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Studies on Sphagnum Peat

III *. A Quantitative Study on the Carbohydrate Constituents of Sphagnum Mosses and Sphagnum Peat

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The chemical composition of the chief peat-forming plants, the *Sphagnum* mosses, and of the *Sphagnum* peat is still rather incompletely known [1]. The aim of the present investigation has been to study the variation in the quantity of the various sugars, from which the polysaccharides in *Sphagnum* mosses and *Sphagnum* peat are built up, with huminosity and age.

BOTANICAL AND GEOLOGICAL DATA

Two living mosses and five peat samples have been investigated. One moss, *Sph. fuscum*, W. Klinggr., collected from Bälinge, Uppland, is one of the predominating peat-forming mosses in Sweden. The other moss, *Sph. imbricatum*, Russ., supplied by Professor Einar du Rietz and collected from Kanarp, Småland, is the predominating moss in the peat samples A—E. The peat samples were collected from the central part of Ageröd Mosse, Skåne. The age estimations were made by Laborator Tage Nilsson, and are based upon earlier pollen analytical investigations of the same section of the bog. The botanical investigation and the determination of the huminosity was carried out by Professor Carl Malmström. The data are summarized in Table 1.

FRACTIONATION

The samples were subject to the following subsequent treatments:

1. Continuous extraction with benzene for 2 days.
2. Continuous extraction with methanol for 5 days.
3. Extraction with water at 60—65° C, 3 × 3 hours.
4. Bleaching with sodium chlorite and acetic acid at 60—65° C, 1 h in 2—4 steps.
5. Swelling in liquid ammonia.
6. Extraction with water at 40—45° C, 3 × 3 hours.
7. Extraction with 5 % potassium hydroxide at room temperature, 2 × 4 h.

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Table 1. Botanical and geological data of Sphagnum fuscum, Sphagnum imbricatum and the peat samples A—E.

Sample	Middle depth in centimeters	Age in years	Huminosity *	Details
Sph. fuscum	5	—	0	Collected in September 1951
Sph. imbricatum	5	—	0	Collected in August 1952
A	17	200—400	3	Abundant leaf- and stem remains of *Sph. imbricatum*. Traces of *Cyperaceae*
B	47	700—1 000	4	Abundant remains of *Sph. tenellum*. Traces of *Sph. recurvum*, *Sph. cuspidatum*, *Sph. imbricatum*, *Sph.* of *Acutifolia* type and *Cyperaceae*
C	57	900—1 200	6	Abundant remains of *Sph. imbricatum*. Traces of *Sph. recurvum* and *Cyperaceae*. Some mud without organized structure, with greatest probability from *Sph.*
D	113	1 900—2 200	3	Abundant leaf- and stem remains of *Sph. imbricatum*. Traces of *Sph. recurvum* and *Cyperaceae*.
E	295	4 300—4 800	6—7	Most of the sample is mud, most probably coming from *Sph.*, as a continuous change of *Sph.* remains at various stages of destruction could be seen in it. Some of the remains could be identified as *Sph.* of *Acutifolia* type, *Sph. imbricatum*, *Sph. recurvum* and traces of *Cyperaceae*.

* Estimated by the microscopical investigations, as a measure of the physical destruction on a scale from 0 to 10.

The moisture and ash contents were determined on the original sample and after steps 3 and 4 above. The amount of unbleached holocellulose is estimated as the difference between the weight of the original sample and the weights of the extracts 1, 2 and 3, and the losses by the bleaching are also calculated as the weight difference. Summation of the fraction yields will thus be 100 %, and the accuracy of the recorded holocellulose content is thus artificial. The real values may differ about $\pm$ 1 % from those given in Table 2.

The pH of the press-water from the peat samples was about 4.5. The ash contents of the mosses and the peat do not differ very much. The ash contents of the peat samples appears to vary directly with the huminosity and is probably due to a loss of organic material. On the other hand the living mosses

Table 2. Fractions and ash contents of Sphagnum fuscum, Sphagnum imbricatum and the peat samples A—E. (All the values are given as a percentage of the weight of the total organic material in moss or peat.)

	Sphagnum fuscum	*Sphagnum imbricatum*	A	B	C	D	E
Ash (%)	1.5	1.8	1.0	1.1	1.7	0.7	1.7
Main fractions							
Benzene extract	1.7	1.7	4.0	3.1	5.9	3.3	6.7
Methanol extract	6.3	4.5	3.6	4.5	4.6	3.9	4.9
Water extract	4.6	3.6	1.5	2.0	1.6	1.0	1.1
Losses by bleaching*	3.7	3.7	26.3	24.7	34.5 (43.9)	22.1	36.2 (57.1)
Holocellulose *	83.7	86.5	64.6	65.7	53.4 (44.0)	69.7	51.1 (30.2)
Holocellulose fractions							
Water extract	5.6	6.9	8.0	8.5	8.8	8.3	8.8
Alkali extract	3.7	3.6	5.6	5.5	6.5	5.1	5.2
Residue **	72.5	73.4	48.5	47.8	31.5 (27.8)	53.8	31.7 (20.4)
Losses by the holocellulose fractionation	1.9	2.6	2.5	3.9	6.6	2.5	5.4

* To minimize the loss of carbohydrates the holocellulose fractionation and all quantitative sugar analyses, except that of uronic acids in C and E, were made on the samples bleached in 2 steps only. The samples C and E were bleached a further 2 steps (the values in brackets), which was necessary to get excess of chlorine dioxide in the solution after the bleaching. These values therefore represent the true amounts of bleaching losses and holocellulose.

** The values in brackets represent the amount of residual holocellulose after a further 2 steps bleaching before the analysis of uronic acids.

have greater ash contents than peat of low huminosity, which is natural as they grow on, and get their minerals from the peat.

The quantity of the very complex mixture, bitumen, extracted by benzene, appears to increase with the huminosity. The amounts extracted by both methanol and water do not vary much, but the losses by bleaching increase considerably with the huminosity, in other words the holocellulose content decreases with increasing degree of huminosity. The bleaching losses of the mosses, 3,7 %, agree fairly well with the value of Klason lignin, about 5 %, obtained for some *Sph. mosses* (Part II[2]). The losses by the holocellulose fractionation are partly manipulative, and partly those of non-carbohydrate material during the alkali treatment. The value of the alkali extract in Table 2, represents that part of the extract, precipitated by 75 % ethanol. Especially for C and E, in which the holocelluloses were not bleached sufficiently, a fairly large part of organic material was certainly left in the mother liquors.

THE CARBOHYDRATES IN THE METHANOL AND WATER EXTRACTS

The quantitative estimation of the constituent sugars in the methanol and water extracts after hydrolysis is represented in Table 3.

Table 3. The constituent sugars in the methanol and water extracts of Sphagnum fuscum, Sphagnum imbricatum and the peat samples A—E. (All values are given as a percentage of the weight of the total organic material in moss or peat. + = Traces. — = Not observed.)

Sugars	*Sphagnum fuscum*	*Sphagnum imbricatum*	A	B	C	D	E
Methanol extract							
Uronic acids *	+	+	+	+	+	+	+
Galactose	0.3	0.2	+	0.1	0.1	0.1	+
Glucose	0.8	0.5	+	+	+	+	+
Mannose	0.1	0.1	+	+	+	+	+
Fructose	1.7	1.0	—	—	—	—	—
Arabinose	0.2	0.1	+	+	+	+	+
Xylose	0.3	0.2	+	0.1	0.1	+	+
Rhamnose	+	+	+	+	+	+	+
Water extract							
Uronic acids *	0.4	0.3	0.2	0.1	0.1	0.1	+
Galactose	0.4	0.3	0.2	0.1	0.2	0.1	0.1
Glucose	0.2	0.2	0.2	0.2	0.2	0.1	0.1
Mannose	0.2	0.2	0.1	0.1	0.1	0.1	+
Fructose	1.3	1.1	—	—	—	—	—
Arabinose	0.3	0.2	+	+	+	+	+
Xylose	0.3	0.3	0.2	0.1	0.1	0.1	0.1
Rhamnose	0.1	0.1	—	+	0.1	+	+

* Approximative values visually estimated by paper chromatography.

It may be seen from Table 3 that fructose and glucose predominate in the methanol extracts, and fructose in the aqueous extracts of the mosses, and that fructose is absent in the peat. The methanol and water extracts of the mosses were investigated before the hydrolysis.

In the methanol extract glucose, fructose and sucrose were identified together with fructose containing substances with R_F-values lower than that of sucrose. On the chromatograms the presence of two substances with higher R_F-values and fructose reactions (the red colouration with resorcinol-hydrochloric acid) were obtained. However, these were shown to be artefacts (methyl-fructosides), as the same spots appeared in chromatograms of fructose treated with methanol containing traces of acetic acid. Sucrose was also isolated from the methanol extract of *Sph. balticum* Russow in a pure state in a yield of 1.0 %.

Chromatograms of the water extract, sprayed with the resorcinol reagent, only showed faint spots with R_F-values lower than that of sucrose and obviously identical with the corresponding spots in the methanol extract, in addition to a very strong spot with $R_F = 0$. This indicates the presence of a fructan and its oligosaccharides, which are probably formed by enzymic transglycosidation, as frequently observed in other plants (compare *e.g.* Bacon and Edelman [3]).

The small amounts of the sugars, which build up the holocellulose, present in the hydrolysates of the methanol and water extracts of the mosses and peat samples may come from fragments of polysaccharides formed during

the peat formation. The relatively large amount of these sugars in the moss extracts may be indicative of initial decomposition in the lower part of these plants. A similar investigation was therefore performed with the tops (0.5 cm) of *Sph. fuscum*. Except for traces of other sugars the hydrolysate of the methanol extract contained only fructose and glucose and the hydrolysate of the water extract only fructose, lending further support to the above assumption.

The presence of fucose (in traces only) in the methanol and water extracts of the mosses and peat samples, and also in the holocellulose fractions, was indicated by means of paper chromatography.

THE CARBOHYDRATES IN THE HOLOCELLULOSE FRACTIONS

The analytical data of the holocellulose fractions are given in Table 4.

Losses. It may be seen from Table 4 that about 30 % of the holocellulose is still unaccounted for by the sugar analysis or as losses in connection with the fractionation. There are three possible reasons for this difference: decomposition of sugars during the hydrolysis (compensated for by applying empirical factors), incomplete hydrolysis, by which sugars (with exception of the uronic acids, estimated directly by decarboxylation) remain undetected in the analytical determination, and the occurrence of non-carbohydrate material.

In order to study these possibilities and to obtain information about the acidic sugars, a larger amount of holocellulose from *Sph. fuscum* was hydrolysed. The following data were obtained (all figures are percentages of the holocellulose).

Non-dissolved (a dark-brown material with fragments of pollen)	2 %
Recovered from the solution by concentration to dryness (calculated as polysaccharides)	76 %
Difference	22 %
Acids, precipitated from the hydrolysate as strontium salts in 90 % ethanol	23 %
Total amount of uronic acids in the hydrolysate (Of the uronic acids about 2.5 % were monomeric and 1 % was not precipitated as strontium salts in 90 % ethanol)	14 %

As the decomposition losses of non-acidic sugars, calculated applying empirical factors, plus the analytically estimated losses of uronic acids were about 12 %, which are only partly volatile, at least 10 % of the difference observed, 22 %, must be due to other causes. It has been demonstrated in other experiments that sparingly soluble strontium salts of acidic sugars, formed during the neutralization of the hydrolysate, are very difficult to wash out from the strontium sulphate precipitate. It is, therefore, very probable that at least about 5 % non-acidic sugars, bound to uronic acids, are lost in this way.

From the difference between the precipitated acidic sugars, 23 %, and the uronic acids in this fraction, 13 %, it may be concluded that about a further 10 % non-acidic sugars, bound as aldobiuronides or higher complexes are present in the hydrolysate.

Table 4. The constituent sugars in the holocellulose calculated as polysaccharides. (All values are given as a percentage of the weight of the total organic material in moss and peat. w.h. = water soluble holocellulose, a.h. = alkali soluble holocellulose and r.h. = residue holocellulose).

Sugar component		*Sphagnum fuscum*	*Sphagnum imbricatum*	A	B	C	D	E
Uronic acids	w.h.) a.h.)	3.3	3.4	3.3	3.8	2.5	3.1	1.8
	r.h.	15.6	14.5	7.8	7.3	3.7	8.7	1.1
Galactose	w.h.	0.3	0.3	0.3	0.3	0.3	0.3	0.2
	a.h.	0.2	0.2	0.2	0.2	0.1	0.2	0.1
	r.h.	5.2	5.3	2.6	3.0	1.1	3.4	0.9
Glucose	w.h.	0.3	0.3	0.7	0.6	1.0	0.5	0.5
	a.h.	0.2	0.2	0.4	0.3	0.4	0.3	0.4
	r.h.	26.8	28.4	21.8	18.9	13.6	22.3	11.4
Mannose	w.h.	0.1	0.1	0.2	0.2	0.2	0.2	0.2
	a.h.	0.1	0.1	0.2	0.2	0.2	0.1	0.1
	r.h.	1.3	1.3	2.2	3.0	1.1	2.4	0.6
Arabinose	w.h.	0.1	0.1	0.1	0.1	+	+	−
	a.h.	0.1	0.1	+	+	+	+	−
	r.h.	−	−	−	−	−	−	−
Xylose	w.h.	0.3	0.3	0.4	0.4	0.3	0.4	0.2
	a.h.	0.2	0.2	0.3	0.4	0.3	0.3	0.2
	r.h.	3.9	2.6	2.2	3.0	0.9	2.9	0.3
Rhamnose	w.h.	0.1	0.1	0.1	0.2	0.1	0.1	+
	a.h.	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	r.h.	+	+	+	+	+	+	+
Polysaccharides		58.2	57.6	42.9	42.0	25.9	45.3	18.1
Total amount of holocellulose		83.7	86.5	64.6	65.7	53.4	69.7	51.1
Losses by the fractionation of the holocellulose and by the extra bleaching of the holocellulose residues of C and E		1.9	2.6	2.5	3.9	10.3	2.5	16.7
Holocellulose unaccounted for. (The figures in the brackets are percentage of total holocellulose		23.6 (28)	26.3 (30)	19.2 (30)	19.8 (30)	17.2 (32)	21.9 (31)	16.3 (32)

The non-acidic part of the hydrolysate could be calculated at 52 % and the non-acidic sugars found by analysis amounted to 44 %. The difference between these values, 8 %, represents the upper limit of incompletely hydrolysed non-acidic sugars. The presence of oligosaccharides in the non-acidic part of the hydrolysate was demonstrated by paper chromatography, but the value 8 % is certainly too high, as it also includes non-carbohydrate material.

To sum up, the main losses are due to incomplete hydrolysis of acidic sugar complexes and as the percentages of uronic acids decrease to a greater extent with the huminosity than the total amount of holocellulose, the losses of non-

acidic sugars should also decrease with the huminosity. The fact that the amount of holocellulose unaccounted for is fairly constant may therefore be due to an increase of non-carbohydrate material in the holocellulose.

The analytical determination of the carbohydrate constituents is thus subject to several systematic errors, which probably cannot be reduced with the methods available. The data given in Table 4, for the different samples, however, are obtained under strictly identical experimental conditions, and for a comparison between the samples they are certainly significant.

The acidic sugars. The polymeric acidic sugars in the above mentioned hydrolysate from *Sphagnum fuscum*, isolated from a carbon-Celite column, were further hydrolysed, and qualitative examination of the hydrolysis products by paper chromatography showed uronic acids, galactose, xylose, rhamnose and material with lower R_F-values and sugar reactions. The predominating role of rhamnose is of interest.

In the main part of the neutralized hydrolysate, the acidic components were separated from the neutral by ion exchange and investigated by paper chromatography. About a dozen spots plus some trailing were observed. The spots could be divided into two groups, one with uronic acid reactions (bright red with anisidine reagent) and the other with aldose reactions (yellow, orange or brown).

In the uronic acid group, galacturonic acid was one of the predominating constituents and was isolated in a pure state. The other chief constituent gave a spot somewhat faster than glucuronic acid in ethyl acetate-acetic acid-water (Solvent A), and in butanol-ethanol-water (Solvent B) it gave a spot a little faster than glucose. The substance was isolated by chromatography on thick paper in an impure state and contained 11 % methoxyl, and hence is probably a monomethyl uronic acid. Besides these chief components there were spots with lower R_F-values in which digalacturonic acid seemed to predominate. This and the higher polymers are probably formed from a pectin like polysaccharide built up from galacturonic acid units. There were also two minor spots with higher R_F-values, the first with the same R_F-values as the lactone of glucuronic acid and the second with about the same R_F-values as xylose in the two solvents.

From the other group two crude fractions, which may be aldobiuronic acids, were isolated by chromatography on thick paper. The faster showed the anisidine reaction typical for pentoses (orange) and the slower that of hexoses (brown). After further hydrolysis, paper chromatograms of these hydrolysates showed the presence of xylose and galactose, together with the above mentioned unknown methyl-uronic acid and the lactone with the same R_F-value as glucuronic acid.

A paper chromatographic investigation of the acidic sugars of the peat sample A also showed the above-mentioned components.

In order to demonstrate that the acidic polysaccharides were not of microbial origin (*e.g.* as the polysaccharides in soil studied by Forsyth and Webley [4]) formed by an initial decomposition, the upper parts (0.5 cm) of *Sph. fuscum* were studied. The acidic components in a hydrolysate of the holocellulose showed the same picture as the main moss sample (also the amount of holocellulose and the total amount of uronic acids were equal).

The quantity of uronic acids decreases at a faster rate than the holocellulose with the huminosity. The spot with the R_F-value of glucuronolactone was observed in the hydrolysates of all peat samples. The amount of the uronic acids extracted from the holocellulose by water and alkali is fairly constant for all the samples. The same thing is found with galactose, xylose and rhamnose, the sugars which are linked to uronic acids as mentioned above. It may be mentioned that treatment of the holocellulose with pectinase (Pectinol 10) was practically without effect. By treatment with ammonium oxalate solution (0.5 % at 60° C) or dilute hydrochloric acid (0.5 *N* at room temperature) only small amounts of uronic acid material was extracted and it had no effect on the subsequent extractions with water and alkali.

The neutral sugar constituents. Galactose decreases with the huminosity at about the same rate as the uronic acids.

Glucose, the predominating constituent, is probably present to a greater extent as cellulose, as demonstrated in earlier investigations [5,6]. The decrease of glucose with the huminosity follows that of the holocellulose. The amount of glucose in the water and alkalisoluble holocellulose is small and increases with the huminosity.

Mannose shows a maximum in peat of low huminosity. On the other side the amount of mannose in the methanol and water extracts (Table 3) is greater in the mosses than in the peat.

The amount of xylose shows only a small decrease from mosses to peat of low huminosity but a rapid decrease and a great accumulation in the holocellulose extracts with higher huminosity.

Arabinose is a minor component, which is present in an easily extracted form, and shows a rapid decrease with the huminosity. By the above mentioned comparison between holocellulose hydrolysates from the main sample of *Sphagnum fuscum* and the tops of this moss, the amount of arabinose, which was greater in the tops, was the only significant difference.

Rhamnose seems to be bound to uronic acids in a very difficultly hydrolysable complex and no great decrease of it can be seen with increasing huminosity.

General conclusions. Two general conclusions may be drawn from Table 4. Firstly, no significant difference is noticed between the two mosses and between the samples A and B, formed from *Sph. imbricatum* Russ. and *Sph. tenellum* Pers., respectively. The difficulties in estimating the physical huminosity must be emphasized, and a comparison between the physical huminosity and the chemical composition consequently cannot be stressed too strongly. Secondly, the age of the peat has practically no effect on the composition of the carbohydrates, which seems to depend chiefly upon the huminosity. This fact shows that the chief changes, due to chemical and microbiological processes, take place at the surface of the bog and obviously have already begun in the lower parts of the mosses.

A diagram, describing the changes in composition of moss, peat of low huminosity (A, B and D) and peat of higher huminosity (C and E) is given in Fig. 1.

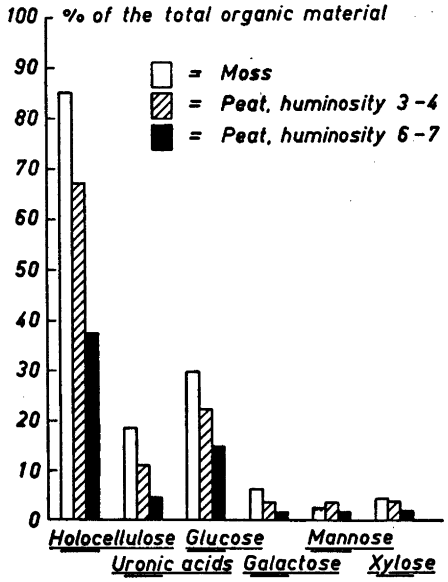

Fig. 1. The holocellulose and the total amount of sugars found, calculated as polysaccharides in Sphagnaœ, Sphagnum peat of huminosity 3—4 and Sphagnum peat of huminosity 6—7.

EXPERIMENTAL

Evaporations were carried out under reduced pressure at 30—40° and all neutralizations were made at room temperature. The melting points are uncorrected.

Fractionation. The samples were dried at room temperature in thin layers, the moss samples after washing with water. Traces of other plants were removed. The dried samples (about 20 g) were continuously extracted with benzene for 2 days and with methanol for 5 × 1 days. The dried residue was then treated with water at 60—65° (25 ml/1 g) with stirring, 3 × 3 h, filtered and washed. For *Sph. imbricatum* the ratio of dissolved material in the 3 steps was 75 : 24 : 1. The extracted samples were bleached according to the method of Wise *et al.*[7] with sodium chlorite and acetic acid. The temperature, however, was only 60—65°. Generally 2 treatments of one hour each were sufficient, but for some samples 4 steps were needed.

Part of the holocellulose (about 1 g) was carefully dried and swollen in liquid ammonia. This treatment followed with some modifications according to the small scale, the procedure of Bishop and Adams [8]. The swollen ammonia-free holocellulose was successively treated with water of 40—45° C, 3 × 3 h, and 5 % potassium hydroxide (in nitrogen atmosphere) at room temperature, 2 × 4 h., followed by washing with water. The solid-solvent ratio in these treatments was about 1:20. The holocellulose residue was carefully washed with water, aqueous acetic acid and acetone before drying. The aqueous extract was directly concentrated to dryness. The alkaline extract was neutralized with hydrochloric acid to pH 6, concentrated, adjusted to pH 4, and ethanol added to a concentration of 75 %. The precipitate was collected by centrifugation and purified by reprecipitation. Practically no carbohydrates were found in the mother liquors.

Hydrolysis. All fractions were hydrolysed with 1 *N* sulphuric acid at 100 ° (oil bath). The holocellulose residue was first swollen in 72 % sulphuric acid at room temperature for 2 hours according to standard methods. The optimal time of hydrolysis, in order to obtain as high a yield of monosaccharides as possible, was determined with the aid of semiquantitative paper chromatography to be 2 hours for the methanol and water extracts and 16 hours for the holocellulose fractions.

The sample, about 20 mg, was hydrolysed with the acid, 2 ml for the methanol and water extracts and the water and alkali soluble parts of the holocellulose, 4 ml for the

holocellulose residue, in centrifuge tubes provided with cold fingers. After hydrolysis the cooled solution was neutralized with barium carbonate, centrifuged and washed three times with water. The combined solutions were adjusted to 5 and 10 ml, respectively.

To ascertain the amounts of sugars destroyed during the hydrolysis, model experiments with pure sugars were performed. The sugars were dissolved in 1 *N* sulphuric acid and heated to 100° for 2 and 16 hours. The amount of sugar in the solutions was then determined by measuring the optical rotation. For the galacturonic acid the amount was estimated by quantitative paper chromatography as below (no traces of arabinose could be observed). The following figures for the losses were found:

Fructose (2 hours)	27 %
Galactose, Glucose, Mannose (16 hours)	6
Xylose, Arabinose, Rhamnose (16 hours)	11
Galacturonic acid (16 hours)	48

The analytical data obtained were corrected for the figures above, except for the uronic acids, which were determined by a separate analysis on unhydrolysed material. These corrections, of course, involve an approximation, as the sugars do not occur as monomers during the whole treatment time with the hydrolysing acid. There might also be some decomposition during the treatment with 72 % sulphuric acid.

Analysis of monomers. The uronic acid content of the holocellulose and holocellulose residue was determined by the semimicro decarboxylation method of Johansson *et al.*[9]

The non-acidic sugars in the hydrolysates were determined by quantitative paper chromatography, according to Hirst and Jones [10]. On a Whatman No 1 paper (50 × 26 cm) a known volume of the solution to be analysed was applied on a line with a micro pipette (Agla) and concentrated if necessary on the paper (the amount of each sugar generally was 0.080—0.300 mg). On each side of this line a reference spot of the same concentration was applied. For the separation of the components, the nature of which had been determined by ordinary paper chromatography different solvent systems had to be used. Generally, multiple development (2 × 18 h at 25°) with butanol-pyridine-water (3 : 1 : 1.5) in which solvent the uronic acids have low R_F-values and do not interfere with the non-acidic sugars, was satisfactory. Mannose and arabinose, however, do not separate well in this solvent, and for solutions with higher amounts of both these sugars, they were determined in a separate analysis, using the solvent system ethyl acetate-acetic acid-water (3 : 1 : 1) (18 h at 25° C). After development the sugars were located on the reference strips with suitable spraying reagents. The strips with the different components plus blank strips were cut out and extracted with water (24 h at 25°) in an ordinary chromatography jar into large test tubes. The eluate was then adjusted to about 5 ml, oxidized and titrated. The recovery by the extraction of the strips was quantitative, and the empirical factors for formic acid formation by the oxidation about the same as those given by Hirst and Jones.

For synthetical mixtures of galactose, glucose and fructose or galactose, glucose, mannose, xylose, arabinose and rhamnose (0.080—0.300 mg of each), which were "hydrolysed", neutralized and analysed just as with the moss and peat fractions, and corrected for decomposition during the "hydrolysis", good separations and recoveries of 92 ± 5 % were observed. The losses, 8 %, are probably mechanical and to a greater extent caused by incomplete washing following the neutralization; the empirical factor 0.92 has been used in all the determinations.

The amount of minor constituents was estimated by visual comparison of the intensity of a spot with those of standard solutions of different concentrations. In addition to the solvent mixtures mentioned above, *n*-butanol-ethanol-water (5 : 1 : 4), alone or combined with the others by successive developments, was used for the qualitative investigations, especially of the acid sugars.

The following spray reagents were used: Silver nitrate-sodium ethoxide, aniline hydrogen phthalate, *p*-anisidine phosphate and resorcinol-hydrochloric acid.

Isolation of sucrose. When the water-soluble part of the methanol extract from *Sphagnum balticum* was fractionated on a hydrocellulose column, sucrose $[\alpha]_D^{20}$ + 64.2 (water, c = 2.2), m.p. 183—184° undepressed on admixture with an authentic sample was isolated in a yield corresponding to 1.0 % of the organic part of the moss.

Investigation of the uronic acids and acid sugars. A sample of *Sphagum fuscum* (30 g) was treated with 1 *N* sulphuric acid at 100° for 16 hours after treatment with 72 % sulphuric acid. The cooled hydrolysate was neutralized with strontium carbonate, the precipitate filtered off, thorougly washed with hot water and the combined solutions after adjustment to pH 5—6 were concentrated to a smaller volume. To part of the filtrate, ethanol was added to a concentration of 90 %, the precipitate centrifuged and washed three times with 90 % ethanol. The strontium salts were dissolved in water and converted to the acids by ion exchange (Amberlite IR 120). After concentration to dryness they were dissolved in 1 % ethanol and added to the top of a carbon-Celite column. Monomers were eluted with 1 % ethanol and compounds of higher molecular weight with 25 % ethanol. The latter eluate after concentration to dryness was hydrolysed with 1 *N* sulphuric acid at 100° for 16 hours, and the hydrolysate studied by paper chromatography.

The main part of the filtrate was treated with the ion exchange resin IR—120, and the total amount of material in the hydrolysate was determined by concentrating part of the solution to dryness. The rest of the hydrolysate after concentration, was neutralized with sodium hydroxide and added to the top of a column of the ion exchange resin Dowex 2 in the acetate form.

The column was eluted with 1 *N* acetic acid, and the eluate, divided into fractions, was investigated by paper chromatography using the solvents discussed above. Only a partial separation was obtained. In this way pure galacturonic acid was isolated by separation on thick paper (Whatman 3 MM) and characterized as its strontium salt $[\alpha]_D^{20}$ + 26.0, authentic: $[\alpha]_D^{20}$ + 29.1 (water $c = 1.3$), as the free acid, $[\alpha]_D^{20}$ + 49.0, authentic: $[\alpha]_D^{20}$ + 51.3 (water $c = 2$) and further by bromine oxidation to mucic acid m.p. 214° (decomp.). The uronic acid also gave the characteristic brick-red precipitate with basic lead acetate [11].

With the aid of thick paper another uronic acid, with higher R_F-values than galacturonic acid in various solvents and a methoxyl value of 11 %, was isolated in an impure state. Two impure aldobiuronic acids were also isolated by the same method. A further investigation of these and other acidic substances, demonstrated by paper chromatography, however, is outside the scope of the present investigation.

SUMMARY

A qualitative and quantitative investigation of the carbohydrates in two *Sphagnum* mosses and five samples of *Sphagnum* peat of different age and degree of huminosity has been performed.

The two mosses investigated showed no significant differences. Samples of very different age but with the same degree of physical huminosity were very similar, indicating that the chief changes occur at the top of the bog and/or are determined by the conditions at the start of the humification. The total amount of carbohydrates was about 90 % of the organic material in the mosses and about 65 % and 35 % in peats with a degree of huminosity of 3—4 and 6—7, respectively.

Of the constituent sugars, fructose which occurred in the mosses, was completely absent in the peat. Another sugar, which occurs in nature as a furanoside, arabinose, disappeared almost completely during the humification.

The uronic acids and galactose decreased faster, while xylose and glucose decreased at about the same rate as the total carbohydrates. Mannose and probably also rhamnose are the most stable components and accumulate during the humification.

The polysaccharides in mosses and peat seem to constitute a very complex mixture. The presence of a fructan in the living moss, of a polyuronide (pectin) and a large amount of more complex polysaccharides built up of galactose,

xylose, rhamnose and uronic acids is indicated. The glucose, the most important constituent, probably occurs chiefly as cellulose, the presence of which has been demonstrated by other workers. Finally the behaviour of mannose during the humification indicates the presence of a stable mannan. There is no evidence of polysaccharides formed by microorganisms in the peat.

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