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QUANTITATIVE GAS CHROMATOGRAPHIC ANALYSIS OF DEGRADATION AND OXIDATION PRODUCTS FROM A FINNISH SPHAGNUM PEAT

SUOMALAISEN SPHAGNUM-TURPEEN HAJOTUS- JA HAPETUSTUOTTEIDEN KVANTITATIIVINEN KAASUKROMATOGRAFINEN ANALYYSI

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A sample of Finnish *Sphagnum* peat and its humic fractions (humin, humic acid and fulvic acid) were oxidized by alkaline CuO and $KMnO_4$ methods and degraded by NaOH hydrolysis and KOH fusion. The oxidation and degradation products were analyzed quantitatively by high resolution gas chromatography.

The results revealed that (1) "lignin" constituents of the peat and peat humic fractions are predominantly p-hydroxyl units which are not typical lignin units; (2) the degree of humification of the peat is low; and (3) a close relation is present among m-hydroxybenzoic acid, p-hydroxybenzoic acid and p-hydroxyacetophenone as the degradation products of peat and peat humic fractions.

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INTRODUCTION

Peat is mainly used for such purposes as energy production, peat rooting media of plant seedlings, to improve soil for agriculture etc. (Fuchsman 1980 and Pihlaja 1982).

According to Ekman (1981a, 1981b, 1982), Fuchsman (1980), Ketola et al. (1979a, 1979b, 1981), Ketola (1981), Luomala (1981) and Pihlaja (1982) it is possible to separate from peat many valuable chemical raw materials. Different types (Luukkanen 1984) and depths (Ketola et al. 1979b) of peatlands contain different quantities of chemical materials such as bitumen per a mass unit. So it is important to know the type and decay level of peat to estimate the quantities of the potential chemical raw materials stored in peatlands.

The chemical structure of the peat and its humic fractions is not well characterized. In order to understand the humification mechanism and the mechanism of metal ions-peat bindings etc., we have to know the chemical structure of the peat. The knowledge of binding mechanism between peat and metal ions would serve biogeochemical prospecting.

The information on the structure of peat humic substances is increasing but the differences in the chemical structures of different humic fractions are not well known. Common instrumental methods are not necessarily sensitive enough to disclose those differences. One of the most effective methods is the analysis of oxidation and degradation products of humic fractions. Oxidation and degradation methods usually transform humic fractions into small molecules which can be easily identified by modern chemical and instrumental methods.

The purpose of this study is to get insight into the chemical structure of Finnish (*Sphagnum*) peat and peat humic fractions by using various chemical oxidation and degradation methods. This paper reports quantitative aspect of the characterization, following our previous paper on the qualitative characterization (Ishiwatari et al. 1983).

EXPERIMENTAL

A commercial peat from the Haukineva peat depot was used for this study. The peat is *Sphagnum* peat of which approximately 95% consists of *Sphagnum acutifolia. Sphagnum cuspidata* and *Sphagnum palustria* are minor. There were very few twigs. The decay level of the peat is 2–3 by v. Post scale. The peat sample was fractionated into humic fractions (humic acid, fulvic acid and humin) by a common method involving extraction of humic acid plus fulvic acid with 0.5 M NaOH and subsequent precipitation of humic acid by addition of HCl. The peat and humic fractions were oxidized by alkaline CuO oxidation or $KMnO_4$ oxidation, or degraded by NaOH or KOH fusion. The details of extraction procedure of humic fractions, methods for oxidation and degradation, and treatments of degradation products were described in Ishiwatari et al. (1983). A gas chromatographic determination of oxidation and degradation products was performed on a Carlo Erba 4160 gas chromatograph equipped with a flame ionization detector (FID). A 0.32 mm i.d. × 25 m glass capillary column coated with SE-52 was used for the analysis under the following conditions: detector and injector temperatures = 300°C, column temperature was programmed from 100°C to 280°C at 6°C/min, nitrogen carrier flow rate = 2 ml/min. The reproducibility of the gas chromatographic determination was checked during analysis by running daily chromatograms of the standard mixture and by means of the internal standard added to the samples and reference compounds. The quantities of the oxidation and degradation products were measured by a Hewlett-Packard 390 A integrator by means of comparison of the peak areas of the products and the standard samples. The detailed analysis of CuO oxidation products was conducted by using a Hewlett-Packard 5880 A gas chromatograph equipped with a FID. A 0.25 mm i.d. × 25 m silica capillary column coated with SE-54 was used. Detector and injector temperatures were programmed from 120°C to 230°C at 4°C/min and then to 300°C at 10°C/min. Helium carrier flow rate was 1.5 ml/min. Cellulose and noncellulose carbohydrates in peat and humic fractions were determined by the procedure reported by Uzaki and Ishiwatari (1983).

RESULTS AND DISCUSSION

As described by Ishiwatari et al. (1983), 12 phenolic compounds, 7 aliphatic α, ω-dicarboxylic acids and 3 monocarboxylic acids were identified or tentatively identified on CuO oxidation products, NaOH hydrolysis and KOH fusion products of peat and peat humic fractions. On the other hand, 3 phenolic compounds, 8 aliphatic α, ω-dicarboxylic acids and 3 monocarboxylic acids were detected for alkaline $KMnO_4$ oxidation products.

Table 1 shows the results of quantitation of major degradation products. By mild oxidation or degradation methods (CuO oxidation or NaOH hydrolysis), the quantities of degradation products of fulvic acid (FA) are higher than those of humic acid (HA). This result is in good accordance to Ruggiero et al. (1980) with that on peracetic oxidation where HA is degraded to a lesser extent than FA. By a strong degradation method (KOH fusion) the result is contrary to the case of the mild oxidation. The result is probably due to more profound degradation of FA than HA, indicating that the structure of HA is more rigid than that of FA.

The yields from the oxidation and degradation procedures used are very sensitive to reaction conditions and furthermore there were only a few values available for the calculations of correlation coefficients. However, we calculated all the possible correlations between the yields of the oxidation or degradation products obtained. This was done in order to get insight into the original structure of peat and peat humic substances from which the oxidation or degradation products have been split. Table 2 gives the results of the calculations.

CuO oxidation

The CuO oxidation produced the highest yields of p-hydroxybenzoic acid, m-hydroxybenzoic acid and p-hydroxyacetophenone from FA. From HA this method produced the highest yields of 3,5-dihydroxybenzoic acid and vanillic acid. Greene and Steelink (1961) found the highest yields of 3,5-dihydroxybenzoic acid, vanillic acid and vanilline from soil HA samples oxidized by CuO and Hedges and Ertel (1982) found high yields of p-hydroxybenzoic acid, vanillic acid and vanilline from lignin oxidized by CuO method.

As shown in Table 2, a strong correlation was obtained between m-hydroxybenzoic acid and p-hydroxybenzoic acid, between m-hydroxybenzoic acid and p-hydroxyacetophe-

Table 1. The quantities of the oxidation and degradation products (mg/g dry sample) produced from peat and peat humic fractions.

Taulukko 1. Turpeesta ja turpeen humusfraktioista saatujen hapetus-ja hajotustuotteiden määrät (mg/g kuivaa näytettä).

Method	Sample	fum	adi	pim	m-OH$_a$	p-OH$_a$	di-OH$_a$	van	p-OH$_o$	cat	phl
CuO oxidation	Peat	+	–	–	0.57	0.51	0.16	0.11	0.86	–	0.29
	Humine	–	–	–	0.17	0.76	0.15	0.11	1.20	–	+
	Humine acid	+	–	–	0.39	0.63	0.82	0.58	0.63	–	–
	Fulvic acid	0.44	–	–	2.40	1.30	0.67	0.30	2.44	–	–
NaOH hydrolysis	Peat	0.08	–	–	0.64	0.26	0.51	0.08	0.71	0.56	–
	Humine	–	–	–	0.63	0.20	0.42	–	0.78	0.32	–
	Humic acid	0.22	–	–	1.00	+	0.59	–	0.37	–	–
	Fulvic acid	1.22	–	–	1.11	0.78	0.57	–	0.84	–	–
KOH fusion	Peat	–	–	–	–	1.86	1.93	0.26	–	–	0.43
	Humine	–	–	–	–	1.93	1.54	0.39	–	–	0.35
	Humic acid	–	–	–	–	5.69	6.68	0.89	–	–	1.73
	Fulvic acid	–	–	–	–	4.86	2.73	–	–	–	–
$KMnO_4$ oxidation	Peat	–	0.10	0.15	–	0.52	–	0.04	–	–	–
	Humine	–	0.15	0.15	–	0.29	–	–	–	–	–
	Humic acid	–	0.91	1.02	–	–	–	–	–	–	–
	Fulvic acid	–	0.37	0.34	–	–	–	–	–	–	–

fum = fumaric acid; adi = adipic acid; pim = pimelic acid; m-OH$_a$ = m-hydroxybenzenecarboxylic acid; pH$_a$ = p-hydroxybenzenecarboxylic acid; di-OH$_a$ = 3,5-dihydroxybenzenecarboxylic acid; van = vanillic acid; p-OH$_o$ = p-hydroxyacetophenone; cat = cathecol; phl = phloroglucine
– = not identified; + = identified but not quantified

none, and between 3,5-dihydroxybenzoic acid and vanillic acid, respectively.

There is no significant correlation between m-hydroxybenzoic acid and vanillic acid, between p-hydroxybenzoic acid and vanillic acid and between p-hydroxyacetophenone and vanillic acid. This fact indicates that vanillyl units do not combine with hydroxyl units in the peat and peat humic molecules.

NaOH hydrolysis

This method produced the highest yields of p-hydroxybenzoic acid, m-hydroxybenzoic acid and p-hydroxyacetophenone from FA. The other products were fumaric acid, cathecol (1,2-dihydroxybenzene) from peat and humin, and vanillic acid from peat. 3,5-dihydroxybenzoic acid was produced in a similar amount from all samples.

The high yield of fumaric acid from FA is probably associated with high content of carbohydrates in FA (approximately 30%: see Table 3).

A strong correlation exists between the quantities of m-hydroxybenzoic acid and 3,5-dihydroxybenzoic acid. A significant correlation was seen between m-hydroxybenzoic acid and p-hydroxyacetophenone.

Table 2. The correlations between the quantities of the oxidation and degradation products from peat and peat humic fractions.

Taulukko 2. Turpeen ja turpeen humusfraktioitten hapetus-ja hajotustuotteiden määrien korrelaatiot laskettuna menetelmäkohtaisesti.

CuO oxidation	p-OH$_a$	di-OH$_a$	van	p-OH$_o$
m-OH$_a$	0.93	0.43	0.08	0.92
p-OH$_a$		0.40	0.07	0.92
di-OH$_a$			0.94	0.20
van				–0.20

NaOH hydrolysis	p-OH$_a$	di-OH$_a$	p-OH$_o$
m-OH$_a$	0.50	0.84	0.63
p-OH$_a$		0.18	
di-OH$_a$			–0.50

KOH fusion	di-OH$_a$	van	phl
p-OH$_a$	0.85	0.35	0.52
di-OH$_a$		0.79	0.90
van			0.97

$KMnO_4$ oxidation	adi
pim	0.99

Table 3. The amounts (mg/g) of carbohydrates in peat and peat humic fractions.

Taulukko 3. Turpeen ja turpeen humusfraktioitten hiilihydraattipitoisuudet (mg/g).

	Non-cellulose carbohydrates	Cellulose	Total
Peat	136	97	233
Humine	142	130	272
Humic acid	53	3	56
Fulvic acid	313	–	313

KOH fusion

By this method we obtained the highest amounts of p-hydroxybenzoic acid and 3,5-dihydroxybenzoic acid from HA and lesser amounts from FA. Vanillic acid and phloroglucin (1,3,5-trihydroxybenzene) were obtained from all samples except FA.

A strong correlation is seen between p-hydroxybenzoic acid and 3,5-dihydroxybenzoic acid, between phloroglucin and 3,5-dihydroxybenzoic acid and between vanillic acid and phloroglucin. A significant correlation exists between 3,5-dihydroxybenzoic acid and vanillic acid.

$KMnO_4$ oxidation

This method produced no aromatic oxidation products from HA and FA. The absence of aromatic oxidation products is due to complete decomposition of phenolic degradation products in a $KMnO_4$ solution (Ishiwatari et al. 1983).

All samples produced adipic and pimelic acid and the highest yield of them was found from HA. The correlation between adipic acid and pimelic acid was strong.

Carbohydrates and detailed analysis of CuO oxidation products

In order to understand in more detail the molecular feature of peat and peat humic fractions, we measured carbohydrates in these samples and carried out their CuO oxidation again in a controlled manner where aromatic aldehydes are preserved during oxidation. The results of carbohydrate analysis shown in Table 3 and 4 indicate that the total (cellulose plus non-cellulose) carbohydrates in the peat amount to approximately 23 %. The non-cellulose carbohydrate (neutral sugars) content in humic fractions decrease in the following order: FA (31 %), humin (14 %), HA (5 %). This fact indicates that in terms of carbohydrate content, the degree of humification of FA is the lowest and that of HA is the highest.

The detailed analysis of CuO oxidation of the peat and peat humic fractions (Table 5) showed that the total products that appeared on gas chromatograms amount to 1.4–1.7 % (by weight) of the initial samples oxidized. Figure 1 gives a representative high resolution gas chromatogram of CuO oxidation products. Aromatic aldehydes (p-hydroxybenzaldehyde, vanillinaldehyde, syringaldehyde), p-coumaric acid and ferulic acid were detected in fair amounts in the degradation products. Aromatic compounds account for 81–85 % of the total products for peat, HA and humin and 52 % for FA.

In agreement with what was already said above p-hydroxyl compounds (p-hydroxybenzaldehyde, p-hydroxyacetophenone and p-hydroxybenzoic acid) account for a large percentage (57–76 % by weight) of the aromatic degradation products of peat, humin and FA, whereas vanillyl and syringyl derivatives, including p-coumaric and ferulic acids, account for 10–31 %. In contrast to this, p-hydroxyl derivatives from HA amount to only 30 % of aromatic degradation products and vanillyl and syringyl compounds, including p-coumaric and ferulic acids amount to 50 %. This fact

Table 4. Relative composition of non-cellulose carbohydrates (% of the total).

Taulukko 4. Ei-selluloosaperäisten hiilihydraattien suhteellinen koostumus (% kokonaismäärästä).

	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose	A*	B*
Peat	10.0	0.0	0.0	1.8	18.2	10.1	19.0	40.5	0.1	0.3
Humine	10.4	0.0	0.0	1.7	19.4	8.5	19.7	40.0	0.0	0.2
Humic acid	12.7	0.0	0.5	4.1	14.8	14.3	15.3	37.2	0.4	0.7
Fulvic acid	11.9	2.7	0.8	4.7	18.2	15.3	19.6	25.7	0.5	0.7

* Unidentified carbohydrates

Table 5. The amounts of alkaline CuO oxidation products in peat and peat humic fractions.

Taulukko 5. Turpeen ja turpeen humusfraktioitten alkaalisen kuparioksidihapetuksen tuotteiden määrät.

Compound	Yields (μg/g dry weight) Peat	Humin	Humic acid	Fulvic acid
p-hydroxybenzaldehyde	1907	1575	838	1122
p-hydroxyacetophenone	4122	3613	1442	2674
p-hydroxybenzoic acid	2505	2322	1658	2107
vanillin	1342	924	831	170
acetovanillone	539	423	755	68
vanillic acid	598	385	1419	158
syringaldehyde	623	371	655	164
acetosyringone	208	126	481	28
syringic acid	289	135	612	79
p-coumaric acid	678	379	1187	62
ferulic acid	369	263	721	25
m-hydroxybenzoic acid	329	334	225	445
3,5-dihydroxybenzoic acid	1337	769	2416	721
Total aromatic acids	14846	11619	13240	7823
$C_{4:0}$ α,ω-dicarboxylic acid	925	905	836	2716
$C_{4:1}$ α,ω-dicarboxylic acid	1634	1825	1967	4381
Total products identified	17405	14349	16043	14920
Total aromatic acids (mg/g)	14.8	11.6	13.2	7.8
Total products (mg/g)	17.4	14.3	16.0	14.9

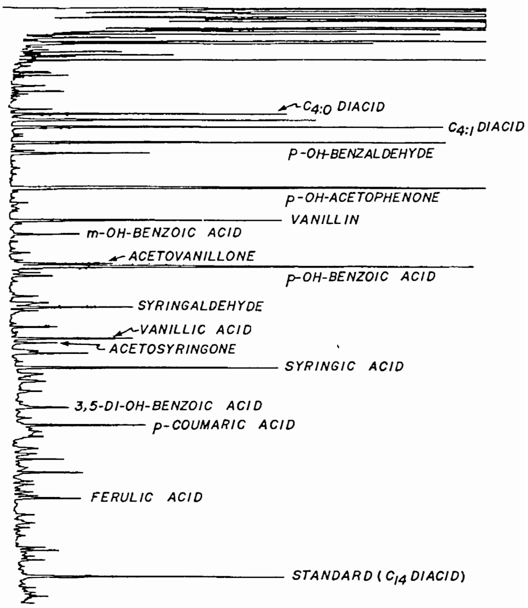

Figure 1. High resolution gas chromatogram of alkaline CuO oxidation products from peat (chromatographic conditions are given in the text).

Kuva 1. Turpeen alkaalisen kuparioksidihapetuksen tuotteiden korkean erottelukyvyn kaasukromatogrammi (ajo-olosuhteet on annettu tekstissä).

indicates that the "typical" lignin is more concentrated in HA than other humic fractions. According to Farmer and Morrison (1964) lignin from fungal and microbial decomposition of the polysaccharides of higher plants would appear in the humic acid.

IMPLICATIONS ON THE CHEMICAL NATURE OF PEAT HUMIC FRACTIONS

The results of this oxidation study clearly indicate that a close relation is present among m-hydroxybenzoic acid, p-hydroxybenzoic acid and p-hydroxyacetophenone. A genetic relation between 3,5-dihydroxybenzoic acid and vanillic acid is not clear, although correlation between these compounds is sometimes observed. Thus the present results support the previous qualitative results by Ishiwatari et al. (1983), where major degradation products are p-hydroxyl monomers which are found from *Sphagnum* degradation products (Farmer & Morrison 1964). P-hydroxyl monomers are not "typical" lignin degradation products and the absence of benzenepolycarboxylic acids in the $KMnO_4$ oxidation products of this peat and its humic fractions seems to be in accordance with the conclusion of this peat being of a low degree of humification. According to Schnitzer (1972, 1978) benzenepolycarboxylic acids are generally observed in $KMnO_4$ degradation products from soil humic substances. The relatively high content of carbohydrates in the peat also supports the above conclusion.

It became also clear from this study that there exist differences in the chemical structure among humic fractions: (1) p-hydroxyl units are much more concentrated in humin than in HA; (2) HA shows characteristics of "typical" lignin containing vanillyl and syringyl units more than humin and FA; (3) the degree of humification of HA is the highest among humic fractions as evidenced by the lowest content of carbohydrates and the highest yield of phenolic degradation products for HA; (4) the degree of humification of FA is the lowest. This is well evidenced by the highest content of carbohydrates and the lowest yield of phenolic degradation products from FA.

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TIIVISTELMÄ

SUOMALAISEN SPHAGNUM-TURPEEN HAJOTUS- JA HAPETUS-TUOTTEIDEN KVANTITATIIVINEN KAASUKROMATOGRAFINEN ANALYYSI

Suomalainen kasvuturve ja sen fraktiot (humiini, humushappo ja fulvohappo) hapetettiin alkaalisilla CuO- ja $KMnO_4$-menetelmillä, sekä hajotettiin NaOH-hydrolyysilla ja KOH-hajotuksella. Hapetus- ja hajotustuotteet analysoitiin korkean erottelukyvyn kaasukromatografilla.

Tuloksista havaittiin, että (1) tutkitun turpeen ja sen humusfraktioitten hapetus- ja hajotustuotteet ovat pääasiassa aromaattisia p-hydroksisubstituoituja yhdisteitä, jotka eivät ole tyypillisiä ligniinin rakenneosia; (2) turvenäytteen maatumisaste oli alhainen, ja (3) m-hydroksibentsoehappo, p-hydroksibentsoehappo ja p-hydroksiasetofenoni liittyvät läheisesti toisiinsa tutkitun turpeen ja sen humusfraktioitten rakenneosina.