CCXXX. SOME CELL-WALL CONSTITUENTS OF *CETRARIA ISLANDICA* ("ICELAND MOSS").

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CHEMICAL investigation of the lichen commonly known as "Iceland Moss" (*Cetraria islandica*) has extended over a considerable period of time, the earliest scientific examination of the material being recorded by Berzelius [1813]. In the main, most attention has been devoted to two groups of substances: (i) the so-called lichen acids and (ii) the water-soluble carbohydrate constituents (lichenin, *isolichenin etc.*), which form the major part of the solids in the lichen. Concerning these latter a very large literature exists. The existence of a number of definite polysaccharides has been established and suggestions put forward for the constitution of certain of them. All appear to be derived from glucose, although Karrer and Joos [1924] reported the isolation of galactose and mannose from one of the fractions. The chemistry of these soluble carbohydrates has been studied so fully that in the present work no attempt has been made to investigate the subject further.

The lichen acids and the soluble polysaccharides comprise in all some 80 % of the total dry matter of the tissue. After their removal, there remains a residue, mainly carbohydrate in nature, consisting of cellulose, various hemicelluloses *etc.* It was this residue which formed the material for the present investigation. Hitherto it seems to have received little attention. Ulander and Tollens [1906] reported the presence of glucose, mannose and galactose therein, an observation confirmed by Hesse [1916]. Salkowski [1921], after hydrolysis of the water-extracted plant by dilute sulphuric acid obtained a resistant xylan-free ashless material, which he showed was not lignin. Poulsson [1906] reported the presence of 3 % of pentosan in the water-insoluble residue, basing his conclusion on a determination of furfuraldehyde. From the purely chemical standpoint, therefore, the present work was designed to furnish further information as to the chemical constituents of an already much-studied plant material. At the same time it was hoped that the results would make it possible to draw a comparison between the cell-wall components of this lichen and those of the higher plants, which have received so much attention in the past few years. The lichens differ so widely from the higher plants in their growth and habit, that it seemed of interest to discover how far the classes resembled one another in the chemical make-up of their cell-walls. It was found that, while there is a resemblance, in that cellulose and the hemicelluloses constitute a considerable proportion of the cell-wall of this particular lichen, the hemicelluloses are of a somewhat unusual type, apparently simpler in structure than those of the higher plants, in being free from pentose residues; while the pectins, of almost universal distribution in the cell-wall of the higher plants, are absent from this lichen. The possible significance of these differences is commented upon later.

EXPERIMENTAL.

1. *Preliminary examination of the lichen.*

In the first place a preliminary survey was made in order to estimate the relative amounts of fractions corresponding roughly to the different groups of carbohydrates present in the lichen. For each experiment, 100 g. of the oven-dried lichen, picked by hand free from extraneous material such as pine needles, were extracted with the following solvents, in the order given:

- (a) Cold water; 12 extractions, each of 24 hours.
- (b) Hot 95 % alcohol; 12 extractions, each of 6 hours.
- (c) Hot water (90°–95°); 20 extractions, each of 6 hours.
- (d) 4 % sodium hydroxide; 3 extractions at room temperature, each of 4 days, followed by 1 extraction at 80° for 6 hours.

At the end of this treatment, the residue was washed with dilute acetic acid until free from alkali, then with hot water and dried. The substances extracted were obtained in solid form, in the first three cases by direct evaporation on the water-bath, and in the case of fraction (d) (corresponding to the hemicelluloses) by precipitation with acetic acid and alcohol.

In the case of the hot water extractions, and to a less extent with the alkali, great difficulty was experienced in handling the material, on account of its extremely gelatinous nature, which made some mechanical loss unavoidable.

Estimations of furfuraldehyde and carbon dioxide yields were made on all the fractions, as well as on the original lichen. The carbon dioxide yields from all fractions were treated as if they arose entirely from uronic anhydride, except in the case of the alcohol extract, which contained the lichen acids—substances which might be expected to yield some amount of carbon dioxide on distillation with 12 % hydrochloric acid. The results of this preliminary examination of the lichen tissue are summarised in Table I.

Table I.

Fraction	Dry weight as % of lichen	Furfuraldehyde yield %	CO ₂ %	Uronic anhydride %
Original lichen	—	1.48	1.65	—
Cold water extract	5.71	0.66	0.95	3.80
Alcohol extract	8.54	Trace	4.72	—
Hot water extract	64.20	1.29	0.85	3.40
NaOH extract	3.20	1.68	1.27	5.08
Residue	10.80	1.52	1.40	5.60

The results indicated that the fractions which it was proposed to study in detail (*i.e.* the “hemicellulose” and cell-residue) contained considerable amounts of furfuraldehyde- and carbon dioxide-yielding constituents, presumably of a “polyuronide” nature. The cell-residue, in fact, contained more of these substances than the hemicellulose fraction, indicating either a partial oxidation of cellulose units or the presence of substances of a “cellulosan” type resistant to extraction by the 4 % alkali usually employed.

It may be noted that the water-soluble fractions (lichenin and isolichenin mainly) gave appreciable amounts of carbon dioxide, and thus appeared to contain uronic anhydride units, a fact which seems to have escaped notice hitherto. (It may be recalled, however, that Ulander and Tollens [1906] reported the presence of traces of pentosan in the lichenin fraction, based upon furfuraldehyde determination; since the uronic anhydrides themselves give

about 16 % of furfuraldehyde on distillation with HCl, it seems possible that the pentosan of Ulander and Tollens may have represented uronic anhydride units. The figures in Table I show that, in the cold water extract, the whole of the furfuraldehyde could be accounted for as derived from uronic anhydride; in the hot water extract about 0.5 % of furfuraldehyde cannot be so accounted for.)

2. Attempted isolation of a pectin fraction.

The preliminary analysis had shown that the amount of furfuraldehyde-yielding bodies in the lichen was small, none of the fractions giving so much as 2 % furfuraldehyde. Thus pectic substances were not expected to be present to any great extent; in view of their possible presence, however, several attempts were made to isolate a pectin fraction. Extractions were carried out on the water-extracted residue with hot 0.5 % ammonium oxalate and the extracts precipitated with alcohol. After redissolving in water and reprecipitating with alcohol the rather gelatinous material was again dissolved in water, and, in aliquot parts of the solution, pectin was estimated by the calcium pectate method. Only a very small amount of an acetic acid-insoluble calcium compound was obtained, and this bore no resemblance to calcium pectate as usually prepared. Further, the calcium content of the substance was found to be 16 % and its carbon dioxide yield 11.3 %, whereas calcium pectate contains 7.4 % Ca and gives 17.6 % CO₂. Two estimations gave a yield respectively of the calcium compound, 0.14 % and 0.42 % of the dry tissue after water extraction. Other experiments on similar lines failed to show the least trace of pectic substance, either in the tissue residue or in the water extracts, and the conclusion was therefore drawn that the lichen contained no pectic substances. The calcium compound obtained was probably the salt of one of the lichen acids.

3. Extraction of hemicelluloses.

The hemicellulose fraction was obtained by extraction of the cell-residue (after complete removal of the water- and alcohol-soluble material) with 4 % sodium hydroxide. The residue was treated, in portions of 200–300 g., with 4 litres of 4 % alkali, twice. The clear extracts, filtered through calico, were then made slightly acid with acetic acid and allowed to stand for some hours. Only in one case was a slight turbidity produced, but not enough solid separated to be removable by filtration or by centrifuging. A hemicellulose of the "A" type thus appeared to be absent.

To the faintly acid solution a half-volume of acetone was added, a copious precipitate being produced; after removal of this, the addition of excess acetone to the filtrate gave no further precipitate, so that the only hemicelluloses found in the sodium hydroxide extract were those of the "B" type [Norris and Preece, 1930]. This fraction was further separated by means of the copper method of Norris and Preece [1930] into fractions B1 and B2. Both were obtained in the form of almost white powders, of low ash content, quite stable in air and not darkening when heated to 100° for 4 hours. The relative yields obtained in different extractions varied somewhat, as shown in Table II.

Table II.

Extraction No.	Weight of lichen residue taken g.	Yield of hemicellulose	
		B 1 g.	B 2 g.
1 <i>a</i>	184	12.0	5.1
1 <i>b</i>	203	16.5	8.8
2	300	31.0	5.0

4. Isolation of a further hemicellulose.

It was noted, in the small-scale extractions, that a considerable amount of furfuraldehyde- and carbon dioxide-yielding material appeared to resist extraction with 4 % alkali (Table I). Examination of the corresponding residues after the large-scale preparation of hemicelluloses B1 and B2 confirmed this observation; further, the material on acid hydrolysis was found to give as much as 12 % mannose (estimated as the phenylhydrazone) and 5 % galactose (estimated as mucic acid). Evidently therefore polysaccharides other than cellulose were still present in this residue. Such resistant "cellulosans" are of course frequently met with in plant tissues, xylose being possibly the sugar most commonly concerned.

An attempt was made to isolate this more firmly combined hemicellulose by extraction with 17 % sodium hydroxide, the extraction being allowed to proceed for several days at room temperature. From the alkaline extract, no precipitate was obtained on acidification, but on subsequent addition of alcohol a bulky yellow-brown precipitate appeared. This was redissolved in 2 % sodium hydroxide and precipitated as a copper compound by addition of an equal volume of Fehling's solution; no further precipitation occurred on addition of acetone. The copper compound was decomposed with dilute acid and the hemicellulose obtained in the usual manner, the yield of the product varying from 7 to 11 % of the alkali-extracted residue taken (*i.e.* approximately 1 % of the original lichen).

5. Examination of the individual hemicelluloses.

Hemicellulose B1. The substance was soluble with difficulty in water, but its solutions were too strongly coloured to allow of a determination of optical rotation. The solution had no reducing action and gave no definite colour with iodine. It was easily hydrolysed to reducing sugars and gave a positive naphthoresorcinol test and a faint green colour with the orcinol reagent.

Determinations of furfuraldehyde and carbon dioxide in the usual manner gave: furfuraldehyde, 2.35 %, 2.32 %; carbon dioxide, 2.39 %, 2.47 %, equivalent to uronic anhydride, 9.56 %, 9.88 %. The furfuraldehyde yield was thus almost wholly accounted for by the uronic anhydride present, the balance corresponding to less than 1 % of pentosan. It was therefore concluded that pentose units were absent from this hemicellulose, which was composed of hexose and uronic anhydride units only.

A study of the rate of hydrolysis of the substance by dilute acid showed that hydrolysis was practically complete within 3 hours, using 3.5 % sulphuric acid (Table III).

Table III.

Time of hydrolysis minutes	% reducing sugar liberated, calc. as glucose (Bertrand)	
	1.4 % sulphuric acid	3.5 % sulphuric acid
30	24.2	40.6
45	45.4	—
60	50.2	59.5
90	50.2	62.7
120	49.9	67.6
160	—	79.8
190	—	84.7
220	—	90.0
250	—	86.2

Accordingly, 2 g. of the hemicellulose were hydrolysed in this manner. During the hydrolysis, a small amount of an insoluble flocculum separated, and this was found to be unhydrolysed by 5 % sulphuric acid in 5 hours. (Some evidence was obtained that the amount of this product formed was dependent upon the conditions of drying the hemicellulose. Samples dried directly in the steam-oven, whereby the hemicellulose was obtained as a horny mass, gave as much as 17 % of the insoluble material, while those dried gradually through alcohol and ether gave only 6 % or less. Anderson [1931, 1, 2], working with hemicelluloses from cottonseed hulls, obtained 19 % of a "body X," apparently an impurity similar to this insoluble product; he considered it to be an essential part of the hemicellulose.) After hydrolysis, the greater part of the acid was removed by means of barium hydroxide; the filtrate was then warmed with excess of barium carbonate, filtered and the filtrate evaporated to a syrup *in vacuo*. The syrup was extracted several times with 85 % alcohol, and in the alcoholic solution the following sugars were identified.

Mannose: as phenylhydrazone. After removal of the phenylhydrazone, no glucosazone was formed on heating with excess of phenylhydrazine; glucose was therefore absent.

Galactose: as osazone, after removal of mannose; as mucic acid, by oxidation with dilute nitric acid.

Tests for other hexoses and pentoses were entirely negative.

The uronic acid present in the hydrolysate was isolated in the form of its barium salt by a method similar to that of Dickson *et al.* [1930]. From 10 g. hemicellulose B1, hydrolysed for 4 hours with a mixture of 12 g. sulphuric acid and 500 cc. water, 1.5 g. of a barium salt were obtained. This was found to contain 24.7 % Ba (barium uronate contains 26.1 % Ba). From this, a cinchonine salt of the uronic acid was prepared by the method of Nanji [1933], and had m.p. 172°, agreeing with that of a cinchonine salt prepared similarly from an authentic sample of galacturonic acid. On hydrolysis by the hydrobromic acid-bromine method of Heidelberger and Goebel [1927], crystals of mucic acid, m.p. 216°, were obtained, thus giving sufficient evidence of the presence of galacturonic acid, since galactose itself is not oxidised to mucic acid by the reagent used.

From these results it was concluded that the hemicellulose B1 of *Cetraria islandica* was a galactose-mannose-galacturonic acid complex. An estimation of the approximate amount of each of these units present in the hydrolysis mixture was made, mannose being estimated as its phenylhydrazone, and (mannose *plus* galactose) by copper reduction, after removal of uronic acid as the barium salt; the details of the methods used were those given in Browne's *Handbook of Sugar Analysis*. The average values obtained were: anhydromannose, 39.6 %; anhydrogalactose, 44.3 % (uronic anhydride, 9.74 %).

Partial hydrolysis of hemicellulose B1. It was noted during the study of the rate of hydrolysis of the hemicellulose by acids (Table III) that with 1.4 % sulphuric acid hydrolysis to the extent of about 50 % took place within 90 minutes, but that further treatment led to no increase in reducing sugars, but rather to a slight fall, due probably to the destruction of uronic acid [*cf.* Preece, 1931]. In order to study this apparent partial hydrolysis more fully, two portions, each of 3 g. of the hemicellulose, were hydrolysed with 2 cc. sulphuric acid and 250 cc. water for 90 minutes. The hydrolysate was in each case neutralised with barium hydroxide followed by barium carbonate, the precipitated barium sulphate with excess carbonate well washed with hot water, and the clear filtrates concentrated to 50 cc. *in vacuo* at low temperature. On

pouring the syrup into two volumes of alcohol, a brownish gum was thrown down, which remained without granulating when left in alcohol at 0° overnight. Two reprecipitations from aqueous solution by alcohol, however, sufficed to granulate the material, which was then dried by means of absolute alcohol and finally *in vacuo*. The amount of the product obtained was (a) 1.05 g.; (b) 1.51 g. From the alcoholic liquors of the second batch, 1.47 g. of sugars remained on evaporation.

(a) *Sugars*. In the alcohol-soluble fraction (sugars) only galactose could be identified. No trace of mannose phenylhydrazone was given under the usual conditions. It therefore appeared that galactose was the only sugar liberated by gentle hydrolysis.

(b) *Insoluble gum*. This fraction, when dry, was found to contain 5.8 % Ba—a figure far too low for a barium uronate and indicating that a fairly complex molecule still remained. After hydrolysis of the gum with 5 % sulphuric acid for 3 hours, mannose and galactose were identified in the hydrolysis mixture, and a quantitative determination showed that they were present roughly in the ratio 3 : 1. A carbon dioxide estimation on the original gum gave 8.6 % uronic anhydride (ash-free). The question of the structure of this gum, and of the parent hemicellulose, is discussed below.

Hemicellulose B2. This fraction resembled B1 in appearance and similarly gave a dark-coloured solution in water. It again had no reducing action but readily gave reducing sugars on gentle hydrolysis. Furfuraldehyde and carbon dioxide determinations gave the following results: furfuraldehyde, 1.72 %; carbon dioxide, 1.97 %, 2.06 %, equivalent to uronic anhydride, 7.88 %, 8.24 %. Again, the furfuraldehyde yield was almost completely accounted for by the uronic anhydride present, so that pentose groups were absent from this hemicellulose also.

The results of a study of the rate of hydrolysis by acids is shown in Table IV.

Table IV.

Time of hydrolysis minutes	% reducing sugar liberated, calc. as glucose (Bertrand)	
	1.4 % sulphuric acid	3.5 % sulphuric acid
30	15.2	32.1
60	24.1	57.1
90	37.1	74.3
120	54.1	87.7
150	54.5	90.1
180	—	89.2
200	74.2	—
240	86.1	—
300	88.2	—

In the case of this substance, there was no evidence of any definite intermediate stage of hydrolysis by the weaker acid; on the whole, the substance seemed less resistant to acid hydrolysis than hemicellulose B1.

The sugars present were identified by the same methods as in the previous case, mannose and galactose being the only sugars found. The small amount of material available made impossible the isolation of the uronic acid as a cinchonine salt; however, a barium compound was obtained, which on oxidation with dilute nitric acid gave mucic acid, so that it was concluded that galacturonic acid was again present.

Estimations of the sugars present were carried out as described above, and the results are shown in Table V. There seemed to be a rather wider variation than usual between the amounts of the sugars found in two samples.

Table V.

Sample	Anhydromannose %	Anhydrogalactose %	Uronic anhydride %
1	21.1, 23.9	64.6	7.88
2	17.6, 17.6	60.1, 62.8	8.24

Hemicellulose extracted by 17 % alkali. This material, after purification by means of its copper compound, was obtained as a light-coloured powder, similar to the other hemicelluloses. It yielded 1.60 % of furfuraldehyde, and carbon dioxide corresponding to 10.7 % uronic anhydride; no pentose was therefore present. The sugars were again identified as mannose and galactose only, and the uronic acid as galacturonic acid by methods similar to those employed in the case of hemicellulose B1. Similar rough estimations of the proportions of the sugar units present gave: anhydromannose, 22.9 % anhydrogalactose, 62.9 %, figures very close to those obtained in the case of hemicellulose B2.

The course of acid hydrolysis of this substance was examined as before, the results being shown in Table VI.

Table VI.

Time of hydrolysis minutes	% reducing sugar liberated, calc. as glucose (Bertrand)	
	1.4 % sulphuric acid	3.5 % sulphuric acid
30	—	26.0
60	11.3	32.0
90	26.0	34.5
105	—	41.0
120	27.3	42.1
150	31.0	46.2
180	33.0	54.8
240	34.1	58.9
300	—	61.3

The substance was evidently much more resistant to acid hydrolysis than either of the hemicelluloses previously studied; it was apparently hydrolysed only to the extent of about one-third by the weaker acid, and again it was found that the only sugar set free during this partial hydrolysis was galactose. On further treatment of the portion unhydrolysed by 1.4 % acid within 4 hours, mannose and galactose were found in the hydrolysis-liquor in the proportion 1 : 2. In all cases examined, the uronic anhydride content of the products of partial hydrolysis was low (3–5 %), suggesting that very extensive decarboxylation had taken place under the influence of the acid, although the hexose units remained to some extent in combination.

Action of enzymes on the hemicelluloses. In view of the large amount of work recorded on the action of diastases on the water-soluble carbohydrates of *Cetraria*, a few experiments were made to test the action of these enzymes on the different hemicelluloses. Malt diastase was without action on any of the substances, but takadiastase produced about 30 % hydrolysis (measured by copper reduction) in 8 days in the case of hemicellulose B1; it was without action on the hemicellulose extracted by 17 % alkali. Attempts to isolate the products of the action of the enzyme failed.

6. *The cellulose residue.*

After very prolonged extraction with 17 % sodium hydroxide (lasting in all about 3 weeks), the residue was well washed with dilute acetic acid, until free from alkali, then with hot 50 % alcohol to remove sodium acetate and finally dried by means of alcohol, giving a dark brown fibrous material.

This substance swelled and dissolved slowly in Schweitzer's reagent and, with the exception of a very small dark residue, was soluble in zinc chloride-hydrochloric acid. On hydrolysis by the method of Monier-Williams [1921] it gave reducing sugars to the extent of 80 % (as glucose); from the hydrolysis mixture, a small amount (about 2 %) of mannose phenylhydrazone was obtained and large amounts of glucosazone, but no evidence of the presence of galactose could be found. It appeared that at least 80 % of this residue gave glucose on hydrolysis and could thus be regarded as cellulose.

A portion of the residue remained unattacked by the acid in the Monier-Williams hydrolysis, 0.4 g. of solid remaining from 5.1 g. of the residue. Similarly, on acetylation of 10 g. of the residue, 0.95 g. of substance remained insoluble in chloroform. It seemed therefore that about 10 % of the cellulose residue, *i.e.* about 0.8 % of the original dried lichen, consisted of this resistant, non-cellulosic material. It seemed possible that this substance was of lignin nature. Its dark colour precluded examination by any of the usual colour tests, and determinations of carbon and hydrogen gave figures intermediate between those required for cellulose and those accepted as an average for lignin, *viz.* C, 58.4 %; H, 6.1 %. A similar product obtained by Monier-Williams hydrolysis of a sample of lichen which had been extracted by water only (to avoid demethoxylation by alkali) was found to contain no methoxyl groups—always present in lignins. Thus the evidence was that the resistant material was not lignin. Finally, sections of the lichen failed to show the presence of lignin by the ordinary staining tests. There seemed no doubt therefore that the lichen contained no lignin, and the nature of the resistant material remained undetermined.

DISCUSSION.

1. *Structure of the hemicelluloses.*

As shown above, approximately 90 % of hemicellulose B1 was accounted for as anhydromannose, anhydrogalactose and galacturonic anhydride, the ratio of the three units being approximately 8 : 10 : 2 (see however below). The nucleus stable to hydrolysis by 1.4 % acid was found to contain anhydromannose and anhydrogalactose in the ratio 3 : 1, with 8.6 % galacturonic anhydride. This product was apparently one of the complex acid substances which have often been isolated from hemicelluloses and gums [*cf.* Norman, 1929], although more complex than the aldobionic acids recognised as the nucleus of certain of the polyuronides. During mild hydrolysis of the hemicellulose, some two-thirds of the galactose was removed, none of the mannose and approximately half the uronic anhydride. That it was impossible to detect galacturonic acid in the hydrolysis mixture was not altogether surprising, in view of the rather unstable nature of these acids and the small amounts of substance used.

Combining the above figures, one may suggest that the original hemicellulose consisted of a resistant nucleus, containing 8 or 9 anhydromannose, 3 anhydrogalactose and 1 galacturonic anhydride units, together with a less resistant portion composed of 6 or 7 units of anhydrogalactose and 1 of galacturonic anhydride. More precise statements as to the proportions of the indi-

vidual units are not warranted, on account of the relatively approximate nature of the experimental methods involved.

A similar consideration of the figures obtained in the case of hemicellulose B2 suggested a molecule based on 9 units of anhydrogalactose, 3 of anhydromannose and 1 of galacturonic anhydride. In this case, however, there was no evidence of the presence of a stable nucleus in the molecule.

The "combined" hemicellulose again was found to be based on the same three units, in approximately the same proportion as in B2. Here, however, not only was the whole molecule rather more stable towards acids, but there was evidence of a more resistant nucleus, as in B1. Since, as shown in Table VI, the apparent end-point of hydrolysis depends on the strength of acid used, there is perhaps little justification for attempting to assign to the resistant nucleus a formula based on data given by a study of the action of acid of an arbitrarily chosen concentration. All that can be said is, that the hemicellulose contains a more resistant portion containing mannose, galactose and galacturonic anhydride, and a less resistant portion consisting of galactose and galacturonic anhydride only.

Three hemicelluloses have therefore been shown to be present in the cell-walls of *Cetraria islandica*, all based upon mannose, galactose and galacturonic acid in varying proportions. The dissimilarity between fractions B1 and B2 affords some evidence for the efficiency of the method of fractionation. It may be noted that, although these products were isolated by the methods used by Norris and Preece [1930], they differed widely in their chemical nature from the products obtained by these authors, which again supports the view, often expressed, that physical nature, rather than ultimate chemical constitution, determines the behaviour of a hemicellulose.

The most notable difference between these polyuronides and those generally obtained from the cell-walls of higher plants was the complete absence from the former of any pentose units. With the exception of certain hemicelluloses of seeds, which may contain hexose or hexose *plus* uronic acid units only, it seems that practically all the hemicelluloses so far isolated from widely different plants contain pentose groups in their molecule. The hemicelluloses of seeds are perhaps of definite "reserve carbohydrate" rather than "structural" nature; it may be that these lichen hemicelluloses combine the functions both of structural and reserve carbohydrate. It is at the moment impossible to amplify this suggestion; it would, however, be strange if the lichen, with its enormous reserves of glucose in the form of lichenin, should need to call upon the mannose and galactose of its hemicelluloses.

The only uronic acid detected was galacturonic acid, and, although mannose was present in quantities comparable with those of galactose, the corresponding uronic acid was not found. This fact, together with the observation that no mannose was liberated on mild hydrolysis, led to the suggestion that the mannose residues, after entering into combination, are in some way protected from oxidation (in the plant) and from hydrolysis (in the liberated hemicelluloses). It may be noted that, although the greater part of the total carbohydrate of *Cetraria islandica* consists of glucosans (lichenin *etc.*), no glycuronic acid has been detected, unless it be that the small amount of carbon dioxide liberated from the lichenin fraction was derived from this acid. One may suggest either that the glucose molecule when present in glucosans is rather less readily oxidised than that of galactose—the apparent non-existence of a highly oxidised glucosan corresponding to pectin is interesting in this connection—or else that glycuronic anhydride complexes pass into pentosans more readily ~~than those of~~

galacturonic anhydride. This second suggestion certainly does not seem to apply in the present case, since pentosans are virtually absent from *Cetraria islandica*.

2. *The cell-wall of Cetraria islandica.*

The work here described, and that previously published, has established the presence of four main well-defined groups of carbohydrate in the cell-wall of *Cetraria islandica*, viz. lichenin, isolichenin, hemicelluloses and cellulose; and the absence of pectins, pentosans and lignin. Of the carbohydrates present, lichenin, isolichenin and cellulose represent condensation products containing glucose only as the parent sugar. The hemicelluloses (using the term in the general sense, to indicate substances resistant to water-extraction, but extracted from the tissue by dilute alkali), on the other hand, are based on mannose and galactose, with partial oxidation of the latter to its uronic acid; glucose is absent. Considerable differences exist, therefore, between the cell-wall constituents of this lichen and those of a typical higher plant. These differences must be accounted for by a different metabolism governing the formation of the cell-wall. The lichens present difficulties, which do not arise in the case of the higher plants, on account of their symbiotic nature; two distinct types of cell-wall, between which considerable differences of nature might be expected to exist, are therefore present. According to Escombe [1896], Wisselingh [1898] and Wester [1909], the cell-walls of the hyphal tissues of lichens contain no cellulose but are frequently composed of chitin; no chitin has, however, been found in *Cetraria islandica*, although it is met with in other members of the genus. The hyphal membranes of this lichen are, in fact, somewhat unusual, in being composed mainly, as Escombe showed, of lichenin and isolichenin. The algal cell-walls were, on the other hand, considered by Escombe to be composed of a type of cellulose. Although it is as yet impossible to demonstrate that the hemicelluloses also occur in these gonidial walls, analogy with the higher plants suggests that such is the case. A small amount of the glucose synthesised by the algal cells becomes converted, by means at present unknown but presumably the same as those effective in the higher green plants, into galactose and mannose, thus giving the nucleus of the hemicelluloses.

In common with all northern lichens, *Cetraria islandica* has a very slow growth. The metabolic demands of the hyphae on the carbohydrate synthesised by the algal cells are thus small, with the result that the major part of the glucose undergoes condensation to lichenin and isolichenin, which are deposited on the much-thickened hyphal walls. In view also of this general low rate of metabolism, oxidation of the carbohydrates deposited in the algal cell-walls only proceeds to a very slight extent, so that the uronic anhydride content of the hemicelluloses is low.

The explanation of the absence from the plant of pentosans and pectic substances probably lies in this low oxidation. In the pectins, oxidation of the sugar units has proceeded to a very considerable extent, some 80 % of the galactose having been oxidised to uronic anhydride; such compounds could only be formed in tissues where the oxidation processes are extensive. Norman [1929] remarks that "the formation of pectin in the plant appears to take place when metabolism is at its highest peak, that is, when growth is most rapid." Further it seems that reactions leading to pectin formation take place only in tissues where there is an abundant water supply. The principal habitat of *Cetraria* is in exposed places in sub-arctic regions; having no root system, it is dependent for its water supply on absorption over its whole surface during rain and storage within its tissues of the water so absorbed. Thus it is pre-

dominantly of xerophytic habit, and another of the conditions for pectin formation is lacking.

The pentosans are generally considered to arise from condensed uronic acids by some process of decarboxylation, either by mild oxidation [Norman and Norris, 1930] or other means. Whatever the means, the process does not take place in this lichen, since pentosans are virtually absent.

3. *The relation between pectins and hemicelluloses.*

The view has frequently been expressed that some at least of the hemicelluloses may arise in the higher plants by partial decarboxylation of the pectins. This suggestion has been based on the observation that in non-lignified tissues pectins predominate over hemicelluloses, while in older, lignified tissues, the reverse is the case, rather than on any direct observations of a change in the amounts of the two classes in the same tissue at different stages of growth. Other support for the view has been found in the production from pectin, *in vitro*, of substances resembling hemicelluloses in having a low uronic anhydride content [Candlin and Schryver, 1928; Norman and Norris, 1930]. So far as *Cetraria islandica* is concerned, however, the facts given in this communication do not support this view. Hemicelluloses containing galacturonic acid occur in fair quantity in the lichen, and if there had been any direct connection between these substances and pectic bodies, as regards their origin, then the large quantities of lichen employed, containing plant specimens of all ages, would have been expected to yield a small quantity of pectin at least; such was not the case. In addition, as no lignin was detected, any pectin present in the young tissues could not have been converted into this substance as has sometimes been suggested.

It might be argued that the absence both of pectins on the one hand, and pentosans and lignin on the other, could be regarded as evidence of a connection between these classes. This may indeed be the case. But the facts presented above make it necessary to emphasise the fact that not all polyuronides, of low uronic anhydride content, arise from pectins by decarboxylation. The only conclusion, then, is that the hemicelluloses of this lichen do not arise from pectic substances, but by feeble oxidation of condensed hexoses—a process distinct from, though largely parallel with, that which leads to the formation of pectins in plants whose general level of metabolism is higher.

SUMMARY.

1. The cell-wall constituents of *Cetraria islandica*, other than lichenin and isolichenin, have been shown to consist mainly of hemicelluloses and cellulose; pectins, pentosans and lignin are absent.

2. Three distinct hemicelluloses have been isolated, one of which is more closely associated with the cellulose and resists extraction with 4 % alkali.

3. All three hemicelluloses give, on hydrolysis, mannose, galactose and galacturonic acid; the amount of the last is in each case low (10 %).

4. Evidence is given to show that in two of the hemicelluloses, the molecule consists of a resistant acid nucleus, consisting of mannose, galactose and galacturonic acid units, and a more loosely combined portion, containing no mannose.

5. The relation between the chemical nature of the cell-wall constituents and the habit of growth of the lichen is discussed.

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