

Share of Carbohydrates in the Humus Substances Composition

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Received April 6, 1966

Abstract. The effect of different concentrations of hydrochloric acid on the qualitative and quantitative content of carbohydrates in humus fraction hydrolyzates was investigated. Three humic acid preparations were subjected to the usual procedure for obtaining fractions of humic and humatomelanic acids. The carbohydrate content of the hydrolyzates was determined after their chromatographic separation on Whatman 1, using the Partridge system and detection with acid anilin phthalate. The results showed that the qualitative carbohydrate content in the hydrolyzates of most humus fractions of samples used was identical. With increasing acid concentrations the amount of hydrolyzable carbohydrates usually increased, the total amount of carbohydrate in the hydrolyzate not exceeding 4%. The difference in the carbohydrate content as between hydrolysis with 0.9N and 5.3N HCl was sometimes fourfold. A higher content of hexoses than of pentoses was generally found.

The problem of composition as well as formation of humus substances has been frequently approached in the past and even at present receives considerable attention. Among the variety of compounds found in samples of humic acids, one should also mention the carbohydrates. The question of the presence of carbohydrates in humus substances is still not quite clear. The carbohydrate content found is generally low, most authors considering it as an admixture (KONOVÁ 1963).

The results of our earlier work (HAVRÁNKOVÁ, TICHÝ 1966) where we examined the dynamic changes in the carbohydrate fraction of humus fractions, as they are formed by gradual humification of spruce wood by the fungus *Fomes marginatus*, point to the important role of carbohydrates in the humification process. It is the aim of the present communication to consider in detail the content of carbohydrates in the high-molecular fractions of humus substances.

Material and Methods

Three samples of humus substances were chosen for this work:
Sample I: humus acid (produced by the Institute for Scientific Research of Coal, using northern Bohemian humolite).

Address: Ostrava I, Tyršova 9, Czechoslovakia

Sample II: Humussäure Riedel-de-Haen, Batch Nos. 58217 11047 27651.
 Sample III: Humussäure Riedel-de-Haen, Batch Nos. 11657 11048 27651
 (the starting material for these preparations is unknown).

For estimation we used 1 g of thoroughly ground sample. After dissolving in 150 ml 0.1N-NaOH, the humic and hymatomelanic acids were precipitated by an addition of 0.5N-H₂SO₄ (pH 3). After filtration the components were separated on the basis of their different solubility in 96% ethanol. The fractions were converted to sodium salts and dialyzed against distilled water until the disappearance of alkaline reaction.

Five ml of the fraction solution were mixed with 1, 2 or 6 ml of 36% HCl and distilled water added to make the volume 11 ml. In this way, a series of solutions of rising HCl concentrations was obtained (0.9N, 1.8N, 5.3N). Hydrolysis took place in a sealed glass ampoule in a boiling-water bath for 4 h. After filtration the hydrolyzates were dried in vacuo over KOH. Sugars were extracted from the residue with anhydrous pyridine (MALPRESS, MORRISON 1949). After removing pyridine, the sugars were dissolved in 1 ml of 10% isopropanol. The carbohydrate content in the hydrolyzates was followed by paper chromatography; they were separated on Whatman

Table 1

Sugar content of hydrolyzates of humus fractions

Sample	Fraction	HCl N	Sugar content of fraction in % of dry weight					% con- tent of pentose	% con- tent of hexose	Total carbo- hydrate in %
			XY	AR	MA	GL	GA			
I	HU	0.9	0.10	0.01	0.05	0.09	0.02	0.11	0.16	0.27
		1.8	0.13	0.01	0.06	0.12	0.03	0.14	0.21	0.35
		5.3	0.22	0.08	0.23	0.24	0.08	0.30	0.55	0.85
	HY	0.9	0.18	0.03	0.18	0.13	0.03	0.21	0.34	0.55
		1.8	0.27	0.06	0.08	0.18	0.08	0.33	0.34	0.67
		5.3	0.67	0.20	0.40	0.67	0.13	0.87	1.20	2.07
II	HU	0.9	0.94	0.12	0.31	0.94	0.12	1.06	1.37	2.43
		1.8	0.88	0.11	0.28	1.15	0.11	0.99	1.54	2.53
		5.3	1.40	0.31	1.09	1.16	0.12	1.71	2.37	4.08
	HY	0.9	0.06	0.02	0.10	0.29	0.01	0.08	0.40	0.48
		1.8	0.20	0.04	0.23	0.31	0.10	0.24	0.64	0.88
		5.3	0.51	0.10	0.64	0.70	0.26	0.61	1.60	2.21
III	HU	0.9	0.38	0.06	0.06	0.14	—	0.44	0.20	0.64
		1.8	0.55	0.05	0.10	0.28	—	0.60	0.38	0.98
		5.3	0.92	0.18	0.36	0.55	0.09	1.10	1.00	2.10
	HY	0.9	0.09	0.02	0.07	0.09	0.06	0.11	0.22	0.33
		1.8	0.12	0.03	0.09	0.11	0.08	0.15	0.28	0.43
		5.3	0.30	0.09	0.17	0.23	0.10	0.39	0.50	0.89

Abbreviations: HU humic acids, HY hymatomelanic acids, XY xylose, GL glucose, AR arabinose, GA galactose, MA mannose.

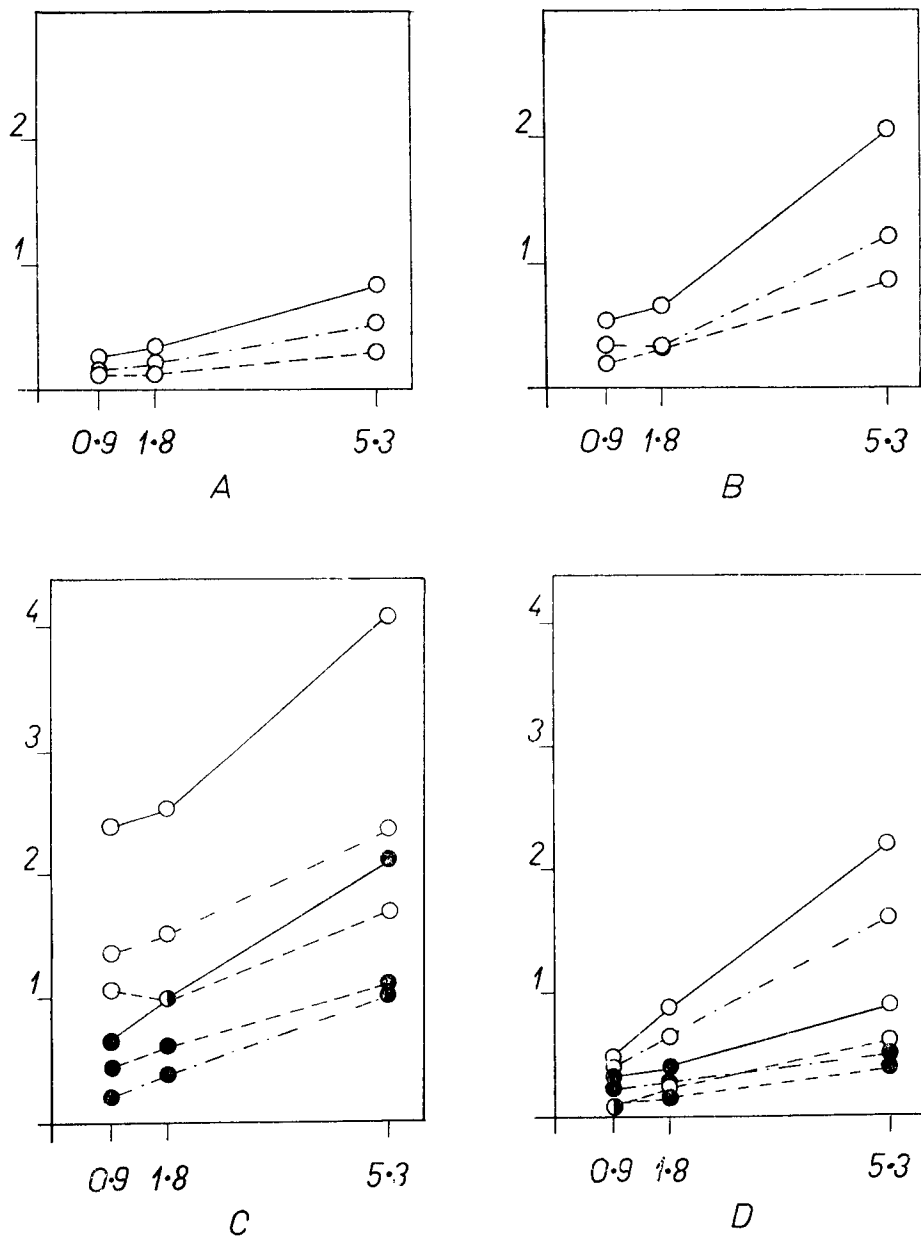
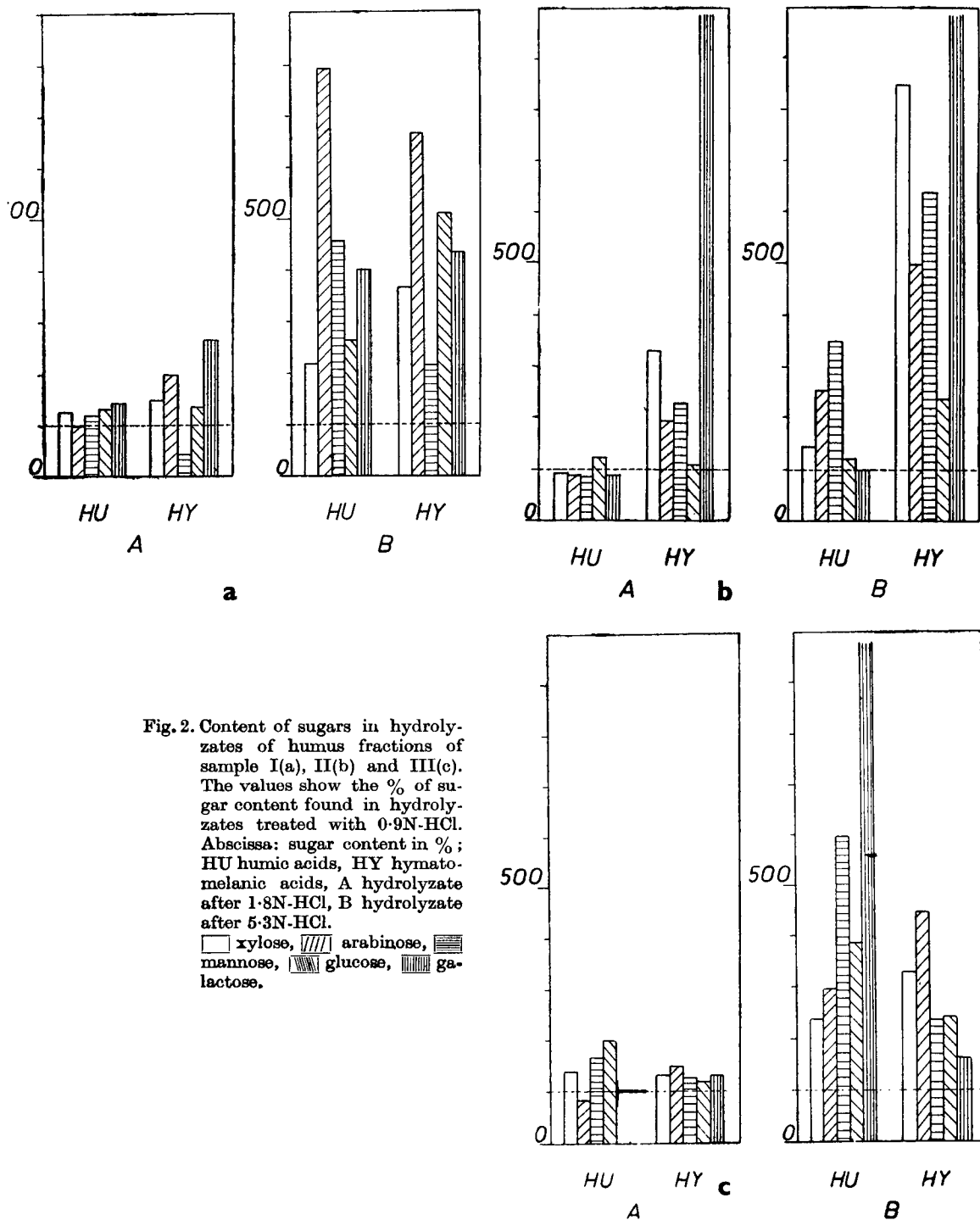


Fig. 1. Changes in the content of pentoses, hexoses and total carbohydrate in hydrolyzates of humus fractions in dependence on the concentration of the acid used. — A: fraction of humic acids, sample I; B: fraction of humatmelanic acids, sample I; C: fraction of humic acids, samples II and III; D: fraction of humatmelanic acids, samples II and III. Abscissa: HCl (N); ordinate: sugar content in % of fraction dry weight. ○ sample I, II ● sample III, — — — pentoses, — . — . hexoses, ————— total carbohydrate.



No. 1 paper in n-butanol-glacial acetic acid-water (4 : 1 : 5), developing four times in the descending direction. Carbohydrates were detected with acid anilin phthalate: during the fourth run 2% phthalic acid was added to the mobile phase, the dry chromatogram was passed through 2% aniline in ether and heated in an oven for 3–4 min at 105° C. The quantitative sugar content was assessed by visual comparison of the colour intensity and the size of the spot with a standard scale of a mixture of known sugars (HAIS, MACEK 1959). The error of the method used was 12–15%.

Results

The sugar content of hydrolyzates of humus fractions is shown in Table 1. The qualitative monosaccharide content was almost the same in all fractions. The hydrolyzates were found to contain xylose, arabinose, mannose, glucose and galactose. In addition to the identified sugars, the chromatograms contained 1–3 spots with a low R_F which could not be identified. With the detecting agent they gave a red-brown colour. The substantial fraction of pentoses was represented by xylose, that of hexoses by glucose.

Fig. 1 shows the content of pentoses, hexoses and the total carbohydrate in hydrolyzates of fractions of Samples I–III in dependence on the extent of hydrolysis. The sugar content is expressed as a percent of fraction dry weight. The carbohydrate content in hydrolyzates of fractions of Sample II was higher than in the corresponding fractions of Sample III, even though the preparations had the same commercial designation and were produced by the same firm. Both samples were characterized by a higher content of hydrolyzable carbohydrates in humic acid fractions. On the other hand, Sample I contained more sugars in the fraction of hymatomelanic acids.

A number of fractions contained more hexoses than pentoses. The ratio remains identical even after more potent hydrolysis, with the exception of humic acid fractions of Sample III. The same ratio of hexoses and pentoses was found in our previous paper (HAVRÁNKOVÁ, TICHÝ 1966) in the hydrolyzates of most high-molecular fractions of humus substances obtained from spruce wood at various stages of decomposition. The material was decomposed by the fungus *Fomes marginatus* under laboratory conditions.

The rise in sugar content of hydrolyzates with increasing HCl concentrations is best seen in Fig. 2. The sugar content in the sample with the lowest amount of added HCl (1 ml) was taken as a basis for the per cent calculation of the sugar content in each fraction. The content of the individual sugars in fractions did not rise parallelly with the increase in the concentration of the acid used for hydrolysis. In some hydrolyzates of Samples II and III the content of galactose increased substantially when using this type of calculation.

Discussion

The content of sugars in hydrolyzates of high-molecular humus substances found in the present paper was low: it attained at most 4% (fraction of humic acids, Sample II). In most cases it varied between 0.3- and 2.5%, depending on the extent of hydrolysis. If one considers the possibility of decomposition of split sugars in the presence of the acid, on the one hand,

and the relatively high stability of humus substances toward the effect of acid, on the other, it is disputable whether the conditions used were sufficient for releasing most of the sugars intact and it is not clear what fraction of sugars may have been destroyed during hydrolysis. This circumstance is to be pointed out in connection with the problem of non-uniform increase in the sugar content of hydrolyzates with the acid concentration increase.

The work of some authors indicates that sugars represent a component, the presence of which in the hydrolyzates of humus substances of different origin is characteristic for the sample. We should cite here the works by FORSYTH (1947) and by ROULET (1963) who determined sugar content in low-molecular fractions of humus substances. The sugar content in the high-molecular fractions of humus substances from soil was determined by COULSON and co-workers (1959); LYNCH and collaborators (1957) determined, in addition to a number of monosaccharides, several related compounds (uronic acids, sugar alcohols, amino sugars and sugar methyl derivatives). HAVRÁNKOVÁ and TICHÝ (1966) pointed out the presence of sugars in hydrolyzates of humus substances obtained from decomposed wood. The sugar content found in humus substances is usually very low. If one considers the theories accounting for the origin of humus substances in nature from carbohydrates (e.g. from carbohydrates and amino acids; ENDERS 1942) or from oxycellulose (MARCUSON 1926), one might assume that the fraction of sugars in the composition of humus substances will be higher. MARCUSON (1926) states that treatment of humic acids of decomposed wood with concentrated hydrochloric acid yields as much as 20% of sugars, i.e. a much higher content than could be established in the present and analogous papers on humus substances of different origin.

When studying the properties of humus substances one need not know their source with much accuracy. What we are interested in is to establish the properties that are common to all substances considered as humus ones and on the basis of such properties to define more closely the humus substances proper.

The results of this work showed again the difficulties in looking for a suitable standard to be used for studying the properties of humus substances. With commercial preparations, even of the same name and producer, one should not assume an identical quantitative content of the various components.

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Byl sledován vliv vzrůstající koncentrace kyseliny chlorovodíkové na kvalitativní a kvantitativní obsah glycidů v hydrolyzátech humusových frakcí. Ze tří preparátů humusových kyselin byly obvyklým způsobem získány frakce huminových a hymatomelanových kyselin. Obsah glycidů v hydrolyzátech byl určen po jejich chromatografickém rozdělení na papíře Whatman 1 za použití Partridgeovy soustavy po detekci kyselým anilinfталátem. Výsledky ukázaly, že kvalitativní obsah glycidů v hydrolyzátech většiny humusových frakcí uvedených vzorků byl stejný. Se vzrůstající koncentrací kyseliny se obsah hydrolyzovatelných glycidů obvykle zvyšoval, při čemž celkový obsah cukrů v hydrolyzátu nepřesáhl 4 %. Rozdíl v obsahu glycidů při hydrolyze 0,9 a 5,3 n HCl byl v některých případech až čtyřnásobný. Obvykle byl zjištěn vyšší obsah hexos než pentos.

Я. Гавранкова, Кафедра физиологии растений и биологии почвы, Карлов университет, Прага: **Относительное содержание углеводов в составе веществ гумуса.** — *Biol. Plant.* **9** : 102—108, 1967.

Исследовалось влияние возрастающих концентраций хлористоводородной кислоты на качественное и количественное содержание углеводов в гидролизатах гумусных фракций. Из трех препаратов гумусных кислот были обычным способом получены фракции гуминовых и гиматомелановых кислот. Содержание углеводов в гидролизатах было определено после их хроматографического разделения на бумаге Уотман 1 с применением системы растворителей Патриджа и детекции анилинфталатом. Результаты, приведенные в таблице и на графиках показали, что качественный состав углеводов в гидролизатах большинства гумусных фракций образцов был одинаковым. С возрастающей концентрацией кислоты количество углеводов обычно повышалось, причем общее содержание углеводов в гидролизате не превысило 4 %. Содержание углеводов при гидролизе 5,3 нормальной хлористоводородной кислотой было в некоторых случаях в 4 раза большим, чем при гидролизе 0,9 нормальной кислотой. Обычно было установлено большее содержание гексоз, чем пентоз.