Alkaline Degradation of Peat Humic Acids. Part I. Identification of Lipophilic Products

Kari Hänninen*,[a] and Klaus Niemelä[b]

[a]Department of Chemistry, University of Joensuu, P.O. Box 111, SF-80101 Joensuu, Finland and [b]Laboratory of Wood Chemistry, Helsinki University of Technology, SF-02150 Espoo, Finland

Hänninen, K. and Niemelä, K., 1991. Alkaline Degradation of Peat Humic Acids. Part I. Identification of Lipophilic Products. – Acta Chem. Scand. 45: 193–199.

Alkaline degradation of peat humic acids at 185 °C gives rise to a large number of lipophilic, chloroform-soluble compounds, which have been analyzed by capillary gas–liquid chromatography – mass spectrometry. Identified compounds included 50 aromatic compounds, 20 aliphatic mono- and di-carboxylic acids, 6 alkyl-substituted 2-cyclopentenones and 5 alkyl-substituted 2-hydroxy-2-cyclopentenones. Degradation in the presence of sodium sulfide (Na_2S) produces some thiophenes and increases the number of phenolic compounds.

The total yields of identified lipophilic compounds were 3.2 % in both the presence and absence of Na_2S. The formation of thiophenes and alkylcyclopentenones suggests the presence of aliphatic 1,4-diketonic structures in peat humic acids.

Humic acids (HA) are polymeric, brown compounds occurring in aquatic, terrestrial and sedimentary environments.[1] Despite an extensive number of investigations, their chemical nature is far from being fully understood. Opinions differ, for example, about the relative amounts of aromatic and aliphatic constituents in their structures,[1–10] and the nature and amounts of their unsaturation.[11–13] The importance of the elucidation of the structural characteristics of HA is generally recognized.[14]

The degradation methods used to investigate the structural elements of HA fall into six main groups: (1) oxidative, (2) reductive, (3) hydrolytic, (4) thermal, (5) radiochemical and (6) biological.[15] The most commonly used methods include reactions in alkaline media: hydrolysis, oxidative cleavage and methylation with subsequent oxidation.[16–31]

In this study, samples of peat HA were degraded by non-oxidative alkaline hydrolysis in the absence and presence of sodium sulfide (Na_2S). These degradation methods reportedly preserve the aliphatic structures of HA,[29] while the addition of Na_2S has a well known catalytic effect on the degradation of lignin-type structural units.[32] Degradation products were analyzed by capillary gas–liquid chromatography (GLC) and mass spectrometry (MS). Origins of the compounds are discussed.

The HA investigated in this study was previously analyzed by means of cupric oxide (CuO) oxidation[31] and $KMnO_4$ oxidation.[12] At that time the primary interest was, in the first case, in the fractionation of the HA material during the procedure and, in the second case, in the analysis of phenolic and benzenecarboxylic acids.

* To whom correspondence should be addressed.

Experimental

The peat sample from which the HA was extracted was a commercial milled peat, a product of the State Fuel Company, harvested from Haukineva bog in Peräseinäjoki. The age of the peat was estimated to be 2000 years by ^{14}C dating;[33] the degree of humification was H_6–H_7 on von Post's scale.[33] Only 30 % of the plant cells were identifiable by botanical analysis; of these, 70 % belonged to coniferous trees, 10 % to deciduous trees, 10 % to *Eriophorum* and 6 % to *Sphagnum*.[33] In extractions[13] with hot water, chloroform and 0.1 M NaOH the peat was fractionated into five fractions: hot-water soluble, chloroform soluble (bitumens), base and acid soluble (fulvic acids), base-soluble and acid-insoluble (HA) and insoluble (humin) fractions. The yield of HA was ca. 46 %.

Alkaline hydrolysis. HA samples (300 mg) were treated in a nitrogen atmosphere, in an initial series of experiments, with 50 ml of 2 M NaOH and, in a second series of experiments, with 2 M NaOH and 0.5 M Na_2S. Treatments were made at 185 °C for 2 h in a polyethyleneglycol bath in a rotating autoclave.

Fractionation and derivatization. To 15 ml aliquots taken from the reaction mixtures was added 1 ml of an aqueous solution of 2-hydroxy-3-methoxybenzaldehyde (7 mg/100 ml) and *meso*-erythritol (13 mg/100 ml), which were used as internal standards for lipophilic and hydrophilic compounds, respectively. The aliquots were then cation exchanged (Dowex 50 WX8, H^+), filtered, extracted with 30 ml of chloroform and the chloroform extracts were divided into two equal parts. One part was evaporated to dryness under reduced pressure and the residue was dissolved in 0.2

ml of pyridine and trimethylsilylated with a mixture[34] of 95 % *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and 5 % chloro(trimethyl)silane (CTMS) for GLC and MS analysis. The other part was concentrated to a volume of ca. 0.3 ml and analyzed with GLC and MS without silylation.

As soon as it was evident that the chloroform extracts contained compounds which had no hydroxy groups, additional 20 ml aliquots from the reaction mixture were taken and extracted with chloroform without lowering the pH. These extracts were concentrated to a volume of 0.5 ml and analyzed with GLC and MS in the absence of an internal standard.

The analysis of the cation-exchanged and chloroform-extracted water phase containing the hydrophilic components will be reported in a separate paper.

GLC and MS. Separations were performed with a Hewlett-Packard 5890 A gas chromatograph, equipped with a flame-ionization detector. For the trimethylsilylated samples the temperature program was 2 min at 90 °C, 15 °C min^{-1} to 240 °C and 10 min at 240 °C. For the underivatized samples the program was 2 min at 65 °C, 12 °C min^{-1} to 260 °C and 5 min at 260 °C. Two fused silica capillary columns (SE-30 and SE-54, 25 m×0.32 mm id) were used for each separation. The temperature of both the injection port and the detector was 265 °C. Hydrogen was the carrier gas at a rate of 2 ml min^{-1}. Fig. 1 presents an example of the separation of trimethylsilylated samples.

The electron ionization mass spectra were recorded[35] at 70 eV with a Jeol JMS-DX303 instrument combined with a Hewlett-Packard 5790 A gas chromatograph and the above-mentioned columns. The temperature programs were similar to those used in GLC. For the trimethylsilylated and underivatized samples, the scanning ranges were 60–600 and 30–400, respectively.

Most of the aromatic compounds, including phenol, 4-hydroxybenzaldehyde, benzoic acid, catechol, 3,4-dihydroxybenzaldehyde, and the guaiacyl and syringyl compounds, were easily identified as their trimethylsilyl (TMS) derivatives on the basis of the earlier studies.[35,36] Additional mass spectral data were required for the identification of the TMS derivatives of 4-hydroxyphenylacetic acid,[37] 4-vinylphenol,[38] methylphenols,[38] hydroxyacetophenones[39] and terephthalic acid.[40] Identification of the TMS ester of phenylpropanoic acid was based on the automatic library search (NIH/EPA, 31,000 spectra) only. Identification of the isomeric methylphenols was further supported by the published[41] retention time (index) data.

Identifications of underivatized aromatic compounds were based on the use of the computerized library search and were confirmed by relevant literature data.[42,43]

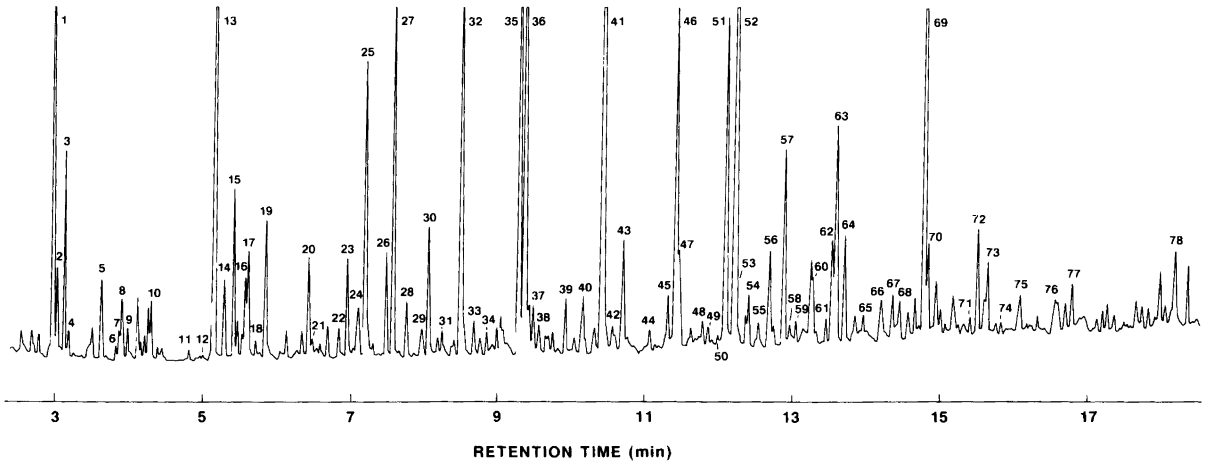

Fig. 1. Separation on an SE-54 fused silica capillary column of the trimethylsilylated compounds obtained after alkaline treatment of HA in the presence of Na_2S: 1, phenol; 2, lactic acid; 3, hexanoic acid; 4, glycolic acid; 5, a branched octanol; 6, 2-methylphenol; 7, 3-methylphenol; 8, levulinic acid; 9, 4-methylphenol; 10, heptanoic acid; 11, 2-hydroxy-3-methyl-2-cyclopentenone; 12, 2-hydroxy-3,4-dimethyl-2-cyclopentenone; 13, guaiacol; 14, 4-ethylphenol; 15, benzoic acid; 16, 3-thiophenecarboxylic acid; 17, octanoic acid; 18, 3-ethyl-2-hydroxy-2-cyclopentenone; 19, 4-vinylphenol; 20, catechol; 21, 4-methylguaiacol; 22, methylthiophenecarboxylic acid I; 23, nonanoic acid; 24, methylthiophenecarboxylic acid II; 25, 4-hydroxybenzaldehyde; 26, 4-ethylguaiacol; 27, syringol; 28, 3-hydroxyacetophenone; 29, 3-methoxycatechol; 30, 4-vinylguaiacol; 31, decanoic acid; 32, 4-hydroxyacetophenone; 33, 4-methylsyringol; 34, (4-hydroxyphenyl)acetone; 35, 2-hydroxy-3-methoxybenzaldehyde (internal standard); 36, vanillin; 37, undecanoic acid; 38, 4-ethylsyringol; 39, 1-guaiacylethanol; 40, a dihydroxyacetophenone; 41, acetovanillone; 42, 3,4-dihydroxybenzaldehyde; 43, lauric acid; 44, a dihydroxyacetophenone; 45, 4-hydroxyphenylacetic acid; 46, syringaldehyde; 47, suberic acid; 48, 4-(1-propenyl) syringol; 49, tridecanoic acid; 50, terephthalic acid; 51, vanillic acid; 52, acetosyringone; 53, homovanillic acid; 54, azelaic acid; 55, a butylthiophenecarboxylic acid; 56, dihydroconiferyl alcohol; 57, unknown isomer of 56; 58, myristic acid; 59, propiosyringone; 60, *cis*-ferulic acid; 61, sebacic acid; 62, unknown guaiacyl compound; 63, syringic acid; 64, homosyringic acid; 65, pentadecanoic acid; 66, unknown phthalate; 67, guaiacylglyoxylic acid; 68, undecanedioic acid; 69, unknown A; 70, palmitic acid; 71, dodecanedioic acid; 72, *trans*-ferulic acid; 73, syringylglyoxylic acid; 74, heptadecanoic acid; 75, unknown syringyl compound; 76, tridecanedioic acid; 77, stearic acid; 78, 1,1-diguaiacylethane.

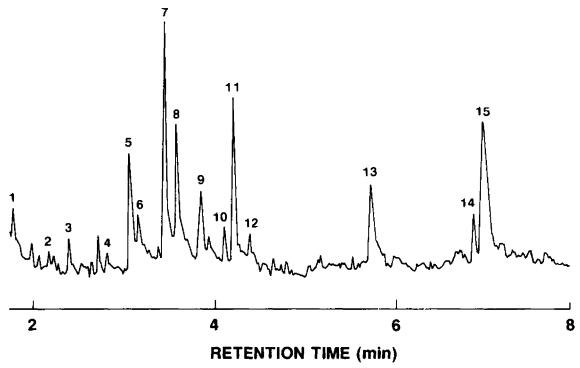

Fig. 2. Separation (total ion chromatogram) on an SE-54 fused silica capillary column of some neutral compounds obtained on treatment of HA with alkali in the presence of Na_2S: 1, 2-methyl-2-cyclopentenone; 2, 2,5-dimethyl-2-cyclopentenone; 3, 3-methyl-2-cyclopentenone; 4, 3,4-dimethyl-2-cyclopentenone; 5, a hydrocarbon; 6, a dimethyl-2-cyclopentenone; 7, 2,3-dimethyl-2-cyclopentenone; 8, a branched octanol; 9, acetophenone; 10, 2-acetylthiophene; 11, 2,3,4-trimethyl-2-cyclopentenone; 12, unknown (*m/z* 141 100 %); 13, a hydrocarbon; 14, a C_4-substituted-2-cyclopentenone; 15, indole.

Identification of the TMS esters of fatty and alkanedioic acids was also based on a recent study[44] on their formation (liberation) from wood during mild alkaline treatment. Levulinic acid was identified by means of the library search and published mass spectrum[45] and finally confirmed by comparison with a commercial reference sample. The branched octanol, according to the library search, was 2-ethyl-1-hexanol.

Identifications of 2-cyclopentenones were based on earlier work[46,47] on their formation from polysaccharides during hot alkaline treatments. Fig. 2 shows part of a total ion chromatogram recorded during a GLC–MS run of the chloroform extract of the alkaline reaction mixture, demonstrating the separation of alkyl-substituted 2-cyclopentenones.

The mass spectra of the TMS esters of 3-thiophenecarboxylic acid and two (unidentified) methylthiophenecarboxylic acids were interpreted with the aid of literature data.[48] Other compounds of this type were identified as the TMS ester of a propylthiophenecarboxylic acid (*m/z* and % of the base peak): 242 (M^+, 16), 227 (57), 199 (5), 183 ($M^+-15-44$, 100), 153 (18), 111 (27) and 73 (27) and butylthiophenecarboxylic acid (*m/z* and % of the base peak): 256 (M^+, 22), 241 (43), 219 (32), 197 ($M^+-15-44$; 100), 167 (41), 139 022) and 73 (17). The mass spectrum of 2-acetylthiophene was taken from Ref. 49.

Uncorrected peak areas from the GLC analysis of the trimethylsilyl derivatized samples were used for quantitative calculations.

Results and discussion

The conditions used in this study were somewhat stronger than those usually used in kraft pulping; on the other hand the conditions were relatively mild compared with those usually used in the HA degradations.

In all, more than 100 peaks appeared in the chromatograms (Figs. 1 and 2), from which some 80 compounds could be identified. The total amount of these compounds corresponded to 3.2 % of the starting material; the proportion of aromatic products was 2.7 % and that of the aliphatic compounds 0.5 %. In addition over 20 diaryl compounds were found, which do not appear in Fig. 1, owing to their longer retention times.

Aromatic compounds. The main part of the aromatics were phenolics, which were classified into guaiacyl (G), syringyl (S) and 4-hydroxyphenyl (H) compounds. Others were classified into benzenecarboxylic acids and miscellaneous compounds (Table 1).

Quantitatively the major degradation products were vanillin, acetovanillone (acetoguaiacone), vanillic acid, ferulic acid, syringaldehyde, acetosyringone, syringic acid, 4-hydroxybenzaldehyde, 4-hydroxyacetophenone and 4-hydroxybenzoic acid, which have frequently been obtained in similar degradation treatments.[15,18,25–29] The minor degradation products were the occasionally reported[50] phenol, guaiacol, ethylphenol, methylguaiacol, vinylphenol, ethylguaiacol, eugenol, *cis*-isoeugenol, *trans*-isoeugenol, propioguaiacone (propiovanillone) and guaiacylpropane.[51]

Also among the minor products were guaiacylethanols and guaiacylglyoxylic acid, syringylethanols, propiosyringone, homosyringic acid and syringylglyoxylic acid. As far as we know these compounds have not been reported previously among the alkaline hydrolysis products of peat HA. The glyoxylic acids and propiosyringone were found only in the presence of Na_2S.

The presence of carboxyphenylglyoxylic acids among the $KMnO_4$ oxidation products of aquatic humic materials has been reported by Liao *et al.*[4] and the presence of 4-methyl-, 4-ethyl- and 4-propyl-guaiacol and 4-methyl-, 4-ethyl- and 4-propyl-syringol among the catalytic hydrogenation products of river humic material by Mycke and Michaelis.[52] Burges *et al.*[53] found guaiacylpropionic and syringylpropionic acids in the sodium amalgam reduction of HA in NaOH.

Catechol, 3,4-dihydroxybenzaldehyde and 3-methoxycatechol are here considered to be sulfidolytic demethylation products of guaiacol, vanillin and syringol, respectively.[54]

Some compounds such as 3,5-dihydroxybenzoic acid, which are reported[53] to be important HA degradation products, were not identified in the present study.

The G, S and H phenolics are present in the lignins of trees[55] and grasses[56] and the H phenolics in the phenolic cell wall polymer of *Sphagnum*.[57,58] The building blocks of the cell wall polymer of *Sphagnum* are 4-hydroxyphenyl compounds. The polymer is called Sphagnol,[59,60] and depending on the definition, may[57] or may not[58] be regarded as lignin. Phenolics are recognized as important tracer substances in terrestrial plant vegetation:[31,51,61] syringyl compounds are

Table 1. Yields (mg g^{-1} charge) of aromatic compounds obtained on treatment of HA with sodium hydroxide solution.

Compound	NaOH treatment	Na_2S/NaOH treatment
Guaiacyl compounds		
Guaiacol	2.3	1.6
4-Methylguaiacol	–	<0.1
4-Ethylguaiacol	0.1	0.3
4-Vinylguaiacol	–	0.3
Eugenol	0.1	0.3
trans-Isoeugenol	0.2	0.1
1-Guaiacylethanol	0.1	0.2
2-Guaiacylethanol	–	<0.1
Dihydroconiferyl alcohol	0.7	0.3
Vanillin	1.4	2.3
Acetovanillone	3.7	4.9
Propiovanillone	–	0.1
Guaiacylacetone	0.1	–
Vanillic acid	2.6	1.1
Homovanillic acid	0.4	0.3
cis-Ferulic acid	–	0.2
trans-Ferulic acid	0.7	0.4
Guaiacylglyoxylic acid	–	0.2
3,4–Dihydroxybenzaldehyde	0.1	0.3
1,1-Diguaiacylethane	0.2	0.4
Catechol	–	0.1
Syringyl compounds		
Syringol	1.4	1.3
4-Methylsyringol	–	0.1
4-Ethylsyringol	0.1	0.1
4-Vinylsyringol	–	0.1
4-(1-Propenyl)syringol	–	0.1
1-Syringylethanol	0.1	<0.1
2-Syringylethanol	–	<0.1
Dihydrosinapyl alcohol	1.0	–
Syringaldehyde	1.6	1.3
Acetosyringone	1.9	2.7
Propiosyringone	–	<0.1
Syringic acid	1.2	0.8
Homosyringic acid	0.4	0.8
Syringylglyoxylic acid	0.1	0.3
3-Methoxycatechol	–	0.1
p-Hydroxyphenyl compounds		
Phenol	2.4	1.4
4-Methylphenol	0.1	0.1
4-Ethylphenol	0.2	0.2
4-Vinylphenol	0.1	0.5
4-Hydroxybenzaldehyde	0.9	1.0
4-Hydroxyacetophenone	0.8	1.4
4-Hydroxyphenylacetone	–	0.1
4-Hydroxybenzoic acid	0.1	<0.1
4-Hydroxyphenylacetic acid	0.1	0.2
Benzenecarboxylic acids		
Benzoic acid	1.1	0.6
Phenylpropanoic acid	–	0.1
Terephthalic acid	0.1	<0.1
Miscellaneous		
Indole	<0.1	<0.1
Acetophenone	–	<0.1
2-Methylphenol	0.1	<0.1
3-Methylphenol	0.2	0.1
3-Hydroxyacetophenone	0.2	0.2

from deciduous trees, guaiacyl compounds from coniferous and deciduous trees and 4-hydroxyphenyl compounds from grasses and *Sphagnum* mosses. In the following these compounds are referred to as lignin-derived phenolics.

The major aromatic degradation products of HA originate, without any doubt, from lignin-type structural units, but it is not practicable to attempt to outline more detailed routes for their formation, because there are too many unknown components in the HA structures as yet.

Wallis[62] reported that in alkaline hydrolysis of lignin the reactions are mainly cleavages of the ether linkages between phenylpropane units with the simultaneous formation of phenolic hydroxy groups. To some extent carbon–carbon bonds are cleaved and condensation reactions occur.

The presence of lignin-derived phenolics among the hydrolysis products of peat HA can be taken as evidence of ligning incorporation into the HA structure during the humification process. In attempting to determine the proportion of original, unaltered ligning in the HA, it is important not to overemphasize the monomeric hydrolysis products, since they can be formed from both native and highly modified lignins. The existence of phenylpropane derivatives among peat HA degradation products may, however, indicate that there are linkages between the α- and β-carbon atoms of the propyl side chain, which are typical unaltered lignin units.

Specific analysis of the 20 dimeric phenolics formed in the hydrolysis may be very important in assessing the contribution of unaltered lignin to peat HA. Analysis of the dimeric compounds has been critical to the structural analysis of lignin. In particular, the dimeric phenolics formed in primary reactions have provided specific structural information. In this study was identified 1,1-diguaiacylethane, a novel secondary reaction dimeric compound only recently detected in alkaline pulping spent liquors.[63]

The increased yields of α-ketonic phenylpropane compounds, such as propiovanillone, 4-hydroxyacetophenone and propiosyringone, in the presence of Na_2S emphasize the effects of degradation method; as in lignin[62] β-ether bonds of HA are cleaved more rapidly when hydrogen sulfide ions are used.

The non-lignin aromatic degradation products were benzenecarboxylic acids, indole (originating from proteins), 2- and 3-hydroxyphenols and 3-hydroxyacetophenone.

According to Bland *et al.*[57] *Sphagnum* lignin contains very little ether oxygen, being mainly C–C linked. In the total absence of oxygen alkaline hydrolysis is suggested to be ineffective in breaking C–C bonds.[29] C–C linkages may, however, be cleaved to a certain extent by a retro-aldol reaction. This may explain the low yields of 4-hydroxyphenyl compounds relative to the yields obtained in CuO oxidation.[31] It may also explain the lower yields of benzenecarboxylic acids, formed from the *C*-condensed structures of HA.

Aliphatic mono- and di-carboxylic acids. The aliphatic compounds were classified into mono- and di-carboxylic acids, alkyl-substituted 2-cyclopentenones and alkyl-substituted 2-hydroxy-2-cyclopentenones.

The yields of monocarboxylic acids were 0.39 and 0.35 % in the NaOH and NaOH/Na_2S treatments, respectively, and the yields of dicarboxylic acids 0.08 and 0.1 %, respectively (Table 2).

The mono- and di-carboxylic acids found were relatively long-chain compounds such as are frequently encountered in degradation treatments.[64] Levulinic acid is a well-known carbohydrate cleavage product under acidic conditions[65] and it has been reported to form from HA under such conditions.[66] In alkaline conditions it may not originate from carbohydrates but from some original HA structures. As far as we know, its formation in alkaline conditions has not been reported previously.

Monocarboxylic acids, or fatty acids, are the major components of the peat lipids. Since, however, peat lipids were extracted with chloroform before extraction of the HA, the monocarboxylic acids found here can represent the original lipidic components of peat HA.

There are several possible explanations for the origin of the mono- and di-carboxylic acids: Ogner[67] has postulated that they arise from the oxidation of the aliphatic links between the aromatic components in humic matter, whereas Bayer *et al.*[68] have suggested that aliphatic polyethers could be the basic building blocks of naturally occurring humic substances. As the conditions of the alkaline treatment in this study would not be sufficient to cleave the aliphatic ether linkages, the most probable explanation is that the mono- and di-carboxylic acids found were originally bound with ester bonds to the polymer matrices.

Another explanation for the formation of dicarboxylic acids may be the proposition made by Bracewell *et al.*[69] that the aliphatic dicarboxylic acids ($n = 0$–8) are formed from the unsaturated aliphatic chain structures of HA according to eqn. (1). In order to verify this proposition there should

$$R{-}CH{=}CH{-}(CH_2)_n{-}CH{=}CH{-}R^{\cdot} \rightarrow R{-}COCH + HOOC{-}(CH_2)_n{-}COOH + COOH{-}R^{\cdot} \quad (1)$$

be adequate methods for differentiating the olefinic and the aromatic double bonds from each other in the HA structure.

Table 2. Yields (mg g^{-1} charge) of aliphatic carboxylic acids obtained on treatment of HA with NaOH: (a) without Na_2S; (b) with Na_2S.

Carboxylic acid	(a)	(b)
Monocarboxylic acids		
Levulinic	0.6	0.2
Hexanoic	0.7	0.7
Heptanoic	0.4	0.2
Octanoic	0.4	0.4
Nonanoic	0.3	0.3
Decanoic	0.2	0.1
Undecanoic	0.2	0.2
Lauric	0.2	0.4
Tridecanoic	0.1	0.1
Myristic	0.1	<0.1
Pentadecanoic	0.1	0.1
Palmitic	0.4	0.5
Heptadecanoic	<0.1	<0.1
Stearic	0.2	0.3
Dicarboxylic acids		
Suberic	0.1	0.3
Azelaic	0.2	0.2
Sebacic	0.1	0.1
Undecanedioic	0.2	0.2
Dodecanedioic	0.1	<0.1
Tridecanedioic	0.1	0.2

Table 3. Yields (mg g^{-1} charge) of cyclopentenones obtained on treatment of HA with NaOH: (a) without Na_2S; (b) with Na_2S.

Substituent	(a)	(b)
2-Cyclopentenones		
2-Methyl	<0.1	<0.1
3-Methyl	<0.1	<0.1
2,3-Dimethyl	<0.1	<0.1
3,4-Dimethyl	<0.1	<0.1
2,5-Dimethyl	<0.1	<0.1
2,3,4-Trimethyl	<0.1	<0.1
2-Hydroxy-2-cyclopentenones		
3-Methyl	0.1	0.1
5-Methyl	<0.1	0.1
3,4-Dimethyl	0.1	<0.1
3-Ethyl	<0.1	–
3,5-Diethyl	<0.1	–

The cyclopentenones. The yield of alkyl-substituted cyclopentenones was slightly more than 0.02 % in both degradations (Table 3). Many of the alkylcyclopentenones and hydroxyalkylcyclopentenones have not previously been reported among the alkaline hydrolysis products of peat HA.

Saiz-Jimenez *et al.*[50] have reported the presence of 2- and 3-methyl-2-cyclopentenone and 2-hydroxy-3-methyl-2-cyclopentenone among the pyrolysis products of soil organic matter. The formation of cyclopentenones under alkaline conditions is thoroughly discussed by Niemelä[46] and is suggested here as an indication of 1,4-diketonic structures of the HA polysaccharide constituents.

The thiophenes. The thiophenes were formed only in the presence of Na_2S. The yield was 0.06 % (Table 4), about three times that of the cyclopentenones. To our knowledge the thiophenes have not previously been reported among the alkaline degradation products of peat humic acids, but Saiz-Jimenez *et al.*[50] have found methylthiophene among the pyrolysis products of soil organic matter.

Table 4. Yields (mg g^{-1} charge) of thiophenes obtained on treatment of HA with NaOH: (a) without Na_2S; (b) with Na_2S.

Thiophene	(a)	(b)
2-Acetylthiophene	–	<0.1
3-Thiophenecarboxylic acid	–	0.3
Methylthiophenecarboxylic acid I	–	0.1
Methylthiophenecarboxylic acid II	–	0.1
Propylthiophenecarboxylic acid	–	<0.1
Butylthiophenecarboxylic acid	–	0.1

According to Streitwieser *et al.*[70] the formation of thiophenes can be explained by the Paal–Knorr synthesis in which 1,4-dicarbonyl compounds are heated with an inorganic sulfide. By analogy we assume that the thiophenes formed during the hydrolysis of this study indicate 1,4-diketonic structures in HA. Important to notice is that thiophenes are aromatic rather than aliphatic compounds but their presence is taken as evidence of aliphatic structural features in HA. Their greater yields in comparison with cyclopentenones may be an indication of the relative sensitivity of the 1,4-diketonic structures towards the degradation methods used.

Unknown compounds and contaminants. Unknown compounds formed a relatively large proportion of the products (Table 5). The unknown compound A has molecular weight 336, it contains two OH-groups as shown by trimethylsilylation and its main peak is $M-69$, which is a very unusual fragmentation. Bourbonniere and Meyers[29] also report a major unknown compound among the hydrolysis products. They suggest that their unknown compound might be an unsaturated hydroxy acid.

2-Ethyl-1-hexanol is almost certainly a contaminant. The chief plasticisizers for bottles and minigrip bags used in the extraction and storage of HA may be bis(2-ethylhexyl) phthalate, which on sodium hydroxide saponification yields 2-ethyl-1-hexanol.

Conclusions. This study provides new information on the aromatic lignin-derived characteristics and aliphatic structural features of HA. The identification of α- and β-ether cleavage products, some of which have not to date been reported, indicates the presence of lignin units in 2000-year-old HA. Their low concentrations suggest nevertheless that the lignin may even be a minor contributor to the HA structure. A thorough analysis of the dimeric products should assist in assessing the importance of unaltered lignin in HA.

Table 5. Yields (mg g^{-1} charge) of unknown compounds obtained on treatment of HA with NaOH: (a) in the absence of Na_2S; (b) in the presence of Na_2S.

	(a)	(b)
Unknown A	3.8	4.7
Other unknown monomers	1.3	1.5
Unknown dimers	3.1	4.9

The identification of alkyl-substituted cyclopentenones and thiophenes in peat HA indicates the presence of aliphatic 1,4-diketonic structures so far not demonstrated at the level of individual fragments. Together with the suggestion that the formation of aliphatic dicarboxylic acids is due to the olefinic double bond structure of HA, these findings would seem to support the importance of olefinic double bonds to the unsaturation of HA.

The study shows the need to develop further degradation, isolation and identification techniques in order to obtain more detailed structural information from HA. For example, the use of Na_2S in the alkaline degradation of HA enhanced the production of fragments apparently originating from the 1,4-diketonic structures of HA.

Acknowledgements. Professor Charles H. Fuchsman is thanked for his valuable comments and the Finnish Academy of Sciences for partial financial support.

References

1. Thurman, E. M., Aiken, G. R., Ewald, M., Fischer, W. R., Förstner, U., Hack, A. H., Mantoura, R. F. C., Parsons, J. W., Pocklington, R., Stevenson, F. J., Swift, R. S. and Szpakowska, B. In: Frimmel, F. H. and Christman, R. F., Eds., *Humic Substances and their Role in the Environment,* Wiley, New York 1988, pp. 31–43.
2. Fischer, F. and Schrader, H. *Brenst.-Chem. 2* (1921) 37.
3. Hatcher, P. G., Schnitzer, M., Dennis, L. W. and Maciel, G. E. *Soil Sci. Soc. Am. J. 45* (1981) 1089.
4. Liao, W., Christman, R. F., Johnson, J. D., Millington, D. S. and Hass, J. R. *Environ. Sci. Technol. 16* (1982) 403.
5. Harvey, G. R., Boran, D. A., Chesal, L. A. and Tokar, J. M. *Mar. Chem. 12* (1983) 119.
6. Painter, T. J. *Carbohydr. Res. 124* (1983) C22.
7. Harvey, G. R., Boran, D. A., Piotrowicz, S. R. and Weisel, C. P. *Nature (London) 309* (1984) 244.
8. Flaig, W. In: Fuchsman, C. H., Ed., *Peat and Water,* Elsevier, London 1986, pp. 119–132.
9. Ikan, R., Ioselis, P., Rubinsztain, Y., Aizenshtat, Z., Pugmire, R., Anderson, L. L. and Ishiwatari, R. *Naturwissenschaften 73* (1986) 150.
10. Orlov, D. S., Baranovskaya, V. A. and Okolelova, A. A. *Sov. Soil Sci. 19* (1987) 35.
11. Fog, K. In: Hayes, M. H. B. and Swift, R. S., Eds., *Volunteered Papers 2nd International Conference International Humic Substances Society,* University of Birmingham, UK 1984, pp. 166–168.
12. Hänninen, K. and Paajanen, K. In: *Proc. Int. Peat Symp.,* Bemidji, Minnesota May 16–19, 1989. *In press.*
13. Hänninen, K. *Sci. Tot. Environ. In press.*
14. Fuchsman, C. H. *Telma 10* (1980) 117.
15. Schnitzer, M. In: Schnitzer, M. and Khan, S. U., Eds., *Soil Organic Matter,* Elsevier, Amsterdam 1978.
16. Marcusson, J. *Z. Angew. Chem. 34* (1921) 437.
17. Morrison, R. I. *J. Soil Sci. 9* (1958) 130.
18. Steelink, C., Berry, J. W., Ho, A. and Nordby, H. E. *Sci. Proc. R. Dublin Soc., Ser. A 14* (1960) 59.
19. Greene, G. and Steelink, C. *J. Org. Chem. 27* (1962) 170.

20. Dubach, P. and Mehta, N. C. *Soils Fert. 26* (1963) 293.
21. Jakab, T., Dubach, P., Mehta, N. C. and Deuel, H. *Z. Pflanzenernaehr., Dueng, Bodenkd. 102* (1963) 8.
22. Morrison, R. I. *J. Soil Sci. 14* (1963) 201.
23. Swift, R. S. *Physico-chemical Studies on Soil Organic Matter,* Ph. D. Thesis, University of Birmingham, UK 1968.
24. Hayes, M. H. B., Stacey, M. and Swift, R. S. *Fuel 51* (1972) 211.
25. Neyroud, J. A. and Schnitzer, M. *Can. J. Chem. 52* (1974) 4123.
26. Schnitzer, M. and Ortiz de Serra, M. I. *Can. J. Chem. 51* (1973) 1554.
27. Tsutsuki, K. and Kuwatsuka, S. *Soil Sci. Plant Nutr. 25* (1979) 183.
28. Hedges, J. I. and Ertel, J. R. *Anal. Chem. 54* (1982) 174.
29. Bourbonniere, R. A. and Meyers, P. A. *Org. Geochem. 5* (1983) 131.
30. Ishiwatari, R., Morinaga, S., Yamamoto, S., Machihara, T., Rubinsztain, Y., Ioselis, P., Aizenshtat, Z. and Ikan, R. *Org. Geochem. 9* (1986) 11.
31. Hänninen, K., Mälkönen, P. and Hyvärinen, K. In: Fuchsman, C. H., Ed., *Peat and Water,* Elsevier, London 1986, pp. 241–262.
32. Brunow, G. and Miksche, G. E. *J. Appl. Polym. Sci., Appl. Polym. Symp. 28* (1976) 1155.
33. Hänninen, K. *Ann. Acad. Sci. Fenn., Ser. 2 A 213* (1987) 1.
34. Alén, R., Niemelä, K. and Sjöström, E. *J. Chromatogr. 301* (1984) 273.
35. Niemelä, K. and Sjöström, E. *Holzforschung 40* (1986) 361.
36. Niemelä, K. *Holzforschung 42* (1988) 169.
37. Chapman, D. I., Chapman, J. R. and Clark, J. *Int. J. Biochem. 1* (1970) 465.
38. Wittkowski, R. *Phenole im Räucherrauch. Nachweis und Identifizierung,* VCH-Verlagsgesellschaft, Weinheim 1985.
39. Smith, G. G. and Djerassi, C. *Org. Mass Spectrom. 5* (1971) 505.
40. Herrmann, F., Matoušek, P., Dufka, O. and Churáček, J. *J. Chromatogr. 370* (1986) 49.
41. Mattson, M. and Petersson, G. *J. Chromatogr. Sci. 15* (1977) 546.
42. Kováčik, V., Škamla, J., Joniak, D. and Košiková, B. *Chem. Ber. 102* (1969) 1513.
43. Obst, J. R. *J. Wood Chem. Technol. 3* (1983) 377.
44. Niemelä, K. *J. Chromatogr. 446* (1988) 247.
45. Mamer, O. A., Crawhall, J. C. and Tjoa, S. S. *Clin. Chim. Acta 32* (1971) 171.
46. Niemelä, K. *Carbohydr. Res. 184* (1988) 131.
47. Niemelä, K. *J. Chem. Techn. Biotechnol. 48* (1990) 17.
48. Niemelä, K. *Holzforschung 43* (1989) 169.
49. Nishiwaki, T. *Tetrahedron 23* (1967) 2979.
50. Saiz-Jimenez, C. and de Leeuw, J. W. *J. Anal. Appl. Pyrol. 9* (1986) 99.
51. Whelan, J. K., Tarafa, M. E. and Sherr, E. B. *ACS Symp. Ser. 305* (1986) 62.
52. Mycke, B. and Michaelis, W. *Adv. Org. Geochem. 10* (1986) 847.
53. Burges, N. A., Hurst, H. M. and Walkden, B. *Geochim. Cosmochim. Acta 28* (1964) 1547.
54. Goheen, D. W. In: Sarkanen, K. V. and Ludwig, C. H., Eds., *Lignins, Occurrence, Formation, Structure and Reactions,* Wiley-Interscience, New York 1971, pp. 797–831.
55. Fengel, D. and Wegener, G. *Wood, Chemistry, Ultrastructure, Reactions,* de Gruyter, Berlin 1984.
56. Crawford, R. L. *Lignin Biodegradation and Transformation,* Wiley, New York 1981.
57. Bland, D. E., Logan, A. and Menshun, M. *Phytochemistry 7* (1968) 1373.
58. Erickson, M. and Miksche, G. E. *Phytochemistry 13* (1974) 2295.
59. Czapek, F. *Flora 86* (1889) 361.
60. Engman, B. *Der Sphagnolbegriff und seine Bedeutung für die Frage der Lignifizierung der Sphagnenzellwand,* Ph. D. Thesis, University of Kiel, Germany 1971.
61. Ertel, J. R. and Hedges, J. I. *Geochim. Cosmochim. Acta 48* (1984) 2065.
62. Wallis, A. F. A., In: Sarkanen, K. V. and Ludwig, C. H., Eds., *Lignins, Occurrence, Formation, Structure and Reactions,* Wiley-Interscience, New York 1971, pp. 345–372.
63. Van der Klashorst, G. H. and Strauss, H. F. *J. Wood Chem. Technol. 7* (1987) 325.
64. Spiteller, M. *Z. Pflanzenernaehr. Bodenkd. 144* (1981) 472.
65. Feather, M. S. and Harris, J. F. *Adv. Carbohydr. Chem. Biochem. 28* (1973) 161.
66. Andersson, H. and Hepburn, A. In: Fuchsman, C. H., Ed., *Peat and Water,* Elsevier, London 1986, pp. 177–194.
67. Ogner, G. *Soil Sci. 116* (1973) 93.
68. Bayer, E., Albert, K., Bergmann, W., Jahns, K., Eisener, W. and Peters, H.-K. *Angew. Chem., Int. Ed. Engl. 23* (1984) 147.
69. Bracewell, J. M., Abbt-Brown, G., de Leeuw, J. W., Hayes, M. H. B., Nimz, H. H., Norwood, D. L., Perdue, E. M., Schnitzer, M., Visser, S. A., Wilson, M. A. and Ziechmann, W. In: Frimmel, F. H. and Christman, R. F., Eds., *Humic Substances and their Role in the Environment,* Wiley, NY1988, pp. 151–164.
70. Streitwieser, A., Jr. and Heathcock, C. H. *Introduction to Organic Chemistry,* Macmillan, New York 1981.

Received June 8, 1990.