The small remaining quantity of pectin was then removed by the prolonged action of a hot dilute solution of ammonium oxalate. Finally, the fibre was leached with a cold 4% solution of sodium hydroxide, in order to remove free hemicelluloses.

The fibre-strands, after such purification, still contain some 15—16% of substances which may be removed by the action of boiling dilute alkalis, such as 2% sodium hydroxide or barium hydroxide solution. The nature of these alkali-soluble constituents and their mode of attachment to the cellulose form the main subjects of this communication. Since the soap-cleaned fibre-strands contain no adherent tissues, and also since the middle lamella between the fibre cells clearly represents only a very small proportion by weight of the material, it may be concluded that the bulk of the alkali-soluble substances is associated with the cellulose of the cell-wall. The association of cellulose and alkali-soluble substances in the fibre-strands, purified by the method described, will be referred to as the *cellulose-complex*.

This cellulose-complex was found by Cross and Bevan's method to contain 88·5% of " total cellulose " and 76·5% of " α-cellulose." Since the loss in weight produced by the action of boiling dilute sodium hydroxide solution on the cellulose-complex amounted to some 16%, it would appear that the " total cellulose " estimated by Cross and Bevan's method must include some of the alkali-soluble constituents of the fibre, and that the substances removed

by the 17·5% solution of sodium hydroxide (employed in the α-cellulose estimation) must include all the alkali-soluble constituents. For the estimation of the cellulose content of the cellulose-complex, the alternative method described on p. 724 is to be preferred; this method showed the α-cellulose content to be 82—83%.

The substances extracted from the cellulose-complex by means of boiling dilute alkali solution showed no reducing properties. This method of resolving the cellulose-complex was not considered very promising, because most monosaccharides and polysaccharides are unstable in the presence of dilute alkalis (compare Nef, *Annalen*, 1914, **403**, 204; Nef, Hedenburg, and Glattfeld, *J. Amer. Chem. Soc.*, 1917, **39**, 1618; Glattfeld and Hawke, *ibid.*, 1918, **40**, 973). Subsequent hydrolysis of the alkaline extract with 1% sulphuric acid at 130—140° gave a solution possessing strong reducing properties.

A more promising method of attack was that of hydrolysis with dilute sulphuric acid, and by this means it has been possible to identify the more important constituents of the cellulose-complex. An attempt to break down the complex by means of 5% sulphuric acid at 100° showed that it is very resistant to acid hydrolysis, for the syrup obtained from the neutralised hydrolysis liquors, when dried under diminished pressure, amounted to only 3% of the material taken.

By treatment with 1% sulphuric acid at 130—140° the breakdown was more complete. Thus, in one experiment, 100 g. of air-dried fibre yielded a syrup soluble in 90% alcohol, weighing 12·8 g. after drying. This syrup possessed a total reducing-sugar content of 61·7%, calculated as glucose. It was possible to identify glucose, galactose, xylose, and fucose among the products of hydrolysis. On analysis, the following results were obtained: free pentose (as xylose), 10·4; methylpentose (as fucose), 9·4; galactose, 14·8, 16·3; glucose, 29·7%. With more concentrated sulphuric acid (3 and 5%), the yield of syrup was greater, mainly owing to the more extensive production of glucose. The non-reducing constituents of the syrup obtained in the acid hydrolysis have not been identified. In part, they may arise by the further action of the acid on the sugars produced by hydrolysis, for Davis and Daish (*J. Agric. Sci.*, 1913, **5**, 437) have shown that the hydrolysis of maltose by means of 2—4% hydrochloric acid at 100° is accompanied by the decomposition of a considerable amount of the resulting hexoses.

The glucose produced by hydrolysis at 130—140° in all probability is not entirely due to the breakdown of the cellulose. This

is shown by the comparison, given on p. 727, of the results obtained before and after the cellulose-complex has been extracted with boiling alkali.

The resistance of the cellulose-complex to hydrolysis with 5% sulphuric acid at 100° suggests that the sugar groups are attached to the cellulose by a linkage of a glucosidic nature (compare Mehta, *Biochem. J.*, 1925, **19**, 958. Lignocellulose also is very resistant to hydrolysing agents and from this it is assumed to be a glucoside). The " -uronic anhydride " content of the cellulose complex (compare Ling, Nanji, and Paton, *loc. cit.*) is about 1·5%, amounting therefore to some 10% of the non-cellulosic constituents. Only traces of " -uronic anhydride " were found among the products of hydrolysis, but these groups are stated to be unstable to dilute acids at high temperatures (compare O'Dwyer, *Biochem. J.*, 1926, **20**, 657) and are completely decomposed by 12% hydrochloric acid at 100°.

Finally, the hydrolysis of the cellulose-complex by water has been investigated. At 100°, the action of the water was negligible, but at 140—150° a loss in weight of some 6% was produced, the hydrolysis being completed in less than 3 hours. The water-soluble products of hydrolysis consisted of (*a*) a hemicellulose, insoluble in 70% alcohol, amounting to some 4% of the weight of the fibre, (*b*) a resinous substance soluble in 70% alcohol, amounting to about 1% of the weight of the fibre.

The hemicellulose was finally obtained as a white, hygroscopic powder, containing some 21% of " -uronic anhydride " groups and possessing a neutral reaction. Careful hydrolysis with dilute sulphuric acid showed the presence of galactose and rhamnose groups, but no other sugars could be detected. Proximate analysis yielded the following results: galactose, 60; methylpentose (as rhamnose), 10·8—14·2; free pentose, 0—4; -uronic anhydride, 20·6; ash (mainly CaO), 4·8%.

The hemicellulose was not identical with the pectin extracted from flax-straw by Ehrlich (*loc. cit.*) by the action of water under pressure, as this substance contained more than 60% of " -uronic anhydride."

The soluble resinous substance was not obtained in a pure state, but on hydrolysis with dilute sulphuric acid it yielded about 25% of pentoses, and the residue was a resin of acid nature which has not been further investigated. The pentoses have been identified as fucose and xylose, present in equivalent amounts. The resinous substance contained neither galactose nor " -uronic anhydride " groups.

The fibre after extraction with water at 140—150° was still quite strong, and the cellulose was not apparently attacked. Extraction with water at higher temperatures was carried out, but

even at 170° the subsequent loss in weight amounted to only about 3—4%, although the fibre was weakened considerably. The aqueous extract has been examined, but no appreciable amount of non-cellulosic substances has been isolated. This is somewhat surprising in view of the fact that the fibre after extraction with water at 140—150° still contains some 10% of substances soluble in dilute sodium hydroxide solution at 100°, and also that the " -uronic anhydride " content of the fibre is still about 0·7%.

It would seem that the non-cellulosic portion of the flax fibre is made up, in part, of a hemicellulose bound to the cellulose either by a glucosidic linkage or by means of the carboxyl of the " -uronic anhydride " group. The residual non-cellulose material is evidently very firmly fixed to the cellulose, but its nature has not yet been characterised.

Experimental.

Preparation of the Clean Fibre.—The fibre was obtained from unretted straw (Somerset 1923 crop—good quality) by breaking, scutching, and combing. The last operation removed most of the residual woody tissues. The root and bough ends of the fibre were discarded and the residue was subjected to the action of a dilute (0·5%) soap solution at 80° for 60 minutes, washed in water, and again leached with soap solution at 80° for 90—120 minutes. Washing was carried out by means of successive steeps in hot water until the wash-water was colourless. The loss in weight produced by this soap treatment amounted to some 25—30%, varying according to the thoroughness of the preliminary mechanical treatment. The fibre, after being dried at room temperature, was extracted with ethyl alcohol–benzene for 6 hours, and then with a 0·5% solution of ammonium oxalate at 95° for 24 hours in order to remove the pectin (Norris and Schryver, *Biochem. J.*, 1925, **19**, 676). It was then washed, and kept for 24 hours, with occasional agitation, in a cold 4% solution of sodium hydroxide, in order to remove free hemicelluloses (Norris and Schryver, *Biochem. J.*, 1925, **19**, 676). Finally, it was thoroughly washed and allowed to dry at room temperature. A sample of the fibre was given a further extraction with ammonium oxalate at 95° for 24 hours, but the liquor was free from pectin.

Isolation of a Hemicellulose from the 4% *Aqueous Sodium Hydroxide Extract.*—The alkaline mother-liquor was filtered from the purified fibre-strands, and again through glass wool. The pale yellow liquor possessed no reducing properties; a small portion, acidified with acetic acid, was treated with calcium chloride, but no gel was precipitated. It was therefore assumed that pectic acid was not present in the solution.

The main portion was rendered distinctly acid with acetic acid and 1·5 volumes of 90% alcohol were added (compare O'Dwyer, *Biochem. J.*, 1923, **17**, 500), whereupon a pale brown gel was slowly precipitated. This was filtered off and redissolved in the minimum amount of hot water; the opalescent solution was allowed to cool, and the hemicellulose precipitated by addition of alcohol as before. These operations were repeated and the precipitate was kneaded successively with 70%, 90%, and 98% alcohol and finally with ether. It was then exposed to the air for a short time and finally dried in a vacuum over phosphorus pentoxide. The very pale brown powder thus obtained darkened slowly on exposure to the air, was soluble in hot water, and possessed no reducing properties. It gave the pentose reaction with phloroglucinol and hydrochloric acid, and Tollens's naphtharesorcinol reagent (*Ber.*, 1908, **41**, 1788) also gave a positive reaction, indicating the presence of " -uronic anhydride " residues.

An attempt to purify the hemicellulose by the method of Baker and Pope (J., 1900, **77**, 696) proved unsuccessful, as the blue copper complex dissolved when washed with the dilute alkali.

The yield of the purified hemicellulose was approximately 1% of the weight of the fibre [Found in different preparations : C, 41·8, 41·5; H, 6·5, 5·5 (on ash-free basis); ash, 3·4, 3·6; " -uronic anhydride," 22·8, 22·7, 22·85%].

Cellulose Content of the Fibre-Complex.—(*a*) Estimations by Cross and Bevan's method gave : Total cellulose, 88·5; α-cellulose, 76·5%. (*b*) The purified fibre lost 15—16% in weight by the action of 2% sodium hydroxide solution at 100° for 24 hours. After washing, it was immersed in a cold dilute solution of sodium hypochlorite (containing 0·7 g. of available Cl per litre) for 24 hours, and the residual non-cellulosic substances were removed by the action of 1% sodium carbonate solution at 100° for 2 hours. The loss in weight amounted to less than 2%. From these figures, it appears that the α-cellulose content of the dry purified fibre-strands amounted to 82—83%.

The " -uronic anhydride " content of the cellulose-complex amounted to about 1·8—2·0%; after extraction with 2% sodium hydroxide solution at 100° for 24 hours, the " -uronic anhydride " content, estimated by the method of Ling, Nanji, and Paton, had fallen to about 0·4%.

Extraction of the Cellulose-Complex with Dilute Alkali Solution at 100°.—A sample of the purified fibre-strands lost 15% in weight (allowing for moisture content) after extraction with 2% barium hydroxide solution at 100° for 5 hours.

The air-dried fibre (80 g.) was extracted at 100° for 5 hours with

700 c.c. of the barium hydroxide solution, and the mixture then filtered. The orange-coloured liquor did not reduce Fehling's solution; after neutralisation with sulphuric acid and filtration, the liquor was decolorised with norit and hydrolysed with 1% sulphuric acid at 130—140° for 3 hours. The liquor was then neutralised with barium carbonate, and the filtrate concentrated under diminished pressure to a brown syrup (10·6 g. after drying under diminished pressure over sulphuric acid), which possessed reducing properties. The total reducing-sugars (calculated as glucose) amounted to only 24·5% of the weight of the syrup. The syrup gave positive reactions for pentoses, but only small amounts of these were present. Galactose was isolated from the syrup by means of its α-methylphenylhydrazone (needles, m. p. 190°); a mixed melting point with an authentic specimen showed no depression. The galactose content of the syrup was estimated by oxidation to mucic acid by means of nitric acid (*d* 1·15) [A. W. van der Haar, " Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide und Aldehydsäuren " (Borntraeger, Berlin), 1920, p. 123]: 2·13 g. of dry syrup gave 0·148 g. of mucic acid, m. p. 213° (*op. cit.*, p. 106); galactose, 9·8%. The syrup was not further examined.

Hydrolysis of the Cellulose-Complex with Dilute Sulphuric Acid.—(1) *At* 100° *with* 5% *sulphuric acid.* The air-dried fibre (50 g.) was immersed in 300 c.c. of 5% sulphuric acid, and the mixture was warmed for 2 hours on a boiling water-bath, maintained at 100° under reflux for 5 hours, cooled, and filtered. The residual mass was well washed with water, and the washings and filtrate were warmed on the water-bath and neutralised with powdered barium carbonate. The mixture was filtered, and the mother-liquor concentrated under diminished pressure to a brown syrup. This, together with the dry precipitate, was extracted three times with boiling 90% alcohol, and the extract filtered rapidly; the filtrate was warmed with norit and concentrated to a syrup, which was finally dried in a vacuum over sulphuric acid. The yield was 1·5 g. of a brown syrup which gave the pentose reaction with phloroglucinol and hydrochloric acid.

The pentose content of the purified fibre-strands, estimated by the Kruger–Tollens–Krober method (van der Haar, *op. cit.*, pp. 63—83), amounted to 3·4%; only 180 c.c. of distillate were collected in these pentose estimations, as recommended by Klingstedt (*Z. anal. Chem.*, 1925, **66**, 129).

(2) *At* 130—140° *with* 5% *sulphuric acid.* (a) The air-dried fibre (25 g.) was immersed in 300 c.c. of 5% sulphuric acid and heated in an autoclave for 2½ hours at a pressure of 30—40 lb.

per sq. inch. The mixture was then cooled, filtered, and subjected to the treatment outlined in the previous experiment. The yield of syrup soluble in 90% alcohol was 4·8 g. after drying to constant weight in a vacuum over sulphuric acid [Found : free pentose (as xylose), 6·1; methylpentose (as fucose), 6·2; galactose, 11·5; total reducing-sugars (as glucose), 47%].

Examination of the insoluble barium salts. These were extracted three times with boiling water and the filtrate was nearly neutralised with dilute sulphuric acid, filtered, and concentrated under diminished pressure, yielding a small amount of a syrup which gave a positive reaction for " -uronic anhydride " with Tollens's naphtharesorcinol reagent. Attempts were made to isolate the " -uronic acid " as the cinchonine salt, but without success.

(b) The air-dried fibre (100 g.) was hydrolysed with 600 c.c. of 5% sulphuric acid as before, and after the usual treatment the syrup soluble in 90% alcohol weighed 27 g. It was dark brown, and this colour was not completely removed by boiling the aqueous solution of the syrup with norit [Found : free pentose (as xylose), 10·4; methylpentose (as fucose), 6·8; galactose, 7·0%].

(3) *At* 130—140° *with* 1% *sulphuric acid.* The air-dried fibre (100 g.) was hydrolysed with 600 c.c. of 1% sulphuric acid as before, at a pressure of 30—40 lb. per sq. inch, for 3 hours. The yield of yellowish-brown syrup was 12·8 g. [Found : free pentose (as xylose), 10·4; methylpentose (as fucose), 9·7; galactose, 14·8, 16·3; glucose (by fermentation), 29·7; total reducing-sugars (as glucose), 61·7%]. The glucose was estimated by fermentation with yeast for 3 hours at 35° in a Lohnstein apparatus.

Identification of Monosaccharide Groups.—(a) *The hexose groups.* Glucose was identified as the *p*-nitrophenylosazone, m. p. 250°; galactose as the α-methylphenylhydrazone, m. p. 190°, and as the *o*-tolylhydrazone, m. p. 175°. The absence of fructose in the syrup was ascertained by the fact that Pinoff's reagent (van der Haar, *op. cit.*, p. 91) gave no colour when warmed with the syrup.

(b) *The pentose groups.* Many of the typical pentose reactions are obscured by the presence of hexoses in large amount; the pentoses were accordingly separated from the dry syrup (10 g.) by two extractions with boiling absolute alcohol (20 c.c.), the extract being filtered after cooling. Galactose and glucose are sparingly soluble in this solvent, whereas the pentoses are moderately easily soluble. The extract was concentrated to small bulk, and the product gave positive pentose reactions with Bial's reagent and Neumann's reagent; a spectroscopic examination of the latter colour indicated a strong band in the red, the absorption being weaker in the yellow part of the spectrum. This indicates the pres-

ence of xylose, and confirmation was obtained by means of Bertrand's reaction (*idem, ibid.*, p. 58), whereby the characteristic boat-shaped crystals of the cadmium bromide–cadmium xylonate ($CdBr{\cdot}C_5H_9O_6,H_2O$) were obtained after one crystallisation. Fucose was identified in the extract, by means of the α-methylphenylhydrazone (microcrystalline, rectangular plates, m. p. 181°) and the *as*-diphenylhydrazone (microscopic needles, m. p. 193—194°). The melting points given in the literature (*idem, ibid.*, p. 207) are 180° and 197—198°, respectively. As the m. p. of galactose α-methylphenylhydrazone has been recorded as 180° (compare von Ekenstein and de Bruyn, *Rec. trav. chim.*, 1896, **15**, 97, 225), the melting point of a mixture of galactose α-methylphenylhydrazone and the above α-methylphenylhydrazone was determined; the mixture melted indefinitely at 170—176°. Further confirmation was obtained by analysis of the α-methylphenylhydrazone (Found: C, 58·7; H, 7·1. Calc. for $C_{13}H_{20}O_4N_2$: C, 58·2; H, 7·4%).

The Presence of Glucose among the Products of Hydrolysis of the Non-cellulose Portion of the Complex.—The results summarised in Table I indicate that the glucose produced by the hydrolysis of the cellulose complex is derived in part from the non-cellulose constituents. In experiments (*a*), (*d*), and (*e*), the hydrolysis product was examined for galactose, but no trace of this sugar could be detected.

TABLE I.

Hydrolysis Experiments at 130—140°.

Material.	Hydrolysing agent.	% Glucose produced on dry wt. of material.	% Total reducing sugars (as glucose) on wt. of syrup.
(*a*) Purified fibre-strands after extraction with dilute sodium hydroxide solution at 100°	1% H_2SO_4	2·76	51·6
(*b*) Purified fibre-strands	1% H_2SO_4	4·30	61·7
(*c*) Purified fibre-strands	3% H_2SO_4	9·33	58·3
(*d*) Bleached linen	1% H_2SO_4	2·83	71·0
(*e*) Bleached linen	3% H_2SO_4	3·83	

Action of Water upon the Cellulose-Complex. Preliminary Experiments.—The fibre used in this investigation contained some 3·1% of total pentoses, 1·9% of methylpentose, 2·1% of " -uronic anhydride," and some 13·5% of substances soluble in 2% sodium hydroxide solution at 100°. The results in Table II were obtained on extraction with water at various temperatures.

The residual fibre after extraction at 140—150° for 3 hours contained 1·65% of total pentoses, 0·5% of methylpentoses, 0·7% of

TABLE II.

Temp. of extraction.	Time of extraction (hrs.).	% Loss in weight.
100°	6	0·3
115—120	6	1·0
140—150	3	6·5
140—150	6	5·8
140—150	12	6·3

" -uronic anhydride," and some 10·6% of substances soluble in 2% sodium hydroxide solution at 100°.

Action of Water at 140—150°. *Isolation of Hydrolysis Product Insoluble in* 70% *Alcohol.*—The fibre (about 200 g.) was extracted with water at 100° for 3 hours, washed with water, and then heated in an autoclave with 1200 c.c. of water for 3 hours at a pressure of 50—60 lb. per sq. inch. The aqueous liquors were collected, and concentrated to about 200 c.c. After treatment with norit and the addition of about 600 c.c. of 90% alcohol, a pale brown, flocculent substance was precipitated; this was separated by filtration, the filtrate being retained. The pale brown precipitate was redissolved in warm water, and the yellow solution treated with norit. This removed some of the colour, and after addition of alcohol the precipitate was nearly colourless. The process was repeated, the precipitate next leached successively with 90% alcohol, absolute alcohol, and ether, and finally dried in a vacuum over sulphuric acid. The colourless powder thus obtained was hygroscopic and easily soluble in water, giving a pale yellow solution. The yield of the pure substance amounted to some 3·5% on the weight of the fibre, but the crude substance was obtained in 5% yield. The aqueous solution gave no precipitate with dilute acids, alkalis, or salts such as calcium chloride or lead acetate, either in neutral or in dilute acetic acid solution. It did not reduce Fehling's solution on boiling. The Tollens naphtharesorcinol test gave evidence of the presence of " -uronic anhydride " groups and also a violet colour indicating the presence of rhamnose, which was confirmed by the Rosenthaler test (*Z. anal. Chem.*, 1909, **48**, 167; van der Haar, *op. cit.*, p. 48). Ketose groups were not present, as determined by Pinoff's test. The substance contained some 4·8% of ash which consisted mainly of calcium oxide. This ash was not easily removed, although the aqueous solution of the hemicellulose gave a crystalline precipitate with ammonium oxalate.

Analysis.—(1) The hemicellulose was dried in a vacuum over phosphorus pentoxide for each determination [Found: C, 42·5; H, 6·1 (on ash-free basis); ash, 4·7%].

(2) A sample was dissolved in water and a small amount of dilute acetic acid added; after two precipitations with alcohol, it

was dried as before [Found : C, 42·7, 42·8; H, 6·2, 5·9 (on ash-free basis); ash, 1·8, 1·8%].

" -Uronic anhydride " estimations. Found : " -Uronic anhydride," 20·8, 20·85, 20·3%.

Galactose estimations. The yield of mucic acid corresponded to 78% of galactose and galacturonic anhydride. Assuming that the " -uronic anhydride " group gave a 60% yield of mucic acid * the amount of galactose present in the hemicellulose would be about 60%.

Pentose estimations. In these estimations allowance was made for the " -uronic anhydride " group, which was assumed to give a 30% yield of phloroglucoside (van der Haar, *op. cit.*, p. 75) [Found : free pentose, 4·3, trace; methylpentose (as rhamnose), 10·5, 14·2%].

Hydrolysis of Hemicellulose.—Preliminary experiments were carried out on the action of dilute sulphuric acid at 100° upon the hemicellulose, the total reducing-sugars (calculated as glucose) being estimated by Pavy's method. The maximum yield of total reducing-sugars after 6 hours amounted to less than 60% and the liquor was brown, indicating decomposition of the products of hydrolysis. Hydrolysis for 3 hours with 1% sulphuric acid at 130—140° gave a yield of 83% of reducing sugars (as glucose).

Examination of the Products of Hydrolysis.—A solution of the hemicellulose (8·5 g.) in 250 c.c. of 2% sulphuric acid was heated for 7 hours on the boiling water-bath and at 130—140° for 1½ hours, and the liquor was neutralised with powdered barium carbonate and filtered. The filtrate, pale yellow after treatment with norit, gave a positive reaction for " -uronic anhydride " with Tollens's naphtharesorcinol reagent and reduced Fehling's solution in the cold. It was concentrated to a syrup under diminished pressure, and this syrup extracted twice with boiling absolute alcohol.

The alcoholic extract slowly deposited long, colourless crystals, m. p. 159°; a mixture of this substance with pure *d*-galactose melted at 159°. A small portion was converted into the α-methylphenylhydrazone (m. p. 190°).

The galactose was filtered from the alcoholic liquor, and the latter concentrated to a syrup under diminished pressure. This syrup gave positive tests for rhamnose with Tollens's naphtharesorcinol reagent and Rosenthaler's reagent. Neumann's orcinol test gave a pale yellow coloration, indicating the absence of free pentoses.

Rhamnose was identified as the *p*-nitrophenylosazone, a dark red precipitate which, after being dissolved in pyridine and repre-

* Ehrlich and Schubert (*Biochem. Z.*, 1926, **169**, 57) state that oxidation of *d*-galacturonic acid with bromine water gave a 60% yield of mucic acid.

cipitated with ether, melted at 234—235°. The phenylosazone also was prepared (m. p. 179—180°). Van der Haar (*op. cit.*, p. 227) gives m. p. 236° and 162°, respectively.

No other hexoses or pentoses could be detected among the products of hydrolysis. The nature of the "-uronic anhydride" group was not ascertained with precision, although it is evident, from the yield of mucic acid produced by the oxidation of the hemicellulose, that "galacturonic anhydride" is a component of this substance. The residue from the absolute-alcoholic extraction was dark brown and semi-solid. It was dissolved in water, and to the filtered solution 90% alcohol (4 vols.) was added, whereby a flocculent, pale yellow substance was precipitated. This was washed with absolute alcohol and ether and dried in a vacuum over sulphuric acid. The yield of the pale yellow powder was 0·5 g., the small yield being explained by the fact that *d*-galacturonic acid is unstable under the conditions of hydrolysis (see O'Dwyer, *Biochem. J.*, 1926, **20**, 657) [Found : Ba, 25·7. Calc. for $(C_6H_9O_7)_2Ba$: Ba, 26·2%]. An attempt to isolate crystalline *d*-galacturonic acid by Ehrlich's method (Ehrlich and Schubert, *loc. cit.*) was unsuccessful, a small amount of a syrup being produced, from which no crystalline cinchonine salt could be isolated. The syrup possessed strong reducing properties, even at the ordinary temperature.

Many modifications of the conditions of hydrolysis were made, but in no case was the yield of barium *d*-galacturonate greater than that produced above, and from the accumulated yield it was not possible to prepare crystalline *d*-galacturonic acid or its cinchonine salt. It is possible that a small amount of *d*-glycuronic acid may be present, which may inhibit crystallisation.

Action of Water at 140—150°. *Isolation of a Hydrolysis Product Soluble in* 70% *Alcohol.*—The brown 70% alcoholic mother-liquor, after removal of the insoluble hemicellulose described above, was concentrated to a syrup under diminished pressure, and the syrup dried to constant weight in a vacuum over sulphuric acid. The yield was approximately 1% of the weight of the fibre.

The syrup was dark brown, and gave a negative reaction for "-uronic anhydride." The presence of pentoses was indicated by Neumann's reagent, an olive-green coloration being obtained. Oxidation of a portion of the syrup with nitric acid (*d* 1·15) yielded no mucic acid, and therefore galactose was assumed to be absent [Found : free pentose (as xylose), 12·0; methylpentose (as fucose), 11·9%].

Hydrolysis of the syrup. The syrup was mixed with 100 c.c. of 1% sulphuric acid; it was not completely soluble, a small amount of dark brown substance remaining undissolved at the end of the

hydrolysis. The mixture was heated on the water-bath for 4 hours, norit added, and the mixture filtered. The filtrate was concentrated to a syrup under diminished pressure, and extracted (*a*) with boiling 98% alcohol, (*b*) with 80% alcohol. Fucose and xylose were identified in (*a*) by the methods previously described. No hexoses or pentoses were present in (*b*); the liquor on concentration gave a resinous substance, acid to litmus and insoluble in organic solvents other than aqueous alcohol, which was not further examined.

Action of Water at Higher Temperatures.—The fibre which had already been extracted with water at 130—140° was extracted with water at temperatures up to 170°. The aqueous extract, on concentration to small bulk, yielded only a small amount of a substance insoluble in 80% alcohol. By evaporation of the aqueous extract to dryness under diminished pressure, a small amount of a dark syrup was produced; this possessed slight reducing properties. The fibre lost some 3—4% in weight on extraction with water at 160—175° for 2 hours and was appreciably weakened. At temperatures above 175°, the fibre was disintegrated and the residue was very dark in colour.

It is proposed to examine the products obtained by the action of dilute alkali solution at 100° upon fibre which has been extracted with water at 135—140°. The fibre loses some 10% in weight by this treatment.

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