DEPARTMENT OF CHEMISTRY, UNIVERSITY OF JYVÄSKYLÄ
RESEARCH REPORT No. 69

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Juha Hyötyläinen

Academic Dissertation
for the Degree of
Doctor of Philosophy

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This work is dedicated to Lassi, Tommi and my other relatives and friends,

also to those already dead and those not yet born.

CONTENTS

ABBREVIATIONS

Da	Dalton, unit of molecular weight
CE	Capillary electrophoresis
CE/MS	Capillary electrophoresis-mass spectrometry
CEC	Capillary electrochromatography
CZE	Capillary zone electrophoresis
DAD	Photodiode array detection
DOM	Dissolved organic matter
ECF	Elemental chlorine-free
EI	Electron impact
GC	Gas chromatography
GC/MS	Gas chromatography-mass spectrometry
GC-HRMS	Gas chromatography-high resolution mass spectrometry
GFC	Gel filtration chromatography
GPC	Gel permeation chromatography
HMMs	High-molecular-mass materials
HP	Hewlett Packard
HPLC	High performance liquid chromatography
HPLC/MS	High performance liquid chromatography-mass spectrometry
HRMS	High resolution mass spectrometry
I_{rel}	Relative intensity of peak
M	m/z of a molecular ion
MEKC	Micellar electrokinetic capillary chromatography
MP	Molecular weight corresponding to maximum peak
MSD	Mass selective detector
MS	Mass spectrometry
m/z	Mass-to-charge ratio
NMR	Nuclear magnetic resonance
ODS	Octadecyl silane
PC	Principal component
PCA	Principal component analysis
Py	Pyrolysis
Py-GC/MS	Pyrolysis-gas chromatography-mass spectrometry
RP	Reversed phase
RP-HPLC	Reversed phase-high performance liquid chromatography
SEC	Size exclusion chromatography
TCF	Totally chlorine-free
TIC	Total ion chromatogram
TMAH	Tetramethylammonium hydroxide
TMS	Trimethylsilyl
UV	Ultraviolet

PREFACE

These investigations were carried out during the years 1990-1998 at the Department of Chemistry, University of Jyväskylä, under supervision of Prof. Juha Knuutinen to whom I owe my gratitude for his guidance and encouragement throughout these years.

I would like to thank the Department of Chemistry for providing excellent working conditions and for a most friendly and stimulating atmosphere. I would also like to thank Prof. Raimo Alén for his valuable advice in the writing of this dissertation. My closest co-workers Petteri Malkavaara, Matti Ristolainen, Vesa Miikki and Esa Vilén deserve my thanks for their co-operation and for helping me in the use of analytical apparatus. I thank Michael Freeman for the revision of the language. Finally, I am indebted to both of my families and especially Marika for almost forcing me to finish this work.

Financial support for a part of this study from the Academy of Finland and the Maj and Tor Nessling Foundation is gratefully acknowledged.

Jyväskylä, December 1998

Juha Hyötyläinen

LIST OF ORIGINAL PAPERS

This thesis is based on the following five original papers, which are referred to in the text by their Roman numerals:

I. Hyötyläinen, J. and Knuutinen, J., Chemical Degradation Products of Lignin and Humic Substances. Part I. Synthesis, Structure Verification and Gas Chromatographic Separation of Chlorinated Vanillins and Syringaldehydes. *Chemosphere* **26** (1993) 1843-1858.
https://doi.org/10.1016/0045-6535(93)90078-J

II. Hyötyläinen, J., Chemical Degradation Products of Lignin and Humic Substances. Part II. Synthesis, Structure Verification and Liquid Chromatographic Separation of Chlorinated Protocatechualdehydes (3,4-dihydroxybenzaldehydes), 5-Hydroxyvanillins (3,4-dihydroxy-5-methoxybenzaldehydes) and Gallaldehydes (3,4,5-trihydroxybenzaldehydes). *Chemosphere* **28** (1994) 1641-1656.
https://doi.org/10.1016/0045-6535(94)90423-5

III. Hyötyläinen, J., Knuutinen, J. and Vilén, E., Characterization of High Molecular Mass Fractions of Receiving Waters and Sediments of a Pulp Mill by CuO-oxidation and HPLC. Chemical Degradation Products of Lignin and Humic Substances. Part III. *Chemosphere* **30** (1995) 891-906.
https://doi.org/10.1016/0045-6535(94)00448-4

IV. Hyötyläinen, J., Knuutinen, J., Malkavaara, P. and Siltala, J., Pyrolysis-GC-MS and CuO-Oxidation-HPLC in the Characterization of HMMs from Sediments and Surface Waters Downstream of a Pulp Mill. (Chemical Degradation Products of Lignin and Humic Substances. Part IV.). *Chemosphere* **36** (1998) 297-314.
https://doi.org/10.1016/S0045-6535(97)10006-6

V. Hyötyläinen, J., Knuutinen, J. and Malkavaara, P., Transport of High Molecular Mass Lignin Material In the Receiving Water System of a Mechanical Pulp Mill. (Chemical Degradation Products of Lignin and Humic Substances. Part V.). *Chemosphere* **36** (1998) 577-587.
https://doi.org/10.1016/S0045-6535(97)00366-4

ABSTRACT

A method was developed to monitor qualitatively the transport of lignin-like high-molecular-mass materials (HMMs), such as lignins and humic substances. This method was used to monitor HMMs discharged by two mills producing kraft and mechanical pulps, respectively. Several water and sediment samples were collected up and downstream of these mills. The method included the separation of HMMs from water samples by ultrafiltration followed by the alkaline CuO oxidation of the separated HMMs. Freeze-dried sediment samples were oxidised as such. The degradation products formed were analysed by RP-HPLC and the ratios of 4-hydroxyacetophenone to vanillin or all 4-hydroxyphenyl compounds to guaiacyl-type compounds were used to study the transport of HMMs from different sources. The results were compared to those obtained using pyrolysis-GC/MS. Attempts were also made to analyse the oxidation products using GC/MS instead of HPLC, but the use of HPLC has some advantages over GC/MS and is therefore preferred. Principal component analysis was used to select the compounds suitable for monitoring the transport of HMMs. Several chlorinated and non-chlorinated compounds were synthesized as model compounds for the identification of CuO oxidation products of HMMs. The structures of the model compounds were verified by mass spectrometry and their chromatographic behaviour discussed.

1 INTRODUCTION

Characterization of the different HMMs is a prerequisite for being able to monitor the transport of HMMs produced by a pulp mill. When using degradation methods, like CuO oxidation, this means finding the characteristic degradation products which can be used to selectively distinguish the HMMs produced by industry from naturally occurring humic substances.

The quantitation of the products formed in oxidation does not necessarily give any straightforward information on the material oxidised. In the case of CuO oxidation a large part of the material remains as HMMs and is discarded in preparing the sample for analysis. Also, using UV detection in HPLC, it is possible to detect only UV-absorbing species, i.e., compounds containing multiple conjugated double bonds. Therefore, comparing the peak areas of selected oxidation products is a useful way of identifying HMMs. In the case of gas chromatography, the detection methods are usually less selective and a higher proportion of the oxidation products can be detected. In this case, the restrictions for detecting a specific compound lie in the ability of the derivatising agent used to react with the compound in question and in that of the chromatographic system to separate it from other compounds. In pyrolysis, a larger proportion of the material is transformed into pyrolysis products. On the other hand, the type and amounts of products depend more on the pyrolysis conditions than when using cupric oxide (CuO) oxidation and the pyrolysis products resemble the original structure less than do the CuO oxidation products.

The aim of this study was to develop a method to follow the transport of HMMs in the receiving waters of a pulp mill. The basic idea was to apply CuO oxidation to the HMMs separated from water and sediment samples, and after analysis of their oxidation products consider their origin. Samples were taken from two watercourses having two different sources of industrial HMMs: a kraft pulp mill in Äänekoski using bleaching with chlorine dioxide and a mechanical pulp mill in Lohja using dithionite bleaching. Two degradation methods (Py-GC/MS and CuO oxidation) were tested using the samples collected from the Äänekoski watercourse. In the case of the Lohjanjärvi watercourse, the CuO oxidation products of the collected samples were analysed using both RP-HPLC and GC/MS. The HMMs from the Äänekoski watercourse have previously been characterised using various analytical methods.[1]

2 LIGNINS AND HUMIC SUBSTANCES

Lignins and humic substances are macromolecular materials occurring in nature. The exact structure of these materials remains unresolved.[2,3] Humic substances can be divided into several different groups, all of which have been exhaustively reviewed by Peuravuori.[4] These substances are formed in the nature from decaying plant materials in humification processes. One of the most remarkable characteristics of humic compounds is their wide distribution. They can be found in almost all land and water environments.[5]

Humic substances have many similarities with lignins. Native lignins are essential components in arborescent gymnosperms and angiosperms, and their amounts in wood stems, for example, range from 15 % to 36 %.[6] A variety of different lignin-type materials are released into the receiving water systems from pulp mills. Chlorolignins, formed in the bleaching of pulp with chlorine, the so-called ”TCF lignins”, formed in total chlorine-free (TCF) bleaching and ”ECF lignins”, formed in elemental chlorine-free (ECF) bleaching, still bear a close resemblance to the native lignins in wood materials.[7]

In this report, HMMs are defined as materials separated from water samples using a membrane with a cut-off value of 1000 Da in ultrafiltration, or similar material existing in sediment samples as such. It is usually considered that compounds of this molecular mass range are non-bioaccumulative and non-toxic because of their large molecular size and water solubility.[8,9] However, it has recently been reported that chlorolignin is bioaccumulative in certain organisms and the major toxicant in Echinoderm bioassays of bleached pulp mill effluents.[10] It is also possible that the chlorolignin may decay in the receiving waters, forming hazardous compounds.[11-13]

During the last ten years considerable process improvements have taken place in the pulp industry. Perhaps the most important of these concern the changes in bleaching processes. Bleaching stages based on chlorine gas have been replaced by ECF or by TCF bleaching sequences in many plants in Northern Europe and in North America. Bleaching with chlorine gas ceased in Finland in 1993.[14] This development offers interesting new challenges for scientists in attempts to find out the effects of the low-molecular-mass compounds produced by the new bleaching methods. Generally, the HMMs produced by these bleaching methods have received much less attention than the low-molecular-mass compounds. Recently, Mikkelson and Paasivirta have identified more than 300 different low molecular weight compounds in TCF and ECF wastewater samples.[15]

2.1 Ultrafiltration

Before ultrafiltration the so-called "particulate fraction" is usually separated from the sample.[16] This is traditionally done using a 0.45 μm filter membrane, but can more conveniently be done using a prefiltration membrane pack in the ultrafiltration apparatus. Prefiltration is followed by the actual ultrafiltration, where fractions of HMMs can be separated from the sample using membrane packs of a certain cut-off molecular mass. If the aim is only to separate a presentable fraction of HMMs, it is adviseable to use the membrane pack with the lowest cut-off value. The process of ultrafiltration concentrates the HMMs in a small amount of water, which has to be washed with deionised water in order to get rid of residual low-molecular-mass compounds. Finally, the small amount of water containing the desired compounds is frozen and lyophilised. The latter step is the most time-consuming in the separation process and it yields the HMMs as a light, usually brown powder.

Compared to other methods used to separate HMMs from water, ultrafiltration is an efficient method enabling larger sample sizes than in many other methods. Ultrafiltration has previously been used in the separation and fractionation of HMMs from natural waters,[16-20] pulp mill effluents,[21] process waste waters,[22-24] chlorinated natural humic water[25] and black liquor,[26] in studying the aggregation properties of aquatic fulvic acids[27] and to determine the sources of dissolved organic carbon in lake water.[28] Also, the dependence of chlorolignin removal on pH[29] and the interactions between chlorolignin and the ultrafiltration membranes[30] have been studied.

2.2 Degradation of HMMs to low-molecular-weight compounds using CuO oxidation

The various oxidants applicable to the oxidation of lignin-like materials can be classified by their effects on these materials. Strong oxidants (like permanganate) destroy the aromatic rings, and produce low-molecular-mass acids. On the other hand, mild oxidants do not break the ring and oxidation occurs at the side chain. The group of mild oxidants contains nitrobenzene, oxygen and several metal oxides, such as CuO. All of the mild oxidants are used in alkaline conditions. Alkaline CuO oxidation was first used in 1942 to digest solids in sulphite spent liquors.[31] It has since been applied to several different HMMs, in order to degrade them into low-molecular-mass compounds that are easy to analyse compared to the original HMMs (see references in Paper **III**). The oxidative methods used to degrade humic substances have been reviewed by Christman *et al.*[32,33]

Some attempts to resolve the mechanism of CuO oxidation have also been conducted.[34-36] Compared to other methods applied to degrade HMMs, CuO oxidation appears to be more selective.[37] The reactions in CuO oxidation take place in ~~the~~ those groups of humic or lignin compounds that are able to form phenoxy radicals and only ineffectively degrade other aromatic structures. Oxidation products consist mainly of phenolic aldehydes, ketones and acids.[38,39] Most works related to CuO or nitrobenzene oxidations deal with the oxidation of lignin to obtain phenolics (mainly vanillin) for commercial purposes. These two oxidation methods have recently been reviewed.[40] The yields of oxidation products in these applications are generally about 30 %. Usually the reagent used is copper(II)sulphate, which is transformed to CuO by heating. Temperatures from 160 to 190 °C and reaction times from 120 to 240 minutes are used. The amount of sodium hydroxide used is 65 - 70 ml of 2 - 2.5 molar solution per gram of HMMs and 4 - 14 moles CuO is used per a structural unit of lignin, which is in turn defined as having a molecular weight of 195 g/mol.

The use of CuO oxidation in the structural characterisation of various HMMs has been described in several studies.[41-46] CuO oxidation has also been used to study coal formation,[47] lignins from various plants,[48-52] chlorolignins,[53-58] lignosulphonic acids,[59] humic and fulvic acid fractions separated from peat[60-62] and other humic materials,[37,39,63-69] peat,[70] sediments,[71-78] bitumen separated from composted materials,[79] lignins degraded by fungi,[80] forest floor dissolved organic matter (DOM),[81] and even drilling fluid constituents[82] or organic polymeric material of a meteorite.[83] However, few reports have appeared on the CuO oxidation of HMMs separated from water samples[84-88] or on the analysis of oxidation products using HPLC.[89]

2.3 Synthesis of model compounds

In order to verify the identity of oxidation products a large number of model compounds were required. The appearance of chlorovanillins and chlorovanillic acids among the nitrobenzene[90] and CuO[56-58] oxidation products of chlorolignin prompted the synthesis of these and related model compounds in order to study their existence in CuO oxidation products. Many of them were obtained commercially, but for some the only way was to synthesise authentic model compounds. The routes used were relatively simple but the purification steps were in some cases rather arduous. For example, the purification of some products formed by direct chlorination (e.g., the preparation of 2,5-dichloroprotocatechualdehyde) were difficult and time-consuming because the impurities were usually isomers of the desired compounds.

2.4 Chromatographic analysis of hydroxybenzaldehydes, -benzoic acids and -acetophenones

Many of the phenolics obtained as oxidation products in CuO oxidation exist in nature. Analysis of naturally occurring phenolics have been conducted by several authors by HPLC[44,91-102] or GC.[95,103] Vanillin and related flavour compounds have also been analysed in various food materials[104-106] and beverages[107-109] using HPLC. The analysis of lignin degradation products by HPLC has been a subject of several investigations[68,89,96,110-113] but it has received less attention than the corresponding analysis using GC.[41,45,49,114-120] However, even though the separation and analysis of chlorinated hydroxybenzaldehydes and benzoic acids have been widely studied using GC,[121-128] the chlorinated guaiacyl and syringyl compounds possibly occurring among chlorolignin oxidation products have received less attention.[129] The photodiode array detection (DAD, mistakenly referred to as PAD in Papers I to IV) technique in HPLC offers a possibility to identify oxidation products on the basis of their UV spectra.[130] However, this can be applied to only those compounds that have significant UV absorbance. Modern capillary electrophoretic techniques have recently received some attention as possible methods for the analysis of different phenolics[131,132] and there will no doubt be interesting work done in this field in the future.

2.5 Mass spectrometry

Mass spectra were used to confirm the structures of the synthesized model compounds and to study whether the identification of isomeric oxidation products could be based on their mass spectra. The mass spectra of non-chlorinated model compounds exist in the literature,[133] but the mass spectra of chlorinated model compounds have not previously been studied in detail.

2.6 Py-GC/MS of the HMMs

In the 1970's analytical pyrolysis was shown to give additional information on the chemistry of humic substances and other macromolecular organic matter and has since been applied to the study of such materials.[3,134-139] However, the new pyrolysis-GC/MS (Py-GC/MS) methods developed in the late 1980s and applied in the early 1990s in studies of the aforementioned materials have shown that the information obtained can be misleading, and if not analysed with caution, can lead to misinterpretations about the actual structure of the macromolecules studied.[140-144] The use of improper pyrolysis conditions can lead to aromatisation of the aliphatic structures and thus distort the product distribution. The pyrolytic methods used in lignin analyses have been reviewed by Meier and Faix[145] and the corresponding analyses of humic substances by Bracewell *et al.*[146] There are numerous applications of pyrolytic methods in the analysis of wood[147-149] and other plants,[150] aquatic HMMs,[151-155] compounds in process waters,[156] soil fractions,[157-160] coal[161] and halogenated HMMs.[162-165]

2.7 Size exclusion chromatography

In size exclusion chromatography (SEC) the separation of molecules is based on the molecular size of compounds in the analyte. SEC methods can be classified as gel filtration chromatographic (GFC) or gel permeation chromatographic (GPC) methods, depending on whether the moving phase is aqueous or organic, respectively. Direct analysis of DOM in natural waters has earlier been conducted in our laboratory.[166,167] Aquatic humic substances[25,168-171] and lignins[24] have been analysed using various SEC methods.

3 EXPERIMENTAL

3.1 Samples and separation of HMMs

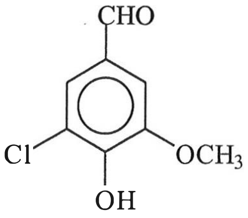
Six water and three sediment samples from the Äänekoski watercourse were collected in March 1993 and March 1994 (Papers III and IV) and thirteen water samples were collected from the Lohjanjärvi watercourse (Paper V) between September and November 1996. Ultrafiltration was used to separate the HMMs from the water samples. In the case of the Äänekoski watercourse two membranes and one prefiltration membrane were used and three fractions obtained from each water sample. In the separation of HMMs from the samples of the Lohjanjärvi watercourse one extra washing stage was used. This was done because some of the samples were sea water containing higher amounts of inorganic salts. Also, only one membrane was used and therefore only one fraction was obtained from these samples. In Paper III the sediment samples were freeze-dried and oxidised as such. The extraction of HMMs from the same sediment samples using 2 M sodium hydroxide was performed as described in Paper IV.

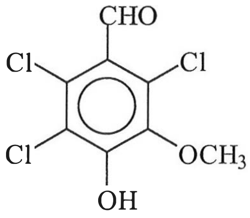
3.2 Degradation methods

The method used in CuO oxidation was a modification of the method of Hedges and Ertel.[51] The Py-GC/MS experiments were carried out using a system consisting of a CDS Pyroprobe 1000 pyrolyser, a HP 5870 Series plus GC and a HP 5892 mass selective detector (MSD). The conditions were as described (Paper V).

3.3 Preparation of model compounds

The model compounds synthesized (Papers I-III) can be divided into five groups (A to E, see below), based on the method of the synthesis used. References mentioned after a method refer to the general procedure of the synthesis and those mentioned after the name of the compound to the data found in the literature referring to the compound in question. Compounds marked with an asterix (*) are compounds for which no synthetical reference was found in the literature.


5-chlorovanillin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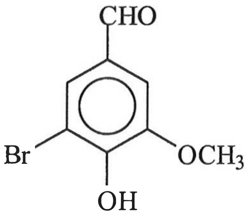
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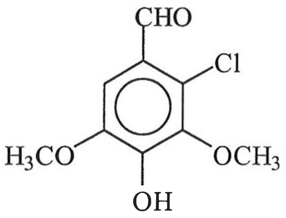
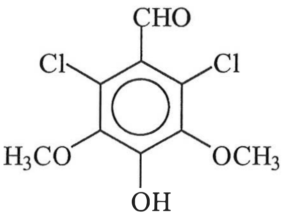

chlorosyringaldehyde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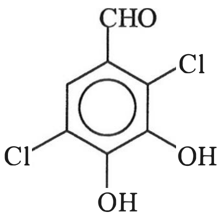
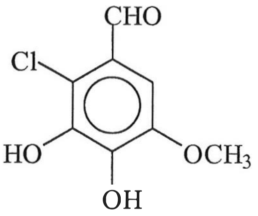

2,5-dichloroproto-
catechualdehyde*


6-chloro-5-hydroxyvanillin*

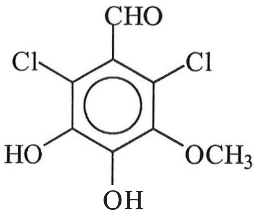
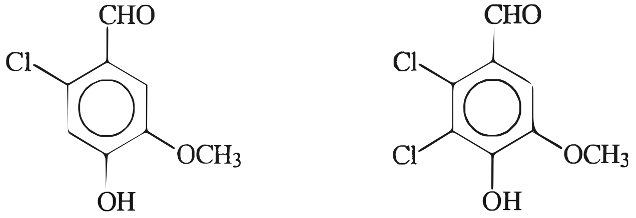

dichloro-5-hydroxyvanillin*

Fig. 1. The model compounds synthesized by direct chlorination or bromination.

A. 1. Direct chlorination[172,173] **or bromination**[174] **of benzaldehydes and benzoic acids or chlorination of their derivatives** were used to prepare the following compounds (Fig. 1): 5-chlorovanillin,[172] trichlorovanillin,[172] 5-bromovanillin,[174] chlorosyringaldehyde,[129] dichlorosyringaldehyde,[129] 2,5-dichloroprotocatechualdehyde,* 6-chloro-5-hydroxyvanillin* and 2,6-dichloro-5-

hydroxyvanillin.* First the starting material was dissolved in a solvent that enhances chlorination and does not dissolve the chlorinated product, so that the material can be separated by filtration after chlorination. Then, to bring about the chlorination, either chlorine gas was bubbled through a solution of the starting material or a calculated amount of sulphuryl chloride was added to the solution. Bromination was done accordingly, adding a calculated amount of bromine to the acetic acid solution of the starting material.

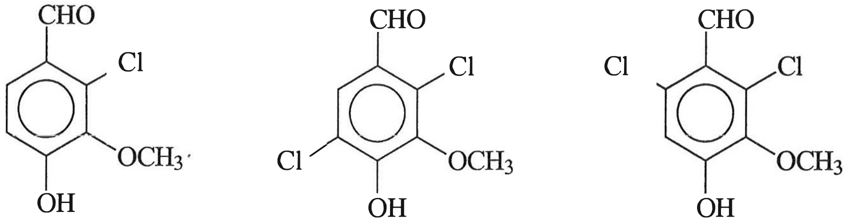
A. 2. Chlorination of triacetates. 6-chlorovanillin[172] and 5,6-dichlorovanillin[172] were synthesized by chlorinating the triacetates of vanillin and 5-chlorovanillin,[172] respectively (Fig. 2). In both cases, chlorine gas was used in the chlorination.



6-chlorovanillin 5,6-dichlorovanillin

Fig. 2. The model compounds synthesized by the chlorination of triacetates.

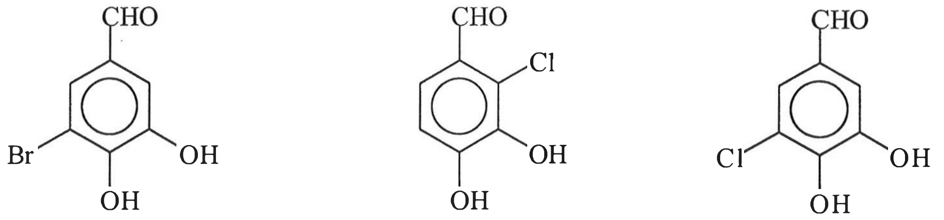
B. Limited methylation[175,176] of dihydroxy compounds to monomethylethers was used to prepare the following compounds (Fig. 3): 2-chlorovanillin,[172] 2,5-dichlorovanillin[172] and 2,6-dichlorovanillin.[172] A calculated amount of dimethyl sulphate was added to the alkaline solution of the starting material. After mixing for 15 minutes, the solution was acidified and the products separated using column chromatography on silica gel.



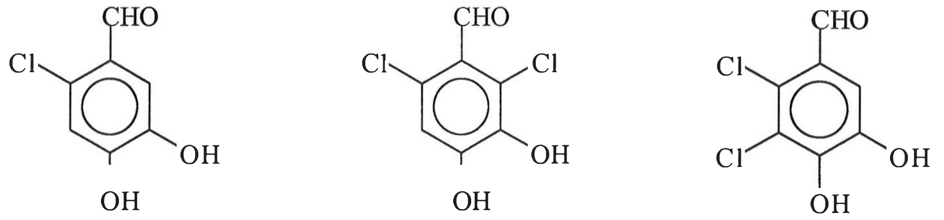
2-chlorovanillin 2,5-dichlorovanillin 2,6-dichlorovanillin

Fig. 3. The model compounds synthesized by the limited methylation of dihydroxybenzaldehydes.

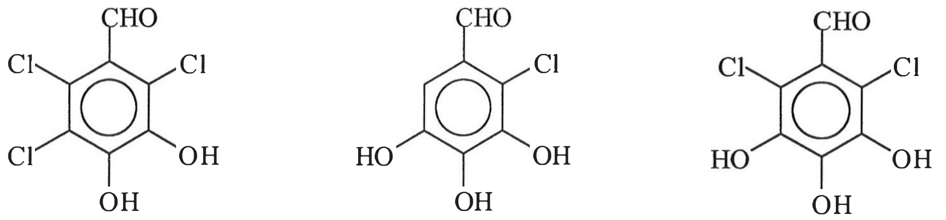
C. Demethylation of compounds[177] containing methoxyl groups was used to prepare the following protocatechualdehydes and gallaldehydes (Fig. 4): 5-bromo-,[178,179] 2-chloro-,[177] 5-chloro-,[177] 6-chloro-,[177] 2,6-dichloro-,* 5,6-dichloro-[180,181] and trichloroprotocatechualdehyde,* chlorogallaldehyde* and dichlorogallaldehyde.* Boron tribromide was added to a slurry of the starting material in dichloromethane and the mixture was allowed to react for six hours. After this period, methanol was added to the solution and it was refluxed for half an hour.



5-bromoprotocatechualdehyde 2-chloroprotocatechualdehyde 5-chloroprotocatechualdehyde



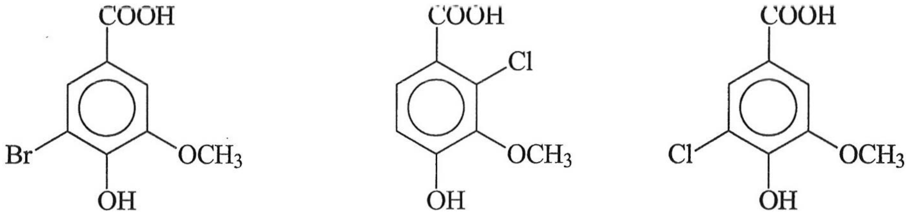
6-chloroprotocatechualdehyde 2,6-dichloroprotocatechualdehyde* 5,6-dichloroprotocatechualdehyde



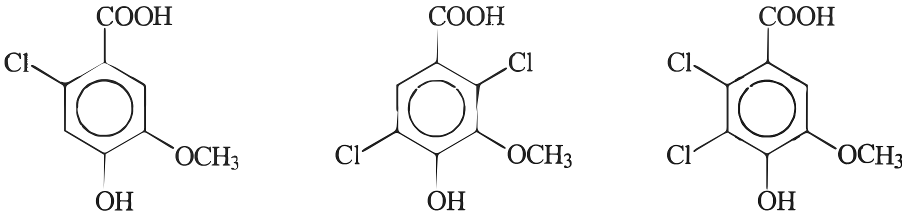
trichloroprotocatechualdehyde* chlorogallaldehyde* dichlorogallaldehyde*

Fig. 4. The model compounds synthesized using demethylation with boron tribromide.

D. Oxidation of benzaldehydes to benzoic acids using either a direct[182-184] or an indirect method.[185] The HPLC separation and synthesis[186,187] of some chlorinated vanillic acids have been reported earlier. The above methods were applied to the synthesis of 5-bromovanillic acid,[185] 2-chlorovanillic acid,[185] 5-chlorovanillic acid,[185,188] 6-chlorovanillic acid,[185] 2,5-dichlorovanillic acid[185] and 5,6-dichlorovanillic acid[185] (Fig. 5). In the direct method, an alkaline solution of hydroxybenzaldehyde was treated with silver(I)oxide and the hydroxybenzoic acid formed was separated from the acidified solution either by filtration or by extraction. In the indirect oxidation, the oximes were first prepared by refluxing the aldehydes with hydroxylamine hydrochloride. Oximes were then converted to the acetates of corresponding vanillonitriles, which could be hydrolysed to vanillic acids.



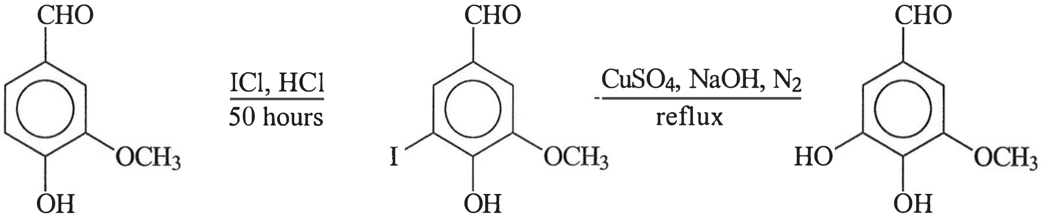
5-bromovanillic acid 2-chlorovanillic acid 5-chlorovanillic acid



6-chlorovanillic acid 2,5-dichlorovanillic acid 5,6-dichlorovanillic acid

Fig. 5. The model compounds synthesized using the oxidation of hydroxybenzaldehydes.

E. Other methods. 5-hydroxyvanillin[189-196] was prepared from 5-iodovanillin using the method of Nishinaga and Matsuura[197] (Fig. 6). To produce the desired compound, 5-iodovanillin was refluxed with copper(II)sulphate under a nitrogen atmosphere and the product was separated by extracting the resulting solution several times with diethyl ether.



vanillin 5-iodovanillin 5-hydroxyvanillin

Fig. 6. The synthesis of 5-hydroxyvanillin.

Fries rearrangement[198,199] was used to prepare 6-chloroacetovanillone[200,201] (Fig. 7). The acetate of 5-chloroguaiacol was heated with aluminium(III)chloride and the mixture of reaction products was separated by column chromatography.

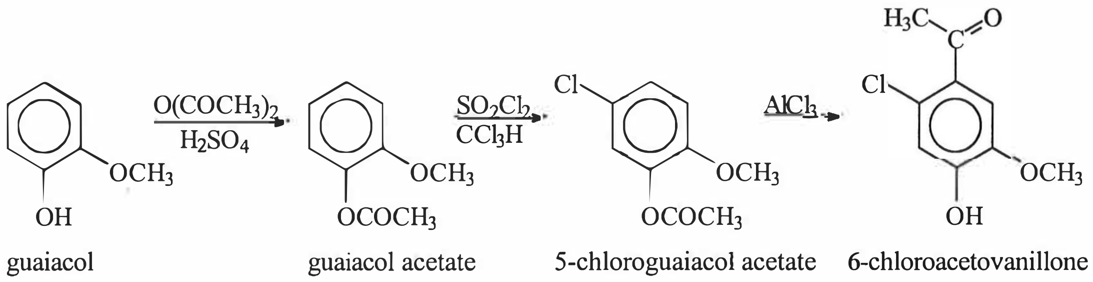


Fig. 7. The synthesis of 6-chloroacetovanillone.

5-formylvanillin[202] was prepared by the formylation of guaiacol[203] (Fig. 8). Guaiacol was heated with hexamethylenetetramine and paraformaldehyde. First acetic acid and later sulphuric acid were added to the mixture. The product was separated from the resulting solution by extraction and recrystallised from toluene.

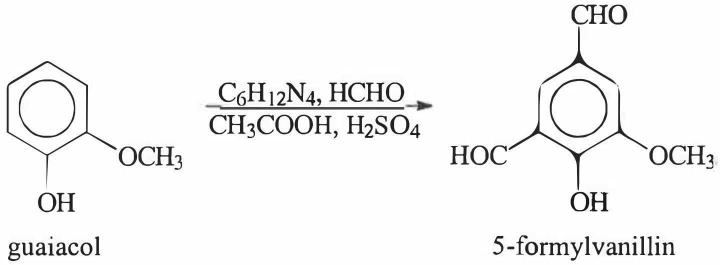
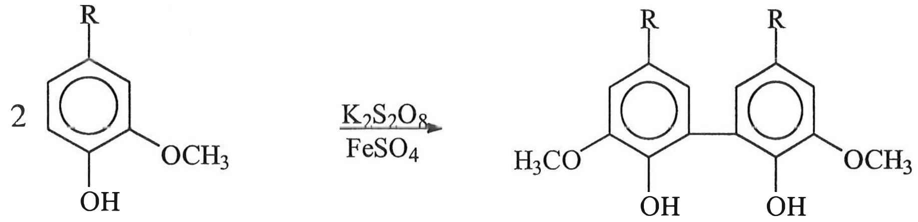


Fig. 8 The synthesis of 5-formylvanillin.

Dehydrodivanillin[204-206] and dehydrodiacetovanillone* were prepared according to the method of Drumond *et al.*[207] (Fig. 9). Vanillin was dissolved in water and a small amount of acetone was added to enhance solubility. $K_2S_2O_8$ and iron(II)sulphate were added and the solution was allowed to mix for one week. The crude product was separated by filtration and purified with recrystallisation from acetic acid.



vanillin, R = CHO
acetovanillone, R = $COCH_3$

dehydrodivanillin, R = CHO
dehydrodiacetovanillone*, R = $COCH_3$

Fig. 9. The syntheses of dehydrodivanillin and dehydrodiacetovanillone.

3.4 Gas chromatographic separation of model compounds and oxidation products

Vanillin, chlorinated vanillins, 5-bromovanillin, syringaldehyde and chlorinated syringaldehydes were separated as their acetyl derivatives (Paper III). The hydroxyl groups can be easily acetylated or silylated to produce volatile derivatives. In the case of hydroxybenzoic acids the analysis using HPLC was preferred because the carboxyl groups can not be acetylated and silylation complicates the prediction of the structure as based on the mass spectra. Several gas chromatographic conditions were tested for the separation experiments (Paper I), for the Py-GC/MS experiments of HMMs from the Äänekoski watercourse (Paper IV) and for the analysis of oxidation products from the Lohjanjärvi watercourse (Paper V). Chromatographic conditions are described in detail in the corresponding papers.

The structures of the model compounds synthesized (Papers I and II) were confirmed by mass spectrometry (MS). In addition to MS, further verification of the structures were obtained by NMR spectroscopy of chlorovanillins[208,209] and some related compounds, such as protocatechualdehydes.[210] However, because NMR spectroscopy can not be used to identify compounds from complex mixtures of oxidation products, the detailed analysis of the NMR-spectra is not reported here. In addition to the direct inlet mass spectra, the mass spectra of their acetylated derivatives were obtained using GC-HRMS. All the mass spectra were obtained by the electron impact (EI) mode. In some cases, the analysis of mother and daughter ions were used to determine the origin of a particular peak. Previously, the fragmentations of vanillin and syringaldehyde based on the fragmentation of deuterated model compounds have been presented.[211] Also, the mass spectra of protocatechualdehyde,[212] trimethylsilyl (TMS) derivatives of several non-chlorinated hydroxybenzoic acids and hydroxybenzaldehydes,[213] and 4-hydroxybenzaldehyde together with all its chloroderivatives[214] have been published earlier.

3.5 HPLC

In the HPLC analysis sample preparation was much easier as no prior derivatisation was needed. Moreover, the problems rising from the inadequate derivatisation of some compounds could be avoided. HPLC separations were carried out using a Waters liquid chromatographic system equipped with a DAD. The column used was Spherisorb ODS 5. The conditions in separating the dihydroxy and trihydroxy compounds synthesized (Paper II) were almost the same as those used in the analysis of oxidation products of HMMs from the Äänekoski watercourse (Papers III and IV) and Lohjanjärvi watercourse (Paper V).

3.6 GPC

GPC was used to analyse the tetrahydrofurane extracts obtained from the sediments of the Äänekoski watercourse (Paper IV). Four 300 mm x 7.8 mm Waters Styragel™ HR columns (HR 0.5, HR 1, HR 2 and HR 3) coupled from highest to lowest pore size so that the exclusion limit was 30,000 Da and the same HPLC system as described above were used.

3.7 Principal component analysis

The analytical data (from the pyrolysis and cupric oxide oxidations in Paper IV and from the cupric oxide oxidations in Paper V) were scaled to unit variance prior to the multivariate calculations. A number of principal components (PCs) was calculated using the singular value decomposition. All chemometric calculations were done using MATLAB[215] software on a personal computer.

4 RESULTS AND DISCUSSION

4.1 Preparation of model compounds

The direct chlorination of different 3,4-disubstituted benzaldehydes gave an illustrative example of the substitution-directing effect of methoxyl groups and phenolic hydroxyl groups. In vanillin, the hydroxyl group in position para (or 4) to the formyl group causes substitution to take place at position 5, or ortho to the hydroxyl group. In the case of 3,4-dihydroxybenzaldehyde (protocatechualdehyde) chlorination easily proceeds to the dichloro stage. However, the resulting 2,5-

dichloroprotocatechualdehyde is rather impure, the resulting mixture containing small amounts of all monochloro and dichloro compounds.

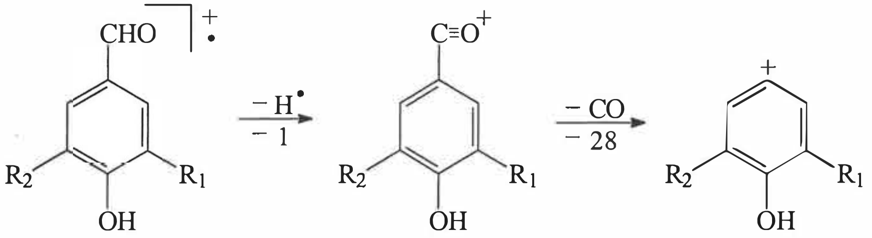
The acetylation of the phenolic hydroxyl group and formyl group causes substitution to take place at position 6. In isovanillin, where the hydroxyl group is in position 3, substitution takes place at position 2, again next to the hydroxyl group and in some extent at position 6, para to the hydroxyl group. In the chlorination of veratraldehyde (3,4-dimethoxybenzaldehyde), substitution takes place at position 6, the reaction being much slower than when an activating phenolic hydroxyl group is attached to the benzene ring. Also, the number of chlorine atoms in the compound has an effect on the reactivity. The oxidation of polychlorinated aldehydes is difficult compared to the oxidation of non- or monochlorinated benzaldehydes.

The compounds studied can also be grouped according to their most important functional groups (benzaldehydes, benzoic acids or phenyl ketones) or according to the type of lignin they represent (4-hydroxyphenyl, guaiacyl and syringyl compounds). Almost all of the compounds synthesized are monomeric aromatic compounds with two to six substituents. An exception to this are the two dimeric guaiacyl compounds, dehydrodivanillin and dehydrodiacetovanillone. In addition to the chlorinated and non-chlorinated compounds, one brominated compound (5-bromovanillin) was synthesized to be used as an internal standard in the quantitative analysis. The chlorinated compounds previously used as model compounds for the oxidation products of chlorolignin will also be of importance in the future, because ECF bleaching continues to produce HMMs containing chlorine and large amounts of chlorolignins still contaminate the sediments downstream of pulp mills.

4.2 Mass spectrometry of model compounds

The mass spectra of chlorinated vanillins and protocatechualdehydes have similarities with the mass spectra of chloroguaiacols[216] and chlorocatechols,[217] respectively. The most important features of the spectra can be summarised as follows:

Isomeric chlorinated vanillins and protocatechualdehydes are difficult to distinguish from other isomers of the same molecular mass on the basis of their electron impact (EI) mass spectra. However, the absence of the $[M\text{-}CH_3\text{-}CO\text{-}HCl]^+$ ion in the spectrum of 6-chlorovanillin at m/z 107 can be used to distinguish this compound from the other monochloro isomers. The same difference can be observed in the mass spectra of three different monochlorinated protocatechualdehydes. The peak m/z 107 is more intense in the mass spectra of 2-chloro- and 5-chloroprotocatechualdehydes than in the spectra of the corresponding chlorovanillins.

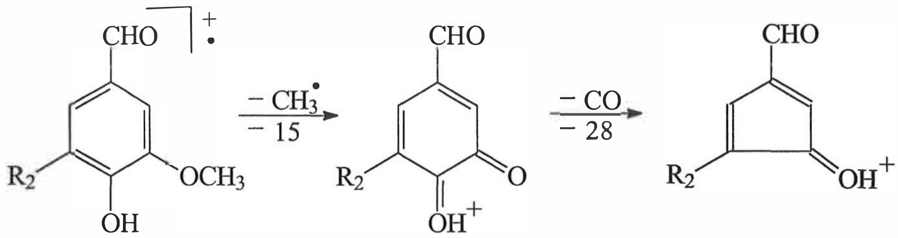


Compound (R_1,R_2)	$[M]^+$ (I_{rel})	$[M-1]^+$ (I_{rel})	$[M-1-28]^+$ (I_{rel})
Vanillin (R_1=OCH_3, R_2=H)	152 (100)	151 (98)	123 (15)
5-chlorovanillin (R_1=OCH_3,R_2=Cl)	186 (100)*	185 (96)	157 (8)
Syringaldehyde (R_1=R_2=OCH_3)	182 (100)	181 (57)	153 (4)
5-hydroxyvanillin (R_1=OCH_3,R_2=OH)	168 (100)	167 (91)	139 (23)
Protocatechualdehyde (R_1=OH,R_2=H)	138 (98)	137 (100)	109 (56)
5-chloroprotocatechualdehyde (R_1=OH,R_2=Cl)	172 (85)*	171 (100)	143 (40)
Gallaldehyde (R_1=R_2=OH)	154 (98)	153 (100)	125 (51)

*The most intensive peaks of isotope patterns are selected and the m/z values of these ions rounded to the closest even number. The m/z values of ions $[M-1]^+$ and $[M-1-28]^+$ are presented accordingly.

Fig. 10. Cleavage of a hydrogen radical followed by cleavage of a carbon monoxide in some selected model compounds.

The abundance of the molecular ion peak in the mass spectrum of an acetylated phenolic model compound is usually below 5 % of the intensity of the base peak, which in turn is usually formed by the loss of one or several ketene molecules from the molecular ion ($[M-CH_2=C=O]^{\cdot+}$), depending on the niumber of acetylated groups. The molecular ions fragment further following the pathways presented in Figures 10 and 11 .



Compound (R_2)	M (I_{rel})	M-15 (I_{rel})	M-15-28 (I_{rel})
Vanillin (R_2=H)	152 (100)	137 (7)	109 (25)
5-chlorovanillin (R_2=Cl)	186 (100)*	171 (6)	143 (24)
Syringaldehyde (R_2=OCH_3)	182 (100)	167 (15)	139 (12)
5-hydroxyvanillin (R_2=OH)	168 (100)	153 (9)	125 (39)

*The most intensive peaks of isotope patterns are selected and the m/z values of these ions rounded to the closest even number. The m/z values of ions $[M-1]^+$ and $[M-1-28]^+$ are presented accordingly.

Fig. 11. Cleavage of methyl radical followed by cleavage of CO in some model compounds.

4.3 Gas chromatographic separation of model compounds and oxidation products

The separation of chlorovanillins and syringaldehydes as their acetyl derivatives using GC and GC/MS can quite easily be accomplished (Paper I). With compounds having two or three phenolic hydroxyl groups, separation may be difficult due to incomplete derivatisation, low volatility and thermal degradation. For such compounds HPLC separation can be recommended (Paper II).

4.4 HPLC

The HPLC separation can be recommended for compounds having more than one phenolic hydroxylic group or for thermally labile ones. Also, in the case of complex mixtures containing both aliphatic and aromatic compounds, detection based on the UV absorption can considerably simplify the analysis, as only aromatic compounds have significant absorbance. On the other hand, differences in response may cause difficulties in the interpretation of results. In this study it was noted that the responses of the compounds analysed at wavelenght 280 nm behaved as follows: benzaldehyde < syringaldehyde < vanillin < 4-hydroxybenzaldehyde and guaiacol < vanillic acid < acetovanillone < vanillin. Thus, the addition of a formyl group has an enhancing effect to the response of the compound. On the other hand, the addition of a methoxyl group or methyl group or the oxidation of a formyl group to carboxylic acid have the opposite effect.

4.5 Pyrolysis-GC/MS

The results of the pyrolysis experiments with the sediments and HMMs separated from the water samples of the Äänekoski watercourse support the results obtained analysing the CuO oxidation products of these samples. However, it is easier to follow the transport of HMMs produced by pulp mills using CuO oxidation. This may partly be due to the reaction and analysis conditions used, so that pyrolysis combined with the derivatisation of pyrolysis products would probably give better results.

4.6 GPC

Gel permeation chromatography of tetrahydrofurane extracts from sediments gave more information on the HMMs in sediments that was supposed. The differences in the UV absorption and in MP values, indicating the position of the highest peak in the chromatogram, hint that this method could also be used to follow the transport of HMMs. However, this clearly can not be proved on the basis of only three samples (Fig. 2 in Paper IV).

4.7 Samples

The six water samples and three surface sediment samples studied in Papers III and IV were collected in March 1993 and March 1994 by the Central Finland Regional Environment Centre. The whole volumes of the water samples were used in the ultrafiltration and most of the HMMs thus obtained were used in the CuO oxidations with a small amount saved for the Py-GC/MS-experiments. In Paper IV the dry weights and the contents of organic matter of the sediment samples were also determined and the amounts of carbon, nitrogen and hydrogen in the samples was also measured. The methods used were standard methods widely used in the wood processing industry. These determinations and the SEC analysis could not be applied to the HMMs separated from the water samples owing to their low amounts. The 12 water samples analysed in Paper V were collected in September to November 1996 by the Environmental Centre of Finland and the effluent sample was donated by the pulp mill. The whole volumes of the river, lake and seawater samples and most of the effluent samples were used in the ultrafiltration. All the HMMs obtained from the seawater samples and most of the other HMMs were used in the CuO oxidations.

It is recommended that the flow rates of a watercourse be analyzed in order to be able to collect the samples representing the HMMs produced at any one time. This would eliminate differences due to results based on different types of HMMs released by the plant. Further still, a suitable sampling season should be selected, because rates of sedimentation and degradation are different at different temperatures. HMMs from other sources may also cause problems in the interpretation of results so that the method should be applied only in cases where there are not too many sources of HMMs present.

4.8 Transport of HMMs in the nature

The ratios of different oxidation products are reproducible, even when the total amounts of oxidation products produced in cupric oxide oxidation often differ between different oxidations of the same starting material. The variation in total amounts may partially be caused by factors such as different amounts of humidity, inorganic material or other non-oxidised material in the sample. Also, measuring the exact weights of samples for oxidation may be difficult owing to their hygrophilic nature and small amounts. The various HMM materials have also a different tendency towards oxidation. Cupric oxide oxidation is therefore a useful method if one wishes to tell whether a sample contains HMMs produced by industry, but the quantitation of different materials becomes difficult. Fortunately, the HMMs produced by pulp mills oxidise more readily than the natural HMMs and their presence in a HMM sample can be pinpointed fairly accurately, especially if suitable uncontaminated samples are available for comparison.

On the basis of the results in Papers III, IV and V, the HMMs produced by the Äänekoski mill seem to be less susceptible to environmental degradation and transport further than the industrial HMMs in the case of the Lohjanjärvi watercourse. On the other hand, this may partly be caused by the larger volume and larger dilution in Lake Lohjanjärvi than in the river that receives the water in the Äänekoski watercourse. Because the presence of chlorine containing compounds is such a clear indication of contamination by pulp plant waste water, the analysis based on oxidation products containing chlorine is easier than one based on non-chlorinated oxidation products.

5 CONCLUSIONS

In addition to the model compounds synthesized in Papers I, II and III and the mass spectrometric and NMR spectroscopic data obtained on them, the most important results relate to the transport of HMMs in the receiving waters. First of all, the method used, even though time-consuming and in need of modifications, enables the transport of these materials to be followed qualitatively. Secondly, the quantitative analysis of different HMMs is complicated mainly by their low amounts in the receiving waters, especially in sea water samples and by their different behaviour in CuO oxidation. On the basis of our results it is evident that the dissolved lignin residues degrade more readily than humic materials in cupric oxide oxidation. Therefore, in oxidising samples where both materials are present, the exact amounts of different HMMs can not be obtained based on the amounts of oxidation products.

The literature cited and the experiments conducted point to the need for further studies of the following topics:

a) SEC of materials extracted from sediments seems to give interesting results. This method could also be applied to aqueous DOM (dissolved organic material). This requires that the DOM is separated using a method that does not fractionate it in the way ultrafiltration does.

b) The conventional Py-GC/MS used indicated differences between different types of samples, but the results could not be applied to following the transport of HMMs in the receiving waters. The use of pyrolysis with tetramethyl ammonium hydroxide (TMAH) methylation in the analysis of HMMs separated from water was not attempted here but remains to be tested.

c) A more detailed comparison of the different oxidation methods and the study of oxidation mechanisms would help to clarify the original structure of the material oxidised.

d) Calibration methods should be developed to obtain the relative amounts of different types of HMMs based on the relative amounts of selected oxidation products.

e) Identification of new compounds from the CuO oxidation products of lignin and humic substances could be accomplished using HPLC/MS and CE/MS.

f) Synthesis of additional model compounds should be carried out to answer the needs caused by new bleaching methods (like TCF, ozone and oxygen bleaching).

g) The use of different capillary electrophoretic (such as capillary zone electrophoresis - CZE and micellar electrokinetic chromatography - MEKC, etc.) and capillary electrochromatographic (CEC) methods in the analysis of CuO oxidation products should be tested. The possible advantages are higher resolution, shorter analysis time, better background and possibly also lower sample amounts needed. In fact, CZE of CuO oxidation products has already given promising results.[218]

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Errata of Papers I to V

Paper/Page	Location	Is:	Should be:
Paper I			
1843	Intro, 14	6-choro	6- and 5,6-dichloro
1855	Table, 1	24(9)	224(9)
Paper II			
1644	L.c., 4	25 x 4.6 mm id	25 cm x 4.6 mm i.d.
1644	L.c., 10	PDA-data	DAD-data
1651	Fig 5.	XI	XII
1656	Ref. 30	Scwarzenbach	Schwarzenbach
Paper III			
891	Intro 8	GC-MS	GC/MS
892	Fig 1.	SEK (lower)	SEV
893	20	10 mg the	10 mg of the
894	5	PDA-data	DAD-data
895	6	ultrafilered	ultrafiltered
Paper IV			
297	Header	PYROLYSIS-GC-MS	PYROLYSIS-GC/MS
297	Intro. 3	(Py-GC-MS)	(Py-GC/MS)
298	4,9,14,36	Py-GC-FID	Py-GC/FID
301	5	Styragel HR cm 300 x 7.8 mm	Styragel^{TM} HR 300 x 7.8 mm
301	6	PDA-data	DAD-data
301	12	dehydrodiisoeugenol (311)	dehydroisoeugenol (326)
301	20	Pyrolysis-GC-MS	Pyrolysis-GC/MS
302	10	dehydrodiisoeugenol	dehydroisoeugenol
307	Pyr. 3	peaks	peaks in mass spectra
Paper V			
583	1. Title	GC-MS	GC/MS
583	5 and 6	GC-MS	GC/MS

PAPER I

https://doi.org/10.1016/0045-6535(93)90078-J

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