

Carbohydrates in Cold Water Extracts of a Pine Forest Soil

ARNE GROV

Chemical Institute, University of Bergen, Bergen, Norway

Water-soluble carbohydrates in a pine forest soil profile from Western Norway have been studied, by a partition chromatography technique with circular filter papers as the stationary phase. The soil samples were first extracted with ether followed by cold water, and the water extracts were concentrated *in vacuo*. Water-soluble polysaccharides were precipitated with ethanol, separated from the mother liquid by filtering, and hydrolyzed. Most of the sugars observed were quantitatively determined, and their amounts and distribution in the profile layers discussed.

With regard to the chemical composition of organic components of soil (humus) there is still considerable obscurity. The humus is formed mostly from plant sediments and its composition is dependent on the vegetation and the amount of micro-organisms in the soil. Therefore the organic matter in soil varies with climate, vegetation and districts.

Earlier studies of organic matter in soil have been limited chiefly to the estimation of the total amount of different groups of compounds, for example carbohydrates, mostly because of unsatisfactory methods of separation. The development of modern chromatographic procedures has revolutionized the carbohydrate analysis. Detection and quantitative estimation of different water-soluble saccharides can be realized only by use of chromatographic procedures.^{1,2}

The differing composition of water-soluble organic matter in various profile layers may be due to leaching processes and different activity of micro-organisms. In the present work the water-soluble components (mono- and polysaccharides) and their amounts and distribution in the profile have been studied.

Investigation of free sugars in water extracts of soil has shown that they exist there in small amounts.³⁻⁵ Humus from uncultivated areas in Western Norway has been previously investigated by Alvsaker⁶ by use of a modified Waksman procedure. Later a paper chromatographic study of carbohydrates in a cold water extract was reported by Alvsaker and Michelsen.⁷ The extracted soil was the combined F-layers in a pine forest soil profile.

EXPERIMENTAL

Soil samples. The soil samples were taken from the same profile as used in the investigations of Alvsaker and Michelsen⁷ (Sandnesset, Söfteland, south of Bergen). A complete description of the profile is given by Alvsaker.⁸ Since, in that profile, no distinct borderline exists neither between the two F-layers nor the two A-layers, only three different layers of the profile were used: Combined F-layers (3 cm thick), H-layer (3 cm) and the combined A-layers (4–5 cm).

Elementary analysis. Preparation of soil samples from H-layer and combined A-layers and determinations of moisture and ash were carried out according to Alvsaker's specifications.⁸ Determinations of total carbon and total hydrogen were performed as described by Reihlen and Weinbrenner.⁸ At the same time ash determinations were also carried out. The results are shown in Table 1.

pH-Measurements. The pH values of the two profile layers were determined according to Alvsaker⁸ (Table 1).

Extractions. 400 g samples of air-dry soil from the H-layer and 900 g samples from the combined A-layers were first extracted with ether and then with water; both extractions were carried out at room temperature (20°C). During the extractions the suspended soils were occasionally stirred in the percolator.

The water extracts were filtered through membrane filters and concentrated *in vacuo* below 40°C. The extracted polysaccharides were precipitated with ethanol (500 ml ethanol per 150 ml of concentrated extract) and filtered on cella filter. The filtrates were further concentrated and then subjected to chromatographic analysis. De-ionization of the concentrates was found to be unnecessary.

The precipitates collected on cella filter (polysaccharide complexes) were dissolved in water. The solution was made 1 M with respect to H₂SO₄ and hydrolyzed for 7 h on a boiling water bath. The hydrolyzates were neutralized with Ba(OH)₂, centrifuged, and the precipitates washed with water until negative carbohydrate reaction (Molisch¹⁰). The solutions were then concentrated *in vacuo*, after which they were subjected to chromatographic analysis.

Pipettes. Micro pipettes for spotting the solutions to the paper were prepared and calibrated as described by Anderson.¹¹

Cabinets. The apparatus (cabinets) used for running the chromatograms were as described by Juvvik and Michelsen.¹²

Paper. The circular filter papers (26, 28, and 30 cm in diameter) were made from Whatman No. 1 and used as such without any further treatment.

Solvents. The following solvent mixtures were investigated with respect to separation of sugars: (a) Ethyl acetate-pyridine-water (40:11:6),¹³ (b) Butanol-acetic acid-water (4:1:5, upper layer),¹⁴ (c) Butanol-pyridine-water (6:4:3),¹⁵ (d) Ethyl acetate-acetic acid-water (3:1:3, upper layer),¹⁶ (e) Ethyl acetate-pyridine-water-acetic acid (5:5:3:1),¹⁷ and (f) Water-saturated phenol.¹⁴ The solvents were distilled before use.

Table 1. Elementary analysis of soils from the H-layer and A-layers.

	H-layer					A-layers				
	Mois- ture %	Ash % of dry (105°C) soil	C % of humus	H % of humus	pH	Mois- ture %	Ash % of dry (105°C) soil	C % of humus	H % of humus	pH
Original soil	7.40	52.37	55.38	5.94	3.62	1.84	88.70	56.59	5.79	3.90
Ether- and water-extr. soil	4.20	52.45	54.95	5.94		1.70	88.80	56.46	5.77	

Reagents. The following reagents were used for the detection and identification of sugars which were separated on the chromatograms: (g) Aniline-diphenylamine phosphate,¹⁸ (h) Triphenyltetrazolium halogenide,¹⁹ (i) Aniline hydrogenphthalate,²⁰ (j) α -Naphthylamine-phosphoric acid,¹⁵ (k) *p*-Anisidine-phosphoric acid,²¹ (l) Periodate oxidation followed by nitro prusseide-piperazine,²² (m) Ninhydrin,²³ and (n) Acetylaceton-*p*-dimethylamino benzaldehyde.¹⁴

The circular paper chromatographic procedures applied, including multi-sector and multiple development techniques, were essentially the same as those described by Giri and Nigam.¹⁵

The sugar bands became completely separated on the chromatograms, and were identified by colour reactions together with comparison of the movements of authentic specimen of sugars run on the same chromatogram.

Natural keto-hexoses became completely separated in the mixture (f). Only fructose was detected in the H-layer, and the ketose-reaction was negative in the A-layer. The solvent mixture (a) gave a clear-cut separation of galactose, glucose, mannose, arabinose, xylose, rhamnose and ribose. The absence of fucose was shown using mixture (d).

One of the bands which occurred on the chromatograms was assumed to be an uronic acid, and the distances moved in different solvent mixtures were identical with those of authentic galacturonic acid. Unfortunately, only solvent mixture (e) was able to separate the galacturonic and glucuronic acids.

The quantitative determinations of the sugars identified, with triphenyltetrazolium bromide, were carried out according to Giri and Nigam's specifications.¹⁵ This method was found more advantageous than other similar procedures.^{24,19} The measurements were performed with a Zeiss Pulfrich photometer (wavelength 4900 Å). The results are listed in Table 2. The values listed represent, in most cases, the average of four or more estimations.

DISCUSSION

It is seen from Table 1 that although great differences in total amount of organic matter is observed in the various profile layers, the chemical composition of humus is relatively constant. The results from Alvsaker's⁶ investigation of a profile from the same locality show great differences with respect to the amount of humus content, but the elementary composition is nearly the same. However, the most surprising observation is the great differences in the amounts of water-soluble saccharides in the corresponding profile layers.

Table 2. Quantitative determination of sugars. All values are expressed as percentage of dry organic matter (humus) $\times 10^2$. Hydrolyzed polysaccharides are calculated as anhydrous sugars.

	GaUa	Ga	Gl	Ma	Fr	Ar	X	Ri	Rh	Total
Water-soluble monosaccharides <i>H</i> -layer	+	trace	18.90	—	1.05	2.72	0.42	0.84	trace	23.93
Hydrolyzed polysaccharides	—	3.59	3.97	3.21	—	1.32	1.51	trace	1.70	15.30
Water-soluble monosaccharides <i>A</i> -layers	—	trace	18.15	trace	—	3.70	trace	trace	—	21.85
Hydrolyzed polysaccharides	—	3.66	4.27	3.05	—	2.03	2.24	—	trace	15.25

Alvsaker found 0.94 % of humus in the H-layer, 1.03 % in A₁ and 0.92 % in A₂. In the present investigation were found 0.39 % in the H-layer and 0.37 % in the combined A-layers. Alvsaker's analyses were performed according to Hagedorn-Jensen (ferricyanide method) and although different profiles with different ages, a great part of the divergence may be due to the difference of the two procedures. The ferricyanide ion is reduced by other reducing components than sugars in soil; proteins and amino acids effect reduction and consequently the method gives too high values when applied to mixtures containing proteins. All water extracts which were investigated gave positive reaction for amino acids, but the error mentioned in the ferricyanide method did not influence the chromatographic method. In any case, the ferricyanide method gives maximum values, whereas the values obtained by chromatographic procedures may be regarded as minimum values.

Fig. 1 illustrates the amounts of the individual sugars in the three different profile layers, expressed as percentage of dry organic matter (humus). The F-layer values are those of Alvsaker and Michelsen.⁷ It is evident that the uppermost layer (F-layer) contains the highest amount of monosaccharides and that the amount decreases downwards in the profile. The decrease from F- to H-layer is approximately one third (0.355 % to 0.24 %). However, from H- to A-layer the decrease is relatively small (0.24 % to 0.22 %). The individual variation of the monosaccharide content from F- to H-layer is approximately similar for the hexoses (galactose, glucose and fructose) and the pentose xylose (0.025 % to 0.03 % absolute decrease). The content of arabinose decreases relatively little, whereas that of ribose increases. The degree of decrease of the individual sugars from H- to A-layer is also rather regular. Arabinose and ribose are exceptions; while the first has increased, the latter has decreased

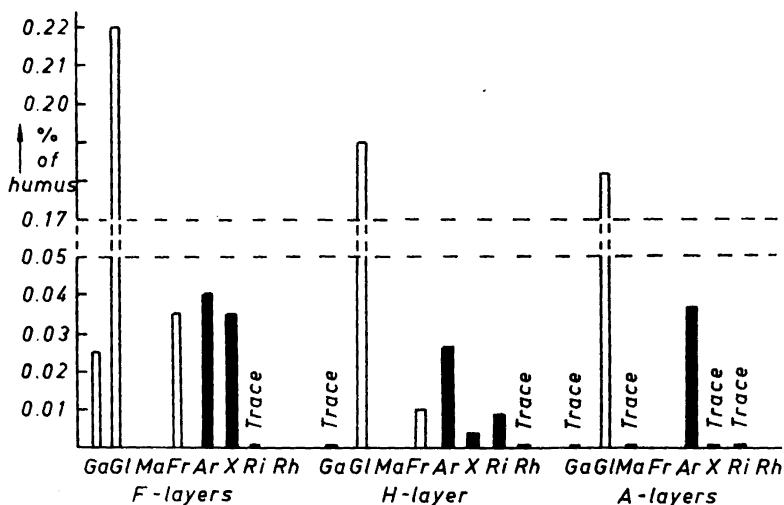


Fig. 1. The amount of individual mono-saccharides in the three profile layers expressed as percentage of dry organic matter (humus).

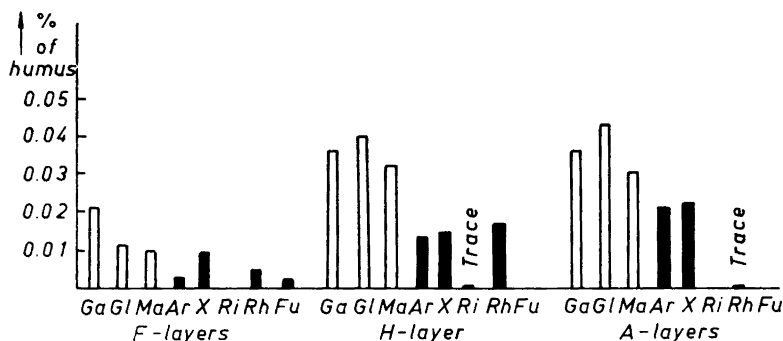


Fig. 2. Polysaccharide complexes in the three profile layers. The amount of individual anhydrous sugars expressed as percentage of dry organic matter (humus).

to trace amounts. It is interesting that the ratios between hexoses and pentoses (in equivalents) in all layers are approximately four to one.

From Fig. 2 which shows the constituents and the amounts of the individual anhydrous sugars in the water-soluble polysaccharide complexes of the three profile layers expressed as percentage of humus, we recognize that the total amount has increased considerable from F- to H-layer (0.058 % to 0.153 %), but is approximately the same in H- and A-layer (0.153 % and 0.1525 %). The individual increase is most pronounced for glucose. Fucose which is found in F-layer, could be detected neither in H- nor in A-layer. Rhamnose decreased to trace amounts from H- to A-layer. Ribose was absent in F- and A-layer and only trace amount could be detected in the H-layer.

None of the hydrolyzed polysaccharide complexes contained fructose. It may be assumed that due to the acid character of the soil the fructosans are most probably hydrolyzed already in the uppermost layer. The ratios between hexosans and pentosans in all of the three profile layers are approximately two to one.

No general conclusion with regard to influences on the composition and distribution of the constituents of organic matter in a soil profile can be based on the present results. Evidently, the microbial activity is greatest in the uppermost layer where the effects are assumed to be the decomposition of complex carbohydrates to more soluble ones. It may be assumed that the activity and distribution of different micro-organisms vary in the profile.

In districts with wet climate, as in Western Norway, it is reasonable to expect that the water-soluble part of humus, partly in colloidal solution, migrates downwards in the profile and that these water-soluble carbohydrates during migration are further decomposed or react to new compounds with greater molecular weight. A possible equilibrium in a profile will be displaced according to seasonal variations of sedimentation, temperature and precipitation.

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