Alkaline Degradation of Peat Humic Acids. Part II. Identification of Hydrophilic Products

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

[a]Technical Research Centre of Finland, Combustion and Thermal Engineering Laboratory, P.O. Box 221, SF-40101 Jyväskylä, Finland and [b]Laboratory of Wood Chemistry, Helsinki University of Technology, SF-02150 Espoo, Finland

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Peat humic acids have been treated at 185 °C with 2 M sodium hydroxide solution, both in the presence and in the absence of sodium sulfide. About 6.3 % of the material was converted into aliphatic low-molecular-weight carboxylic acids, which were analyzed by capillary gas-liquid chromatography – mass spectrometry. In all, nearly 60 carboxylic acids were identified, of which glycolic, lactic, 2-hydroxy-2-methylpropanoic, oxalic, malic and 3,4-dideoxyhexaric acids were the main constituents. Minor products included a wide variety of saccharinic acids and related compounds. The nature of the reaction products suggests that they are derived, to large extent, via a peeling-type reaction from carbohydrates or carbohydrate-related structural units of the starting material. The presence of carbohydrate structures as integral parts of peat humic acids is discussed.

The main interest in chemical degradation studies of humic acids (HA) has been their lipophilic aromatic degradation products.[1-3] Little attention has been given to the analysis of hydrophilic degradation products of HA, particularly those resulting from alkaline degradation. However, since most chemical treatments of HA also result in the formation of hydrophilic compounds, any structural analysis limited to the lipophilic compounds will result in an inadequate description of HA.

As a continuation of our studies on low-molecular-weight degradation products of peat HA, we now report determinations of the hydrophilic products of the alkaline degradation of HA.

Experimental

The peat and the extraction procedure is described in part I.[3] Treatment of HA samples at 185 °C with 2 M NaOH or 2 M NaOH + 0.5 M Na_2S solution, followed by fractionation of the reaction mixture to lipophilic and hydrophilic compounds, has also been described in part I.[3]

Gas–liquid chromatography and mass spectrometry. The samples of hydrophilic compounds (containing *meso*-erythritol as the internal standard) were made alkaline with 2 M NH_4OH in order to convert the carboxylic acids into their ammonium salts. The solutions were evaporated to dryness and trimethylsilylated.[3] Analyses were done with a Hewlett–Packard 5890 A gas chromatograph equipped with a flame-ionization detector and SE-30 or SE-54 fused-silica capillary columns (0.32 mm i.d. × 25 m). The SE-54 column was mainly used to separate 2-hydroxy-2-methylpropanoic acid from glycolic acid.[4,5] The temperature program was 2 min at 95 °C, 15 °C min^{-1} to 245 °C, and 10 min at 245 °C. The temperature of both the injection port and the detector was 265 °C, and hydrogen was used as the carrier gas at a rate of 2 ml min^{-1}. Fig. 1 presents an example of the separation thus obtained.

The electron ionization mass spectra were recorded[3] at 70 eV with a Jeol JMS-DX303 instrument combined with a Hewlett–Packard 5790 A gas chromatograph and the above SE-30 column. The scanning range was 60 to 600 mass units.

Nearly all hydroxy monocarboxylic acids and dicarboxylic acids were identified on the basis of the abundant literature data and of earlier studies on their formation from various carbohydrates.[4-10] One pentenedioic acid (peak No. 15) exhibited a mass spectrum [*m/z* 274 (M^+, 6 %), 259 (24), 231 (3), 215 (9), 184 (7), 169 (3), 156 (11), 147 (100), 141 (7) and 73 (77)] nearly identical with that of authentic itaconic acid, but it eluted well before itaconic acid, making vinylmalonic acid a likely alternative.

Identification of benzenetricarboxylic acids was confirmed through comparison with commercial reference compounds. Their mass spectra were as follows (isomer, *m/z* and % of the base peak): **1**, **2**, **3**, 411 (M^+ − 15, 51), 381 (7), 337 (30), 323 (6), 309 (4), 249 (21), 219 (11), 175 (25), 147 (100), 133 (9), 103 (6); **1**, **2**, **4**, 426 (M^+, 4), 411 (47), 337 (17), 309 (9), 249 (10), 219 (5), 175 (8), 147 (100), 133 (9), 103 (5); **1**, **3**, **5**, 426 (M^+, 15), 411 (100), 396 (6), 339 (13), 337 (13), 321 (6), 309 (4), 293 (6), 249 (6), 219 (7), 198 (22), 177 (16), 147 (13), 103 (7). Apparently, only the

* Author to whom correspondence should be addressed.
† Present address: Alcoa of Australia Ltd, R&D Department, P.O. Box 161, Kwinana, WA 6167, Australia.

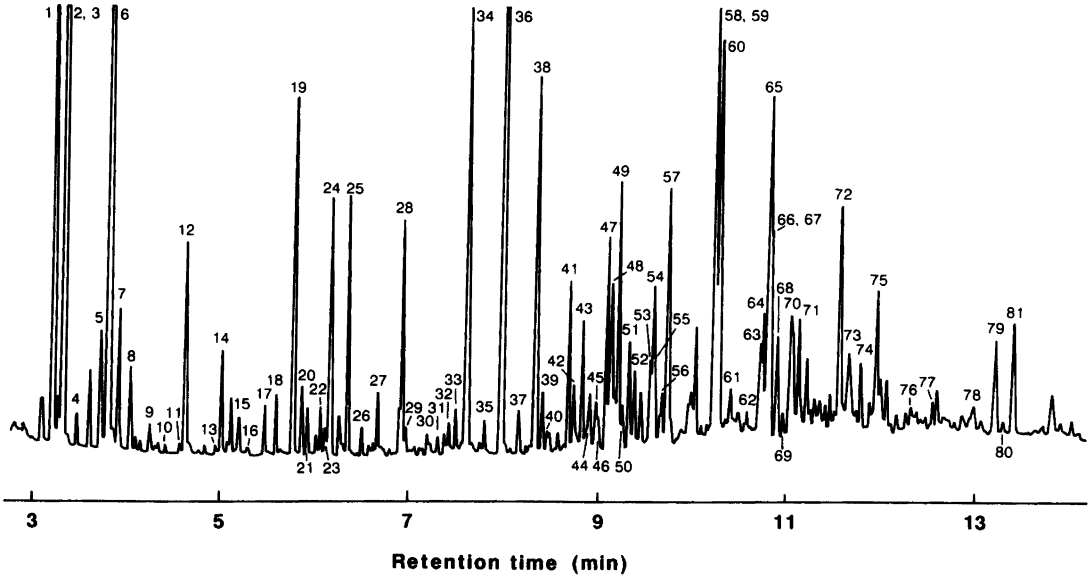

Fig. 1. Separation, on an SE-30 fused-silica capillary column, of trimethylsilylated carboxylic acids obtained after the treatment of humic acid with 2 M NaOH at 185 °C. List of acids: 1, lactic; 2, 2-hydroxy-2-methylpropanoic; 3, glycolic; 4, pyruvic (enolic); 5, levulinic; 6, oxalic; 7, 2-hydroxybutanoic; 8, 3-hydroxypropanoic; 9, 3-hydroxybutanoic; 10, 2-hydroxy-3-methylbutanoic; 11, a hydroxybutenoic; 12, malonic; 13, benzoic; 14, 4-hydroxybutanoic; 15, vinylmalonic (tentative identification); 16, maleic; 17, phosphoric; 18, glycerol; 19, succinic; 20, a branched hydroxyheptanoic; 21, methylsuccinic; 22, 2-*C*-methylglyceric; 23, glyceric; 24, fumaric; 25, *C*-methyltartronic; 26, resorcinol; 27, glutaric; 28, 3-deoxytetronic; 29, 3,5-dideoxy-*erythro*-pentonic; 30, 3,5-dideoxy-*threo*-pentonic; 31, a methylglutaric; 32, a dihydroxybenzaldehyde; 33, 2-deoxy-3-*C*-methyltetraric; 34, malic; 35, 3,4-dideoxypentonic; 36, erythritol (internal standard); 37, 3-hydroxybenzoic; 38, 2,3-dideoxypentaric; 39, 2,3-dideoxy-4-*C*-methylpentaric; 40, 1-guaiacylethanol; 41, 4-hydroxybenzoic; 42, hydroxyphenylacetic; 43, a branched hydroxyhexanedioic; 44, β-anhydroisosaccharinic; 45, xyloisosaccharinic; 46, α-anhydroisosaccharinic; 47, 3-deoxy-*erythro*-pentonic; 48, 2,3,4-trideoxyhexaric; 49, 3-deoxy-*threo*-pentonic; 50, *C*-(2-hydroxyethyl)-tartronic; 51, 3-deoxy-*erythro*-pentaric (+ unknown); 52, 3-deoxy-*threo*-pentaric; 53, a hydroxyheptanedioic; 54, *C*-(3-hydroxypropyl)-tartronic; 55, a hydroxyoctanedioic; 56, a hydroxyoctanedioic; 57, a dihydroxyheptanedioic; 58, 3,4-dihydroxybenzoic; 59, 3,4-dideoxy-*threo*-hexaric; 60, 3,4-dideoxy-*erythro*-hexaric; 61, a dihydroxyheptanedioic; 62, syringic; 63, 3-deoxy-*lyxo*-hexonic; 64, 3-deoxy-*xylo*-hexonic; 65, β-glucoisosaccharinic; 66, 3-deoxy-*ribo*-hexonic; 67, 3-deoxy-*arabino*-hexonic; 68, α-glucoisosaccharinic; β-glucoisosaccharinaric (3-deoxy-2-*C*-hydroxymethyl-*threo*-pentaric); 70, a dihydroxyoctanedioic; 71, a dihydroxyoctanedioic; 72, a dihydroxynonanedioic; 73, a dihydroxynonanedioic; 74, palmitic; 75, a dihydroxynonanedioic; 76, 1,2,3-benzenetricarboxylic; 77, 1,2,4-benzenetricarboxylic; 78, 1,3,5-benzenetricarboxylic; 79, stearic; 80, a trihydroxydecanedioic, and 82, a trihydroxydecanedioic.

mass spectrum of the derivative of 1,3,5-benzenetricarboxylic acid has been published[11] hitherto, and it differs slightly from that given here.

Results and discussion

Nearly 100 peaks appeared in the gas chromatograms (Fig. 1), of which over 70 compounds could be identified (Tables 1–3 and Fig. 1), including most of the main products. Although compounds of other types were also present, most of the compounds identified were carboxylic acids.

The aliphatics appear to be mainly of carbohydrate origin and their total yield was 6.4 % in the absence of Na_2S and 6.3 % in its presence. The yields were thus about twice as great as the yield of lipophilic, presumably lignin derived compounds (see Part I). All the aliphatic hydroxy monocarboxylic and dicarboxylic acids are frequently encountered among the alkaline hydrolysis products of carbohydrates like starch, xylan[5] and cellulose[9] but in yields more than ten times as great. The yields of the dicarboxylic acids in relation to the hydroxy monocarboxylic acids were greater for HA than for xylan, cellulose and starch.

The nature and the yield of hydrophilic products were slightly affected by the addition of Na_2S. In its presence yields of glycolic, glyceric and dicarboxylic acids increased. This increase may have resulted from oxidation, possibly through the conversion of sulfides into polysulfides.

Being lipophilic the aromatic compounds were only partially removed into the water phase. All twelve compounds identified (Table 3) are frequently reported as HA degradation products,[1,2] recovered mainly from the lipophilic fraction. Their quantity in the hydrophilic fraction was very small and was not included in the yield calculations. The benzenetricarboxylic acids were present in very low amounts.

Aliphatic hydroxy monocarboxylic acids. The yield of aliphatic hydroxy monocarboxylic acids (see Table 1) was 3.0 % in the absence of Na_2S and 2.9 % in its presence. Of the compounds we identified, only the lactic and

Table 1. Yields (mg g^{-1} charge) of hydroxy monocarboxylic acids obtained on treatment of HA with sodium hydroxide.

Carboxylic acid	NaOH	NaOH + Na_2S
Lactic	10.4	9.6
2-Hydroxy-2-methylpropanoic	3.4	2.7
Glycolic	8.8	9.6
2-Hydroxy-2-propenoic (pyruvic)	+[a]	+
2-Hydroxybutanoic	0.7	0.7
3-Hydroxypropanoic	0.3	0.3
3-Hydroxybutanoic	+	+
2-Hydroxy-3-methylbutanoic	+	+
4-Hydroxybutanoic	0.4	0.3
Glyceric	+	0.3
2-*C*-Methylglyceric	+	+
3-Deoxytetronic	0.9	1.0
3,5-Dideoxy-*erythro*-pentonic	+	+
3,5-Dideoxy-*threo*-pentonic	+	+
3,4-Dideoxypentonic	+	0.2
β-Anhydroisosaccharinic[b]	0.2	+
Xyloisosaccharinic	0.2	0.2
α-Anhydroisosaccharinic[b]	+	+
3-Deoxy-*erythro*-pentonic	0.9	0.8
3-Deoxy-*threo*-pentonic	1.1	1.1
3-Deoxy-*lyxo*-hexonic	0.3	0.2
3-Deoxy-*xylo*-hexonic	0.4	0.4
β-Glucoisosaccharinic	1.3	1.1
3-Deoxy-*ribo*- and -*arabino*-hexonic	0.8	0.8
α-Glucoisosaccharinic	0.4	0.4
Total amount mg g^{-1}	30.5	29.4

[a] +, traces (< 0.2 mg g^{-1}). [b] 4-Hydroxy-2-(hydroxymethyl)-tetrahydrofuran-4-carboxylic acids.

Table 2. Yields (mg g^{-1} charge) of dicarboxylic acids obtained on treatment of HA with sodium hydroxide.

Dicarboxylic acid	NaOH	NaOH + Na_2S
Oxalic	14.6	15.2
Malonic	1.0	0.9
Maleic	+[a]	+
Succinic	1.2	1.2
Methylsuccinic	+	+
Fumaric	1.1	1.3
C-Methyltartronic	1.1	1.0
Glutaric	0.3	0.2
Methylglutaric	+	+
2-Deoxy-3-*C*-methyltetraric	+	+
Tartronic	–	+
Malic	2.0	1.8
2,3-Dideoxypentaric	1.2	1.3
2,3-Dideoxy-4-*C*-methylpentaric	0.3	0.2
Hydroxyhexanedioic (branched)	0.5	0.4
2,3,4-Trideoxyhexaric	0.7	0.8
C-(2-Hydroxyethyl)tartronic	+	+
3-Deoxy-*erythro*-pentaric	0.3	0.3
3-Deoxy-*threo*-pentaric	0.3	0.4
C-(3-Hydroxypropyl)tartronic	0.7	0.6
Hydroxyheptanedioic	0.4	0.3
Dihydroxyheptanedioic[b]	1.2	1.2
3,4-Dideoxy-*threo*-hexaric	1.9	1.8
3,4-Dideoxy-*erythro*-hexaric	1.9	1.9
β-Glucoisosaccharinaric	+	+
Dihydroxyoctanedioic[b]	0.8	1.0
Dihydroxynonanedioic[c]	1.4	1.2
Trihydroxydecanedioic[b]	0.5	0.4
Total amount	33.3	33.4

[a] +, traces (< 0.2 mg g^{-1}). [b] Two isomers. [c] Three isomers.

2-hydroxybutanoic acids[12] seem to have been reported earlier among the alkaline degradation products of peat HA.

Glycolic, lactic and 2-hydroxy-2-methylpropanoic acids were the major compounds, comprising over 70% of the yield of the monocarboxylic acids. These acids are typical products of alkaline degradation of several, perhaps all, polysaccharide constituents.[4–10] The same is true also for all other three- and four-carbon-atom carboxylic acids.

Apart from 3-deoxy-*erythro*- and -*threo*-pentonic acids the five-carbon hydroxy acids were formed in low amounts. Of these, 3,4-dideoxypentonic (2,5-dihydroxypentanoic) and xyloisosaccharinic (3-deoxy-2-*C*-hydroxymethyltetronic) acids are characteristic products of 1→4 linked hexosans (cellulose and glucomannans) and pentosans (xylan) respectively.[13] Isomeric 3-deoxypentonic acids are characteristic degradation products of various polysaccharides,[5,7–9,14] even though their generation from numerous low-molecular-weight degradation products, such as glycolaldehyde cannot be excluded.[15] Small amounts of 3,5-dideoxypentonic (2,4-dihydroxypentanoic) acids may have originated[16] from 6-deoxyhexoses (rhamnose and fucose), known[17] to be present in HA, although the possibility of other sources cannot be fully excluded.[5,9]

Numerous six-carbon hydroxy acids, although formed in low amounts only, form an interesting class of degradation products, because their generation can be unambiguously traced to the degradation of hexose polysaccharides.[5,7,14] The formation of 3-deoxy-*xylo*- and 3-deoxy-*lyxo*-hexonic acids indicates the presence of galactose moieties, whereas the *ribo* and *arabino* forms have obviously been formed from glucose and/or mannose units.

Theoretically interesting are the saccharinic acids found among degradation products.[18–20] (See Table 4). They are indicative of the reactions that carbohydrate structures undergo in alkaline conditions – the reactions of peeling[13,21] and termination of peeling.[13,21] The presence of these com-

Table 3. The aromatic compounds identified.

Benzoic acid
Resorcinol
A dihydroxybenzaldehyde
3-Hydroxybenzoic acid
1-Guaiacylethanol
4-Hydroxybenzoic acid
Hydroxyphenylacetic acid
3,4-Dihydroxybenzoic acid
Syringic acid
1,2,3-Benzenetricarboxylic acid
1,2,4-Benzenetricarboxylic acid
1,2,5-Benzenetricarboxylic acid

Table 4. The saccharinic acids identified.

Three carbon atoms	
3-Deoxy-DL-glyceric	or DL-Lactic
Four carbon atoms	
3-Deoxytetronic	or 2,4-Dihydroxybutanoic
2-Methylglyceric	or 2,3-Dihydroxy-2-methylpropanoic
Five carbon atoms	
3-Deoxy-*erythro*-pentonic	
3-Deoxy-*threo*-pentonic	
Xyloisosaccharinic	or 3-Deoxy-2-*C*-hydroxymethyltetronic
Six carbon atoms	
α-Galactometasaccharinic	or 3-Deoxy-*xylo*-hexonic
β-Galactometasaccharinic	or 3-Deoxy-*lyxo*-hexonic
α-Glucometasaccharinic	or 3-Deoxy-*ribo*-hexonic
β-Glucometasaccharinic	or 3-Deoxy-*arabino*-hexonic
α-Glucoisosaccharinic	
β-Glucoisosaccharinic	

pounds suggests that carbohydrate moieties in the HA can undergo a peeling reaction. Metasaccharinic acids are produced during the alkaline degradation of 3-*O* substituted sugars and isosaccharinic acids during the degradation of 4-*O*-substituted sugars respectively.[22]

Aliphatic dicarboxylic acids. More than 30 different dicarboxylic acids were detected (Table 2), of which 23 could be fully identified. Their total amount was 3.3 % both in the presence and in the absence of Na_2S.

The most abundant dicarboxylic acid was oxalic acid, but several other simple alkanedioic acids (without hydroxy groups) were identified as well. All of these compounds have frequently been identified after alkaline hydrolysis or oxidation of different HA samples.[1,2,23] Apparently various substructural units can contribute to their formation, and thus they give limited information on the structure of the starting material.

Straight-chain hydroxy dicarboxylic acids included tartronic, malic, 2,3-dideoxypentaric (2-hydroxyglutaric), 3-deoxypentaric, 2,3,4-trideoxyhexaric (2-hydroxyadipic) and 3,4-dideoxyhexaric (2,5-dihydroxyadipic) acids. All of them may have originated from different carbohydrate structures of the charge material.[5,7,24] It is unclear whether the adipic acid derivatives have been formed by simple degradation reactions or whether condensation reactions are responsible for their formation.

Several branched-chain hydroxy dicarboxylic acids were also identified, including three derivatives of tartronic acid, 2-deoxy-3-*C*-methyltetraric (citramalic), 2,3-dideoxy-4-*C*-methylpentaric (2-hydroxy-2-methylglutaric) and β-glucoisosaccharinaric (3-deoxy-2-*C*-hydroxymethyl-*threo*-pentaric) acid. Without any doubt they all have been formed from polysaccharide materials, obviously mainly from those containing uronic or ulosonic acid structures.[24-26]

Unfortunately, numerous hydroxy dicarboxylic acids with seven to ten carbon atoms remained unidentified. Mass spectral data clearly indicated, however, that most of them were α-hydroxy acids. This type of compound may be condensation products formed during the treatments,[5,9] although the presence of such structural units in original material cannot readily be excluded.

The carbohydrates as structural components of HA. The *Sphagnum* mosses, the primary peat-forming plants, are made up almost entirely (90 %) of carbohydrates (dry matter basis). During humification, the carbohydrate content decreases but does not vanish.[27] Residual carbohydrate structures in peat are found in hot-water extracts,[28] fulvic acids,[29,30] humin[31] and HA.[32,33]

According to Scheffer and Kickuth[32] the HA of transitional peat, obtained by 0.1 M NaOH extraction from samples pre-extracted with ether, contained over 40 % carbohydrates.

It has been suggested that sugars are incorporated into peat HA by a special mechanism.[34] However it is also possible that humification is a biochemical process,[35] in which sugar incorporation occurs universally,[36] so that carbohydrates may also be integral parts of HA from non-peat sources.

Ogner[17] believes the polysaccharide fraction to be strongly associated with the humic matrix of podsol soil, and HA is part of this matrix. He studied methylated HA and observed further that the most important terminal groups are methyl-2,3,4,6-tetra-*O*-methylgluco- plus 2,3,4,6-tetra-*O*-methylmanno-, 2,3,4-tri-*O*-methylxylo- and 2,3,4-tri-*O*-methylfuco-pyranoside. Also present are terminal methyl-2,3,5-tri-*O*-methylxylo-, 2,3,5-tri-*O*-methylarabino- and 2,3,5,6-tetra-*O*-methylgalacto-furanoside units. According to Ogner, the most important chain units are 2,4,6-tri-*O*-methylglucoside, 2,3,- (or 3,4-) di-*O*-methylxylose and 3,4,6-tri-*O*-methylgalactose.

The ^{13}C NMR spectra of soil HA suggest that up to 40 % of the carbon may be bound in *O*-alkyl structures of carbohydrate origin.[37] Using isotope measurements, Dunbar and Wilson[38] were able to show that the oxygen of soil fulvic acids and HA was of carbohydrate origin.

Indeed, the presence in soils of complex structures such as lignocelluloses, and of proteinaceous enzymes, which are also possibly able to incorporate carbohydrate sequences into humic substances seem to make distinctions between humic and non-humic substances arbitrary, and without chemical significance. Unnecessary elimination of structural groups from HA, such as the carbohydrates, on the basis of an arbitrary definition may be one reason for the meagre success in HA structural determinations so far.

Conclusions. This study of the previously neglected hydrophilic fraction of the alkaline degradation products of peat HA provides new information on the aliphatic part of the HA structure, allowing the following conclusions to be drawn: (1) the quantity of identifiable hydrophilic reaction

products of alkaline degradation is more than twice that of lipophilic products; (2) the total yield of identifiable hydrophilic and lipophilic degradation products was ca. 10 %; (3) saccharinic acids produced in the alkaline degradation indicate the presence of the carbohydrate moieties which seem to be integral constituents of the peat HA structure and which probably undergo a peeling reaction; (4) 'humic substances' need to be redefined to take into account the possibility of significant carbohydrate-related sequences in their molecular structure.

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