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Structural characterization of bio- and geo-macromolecules by off-line thermochemolysis with tetramethylammonium hydroxide

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Abstract

A new analytical procedure, tetramethylammonium hydroxide thermochemolysis, was used to structurally characterize a variety of bio-and geo-polymers. The technique cleaves esters and some ethers in macromolecular organic matter, yielding low-molecular-mass monomers such as methyl esters of carboxylic acids and methyl ethers of alcohols that are amenable to gas chromatographic analysis. This procedure can be conducted in sealed glass ampoules, which means that it can be easily implemented in any laboratory having gas chromatographic capabilities, in contrast to other chemolytic or pyrolytic procedures. A set of biogeomacromolecules, ranging from gymnosperm and angiosperm woods, natural polyesters such as cutin, dissolved organic matter in natural and oceanic waters, and humic substances were characterized with this procedure. The information obtained provides molecular-level details which can be used to infer structural composition.

Keywords: Thermochemolysis; Geochemistry; Tetramethylammonium hydroxide; Lignin; Polyesters; Humic substances

1. Introduction

A considerable number of analytical approaches have been used to characterize the structural composition of biogeomacromolecules. The main chemical characterization techniques applied to macromolecular materials found in biological and geological systems can be grouped into two categories: degradative and nondegradative approaches. Nondegradative techniques (e.g., spectroscopic methods) offer a bulk description of the sample, and in particular, ^{13}C-NMR spectroscopy has contributed significantly to our knowledge of the chemical structure of complex macromolecules. However, more precise information at the molecular level is best achieved by degrading the macromolecules into low-molecular-mass compounds that can be analysed by gas chromatographic (GC) methods.

Chemical degradations (oxidation, reduction and hydrolysis) have all been successfully used to degrade macromolecular materials in biological and geological systems. The particular reagents used cleave a variety of different chemical bonds and consequently lead to the formation of different products. In the case of lignin and related materials, the most widely used degradative technique is CuO

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oxidation which has been utilized extensively to characterize lignin in soils and sedimentary mixtures containing multiple types of organic matter [1,2]. This procedure oxidizes the lignin macromolecule and produces a series of *p*-hydroxyphenyl, guaiacyl and syringyl compounds [3]. Quantitative GC analysis of the respective monomers provides quantitative measures of the lignin content. Analysis of the composition and distribution of these phenolic biomarkers allows for chemotaxonomic distinctions between fresh gymnosperm and angiosperm plants [2,4] and has been used to estimate the source of vascular plant detritus in sedimentary mixtures [2–4].

Pyrolytic techniques, in combination with GC and mass spectrometry [pyrolysis (Py)–GC–MS and Py–MS] also provide detailed structural information at the molecular level, without any sample pretreatment and with very small amounts of sample. However, it has been shown that pyrolysis of moieties with underivatized carboxylic groups leads to decarboxylation [5]. In particular, benzenecarboxylic acids and large fatty acids are decarboxylated and not released upon pyrolysis. Also, some other significant structural moieties can be heavily modified by unwanted thermal reactions which may lead to misinterpretation of the structure of the studied biogeomacromolecule.

The pyrolysis in the presence of tetramethylammonium hydroxide (TMAH) has been recently introduced to avoid some of these limitations [6–8]. Py/TMAH prevents decarboxylation, and yields the methyl esters of carboxylic groups and the methyl ethers of phenolic groups, rendering many of the polar compounds volatile enough for GC. Thus, it is possible to separate and detect many more structurally significant products than that observed previously by conventional Py–GC–MS [6,9–13]. Several authors have pointed out that the reaction involved in the TMAH/pyrolysis scheme is one of chemolysis rather than pyrolysis [9,11,12,14,15].

The technique of pyrolysis in the presence of TMAH has already been successfully applied to the structural characterization of different biogeomacromolecules such as polyesters [9,16], humic materials [5,10–12,17–20], asphaltenes and kerogens [21,22] and natural and fossil resins and resinites [23,24]. Pyrolysis/TMAH has also been applied to the structural characterization of lignin [14,25,26]. It has been found that pyrolysis/TMAH cleaves the β-O-4 ether bonds of the lignin macromolecule releasing the monomers which subsequently become methylated.

Sub-pyrolysis temperatures of 300°C in the presence of TMAH have also been found to produce a suite of products similar to that observed at higher pyrolysis temperatures [11,26]. Moreover, in a recent paper by McKinney et al. [27] a procedure was outlined for the characterization of lignin at 300°C in the presence of TMAH using sealed glass ampoules. The TMAH thermochemolysis at sub-pyrolysis temperatures still induces ether β-O-4 bond cleavage in lignin, releasing lignin-derived compounds in much the same way as the CuO oxidation procedure [28].

Most of previous use of TMAH has been in conjunction with pyrolysis; however, in this paper we describe the use of the TMAH/thermochemolysis in sealed glass tubes for the structural characterization of a set of common biogeomacromolecules — lignin, natural polyesters such as cutin, humic substances and dissolved organic matter in natural waters. We focus mostly on the qualitative aspects of the technique in this paper, recognizing, of course, that quantitative yields are obtainable when suitable standards have been found for the products identified. In the case of lignin, we present some quantitative results.

2. Material and methods

The samples, whose origins are listed in Table 1, were weighed (0.5–1.0 mg) and placed in a glass ampoule with a measured amount (100 μl) of TMAH (25% in methanol). The methanol was evaporated under vacuum and the ampoule sealed. The sealed ampoules were then placed in an oven at 250°C for 30 min. After cooling to room temperature, the tubes were cracked open and all inside surfaces washed with methylene chloride (3×1 ml), and the extracts were combined and reduced to dryness under a stream of nitrogen. The sample was then diluted with a known volume of methylene chloride (100 μl), which contained an internal standard (23 ng/μl of *n*-eicosane). The diluted sample (1 μl) was analyzed by capillary GC on a

Table 1
Origin and description of samples

Sample	Description	Origin
Woods		
Fresh pine wood	Freshly cut sample of *Pinus* sp. wood	Obtained from J.I. Hedges
Degraded pine wood	Pine from archaeological site, King Midas tomb	Obtained from J.I. Hedges and described in Nelson et al. [29]
Fresh birch wood	Fresh cut sample of *Betula* sp. wood	Obtained from J.I. Hedges
Degraded birch wood	Sample of birch degraded for 12 weeks by *Phlebia tremellosus* (a white-rot fungi)	Obtained from J.I. Hedges and described in Hedges et al. [33]
Polyesters		
Tomato cutin	Isolated cutin from tomato fruit	Obtained from K. Espelié
Agave americana cutin	Isolated cutin from leaves	Obtained from J.W. de Leeuw and described in Tegelaar et al. [30]
Humic acid		
Minnesota peat HA	Humic acid isolate from peat	Rice Lake, Minnesota
Dissolved organic matter		
Suwannee river	Isolated by reverse osmosis from water of the Suwannee River, Georgia	Obtained from E.M. Perdue
Gulf of Mexico, T1 near shore sample	M_r 1000+ organic matter isolated from surface waters by tangential flow ultrafiltration	Obtained from P. Santschi and T. Bianchi
Gulf of Mexico, T5S offshore sample (surface)	M_r 1000+ organic matter isolated from surface waters by tangential flow ultrafiltration	Obtained from P. Santschi and T. Bianchi
Gulf of Mexico, T5D offshore sample (depth)	M_r 1000+ organic matter isolated from waters at 1600-m depth by tangential flow ultrafiltration	Obtained from P. Santschi and T. Bianchi
Aloha sample	M_r 1000+ organic matter isolated from near-surface waters (5 m) at the Aloha site in the North Pacific ocean by tangential flow ultrafiltration	Obtained from R. Benner and described in Benner et al. [31]

Hewlett-Packard 5890 gas chromatograph. Selected samples were analyzed by GC–MS on a Kratos MS-80 RFA high-resolution gas chromatograph–mass spectrometer system. The columns used for the GC separation were 30 m×0.25 mm, I.D., fused-silica capillary columns (DB-5, J&W Scientific). The column was heated at 30°C/min from an injection temperature of 60°C to a temperature of 100°C, at which point the rate was slowed to 4°C/min to a final temperature of 300°C. For the analysis of lignin, the temperature program was from 60°C to 150°C at 15°C, and then ramped to 280°C at 4°C/min with a 10 min isothermal period. Injector and detector temperatures were set to 300°C.

Mass spectra were obtained at a scan rate of 0.6 s/decade of mass with a 0.2-s magnet settling time added. Compounds were identified by their mass spectra and relative retention times. Most peaks were identified by comparison with the Wiley/NBS library and some were confirmed by comparison with authentic standards. Many of the lignin derivatives having methoxylated side-chains did not have mass spectra present in the library. In these instances, identification is tentative and based solely on analysis of the fragmentation patterns.

3. Results and discussion

3.1. Select biopolymers in plants

3.1.1. Lignin

TMAH/thermochemolysis induces cleavage of β-O-4 ether bonds in the lignin macromolecule, releasing a distribution of products similar to that obtained from the CuO oxidation technique. The chromatograms of the products released after the TMAH-thermochemolysis of a fresh and degraded gymnosperm wood (pine) are shown in Fig. 1. The chromatograms of a fresh and degraded angiosperm (birch) are shown in Fig. 2. Different lignin-derived phenol derivatives were identified among the released products, and these originate from the cleavage of the β-O-4 bonds of the lignin macromolecule.

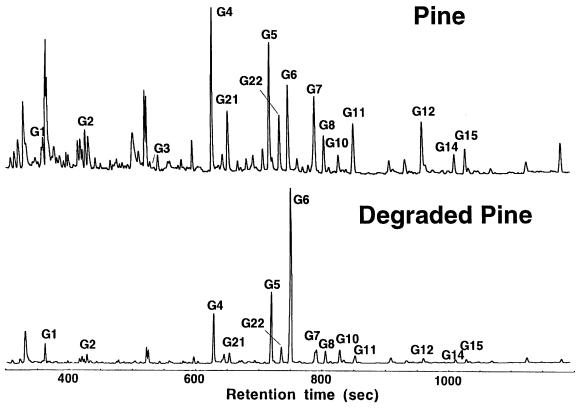

Fig. 1. Gas chromatogram of the products formed from TMAH/thermochemolysis of fresh and degraded pine (after soft-rot fungal degradation). For peak identification refer to Table 1.

The reaction products are similar to those released upon high-temperature pyrolysis in the presence of TMAH [14,25,26]. The compounds identified are listed in Table 2 and the structures are shown in Fig. 3.

The pine wood, a gymnosperm, yielded exclusively compounds having the vanillyl (guaiacyl) structure (noted as G prefixes in Fig. 1), whereas the birch, an angiosperm wood, released both vanillyl and syringyl compounds (noted as S prefixes in Fig. 1). The *p*-hydroxyphenol lignin units are notably absent in TMAH thermochemolysis products of wood samples, consistent with the findings of Hedges and Mann [2]. The presence of lignin-derived phenol derivatives which have an attached trimethoxylated 3-carbon side chain (such as G14/15, S14/15, Table 2) in the studied wood samples is significant because it demonstrates that TMAH/thermochemolysis is a rather mild procedure involving cleavage of the β-O-4 ether linkage and methylation of hydroxyl groups attached to the 3-carbon side chain. This means that the side chain units are preserved during the thermochemolysis procedure, in contrast to the CuO oxidation technique which causes side chain oxidation.

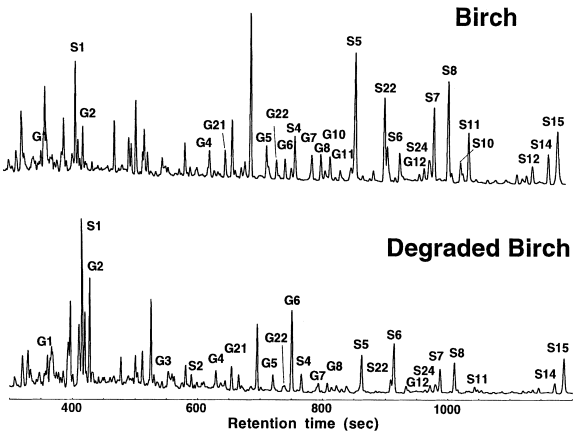

Fig. 2. Gas chromatogram of the products of TMAH/thermochemolysis of fresh and degraded birch (after white-rot fungal degradation). For peak identification refer to Table 1.

The distribution of phenolic compounds allows for chemotaxonomic distinctions (angiosperm, gymnosperms, herbaceous tissues) and for the evaluation of the extent of degradation. Similar parameters as those calculated with the CuO oxidation method (Λ, Ad/Al, C/V, S/V) can be calculated with the TMAH/thermochemolysis. Thus, we can calculate the total yield of lignin-derived phenols normalized to 100 mg of carbon (Λ), the ratios of acid-substituted phenols to aldehyde-substituted phenols which provides a measure of the extent of bacterial oxidation (Ad/Al), the ratios of cinnamyl phenols to vanillyl phenols (C/V) which provides information on the origin of the lignin, and the ratios of syringyl phenols to vanillyl phenols (S/V) which also provides data on lignin sources.

Yields of TMAH thermochemolysis products from lignin were used to determine values of Λ, which are similar to the Λ parameter used by Hedges and Mann [2] and are a measure of the total lignin derived products. Average Λ values for the two fresh and degraded woods studied here are reported in Table 3. The fresh wood samples generally have Λ values of less than 10, and Λ is expected to increase as a function of degradation processes which tend to increase the lignin contents of wood, namely soft-rot and brown-rot fungal degradation. In the case of the degraded pine wood, which had been degraded by a soft-rot fungi, the Λ value increases as expected because the polysaccharides are preferentially removed and the residual wood becomes concentrated in lignin. For the degraded birch wood, decayed for 12 weeks by *P. tremellosus*, a white-rot fungi, the Λ value is lower than in the respective fresh wood due to the fact that white-rot fungi preferentially remove the lignin [32,33].

Table 2
Lignin-derived compounds released after TMAH thermochemolysis

p-Coumaryl structures	
P4	4-Methoxybenzaldehyde
P5	4-Methoxyacetophenone
P6	4-Methoxybenzoic acid methyl ester
P11	*trans*-1-(4-Methoxyphenyl)-2-methoxyprop-1-ene
P12	4-Methoxybenzenepropanoic acid methyl ester
P17	*cis*-3-(4-Methoxyphenyl)-3-propenoic acid methyl ester
P18	*trans*-3-(4-Methoxyphenyl)-3-propenoic acid methyl ester
P22	1-(4-Methoxyphenyl)-2-propanone
Guaiacyl structures	
G1	1,2-Dimethoxybenzene
G2	3,4-Dimethoxytoluene
G3	3,4-Dimethoxystyrene
G4	3,4-Dimethoxybenzaldehyde
G5	3,4-Dimethoxyacetophenone
G6	3,4-Dimethoxybenzoic acid methyl ester
G7	*cis*-1-(3,4-Dimethoxyphenyl)-2-methoxyethylene
G8	*trans*-1-(3,4-Dimethoxyphenyl)-2-methoxyethylene
G9	*cis*-1-(3,4-Dimethoxyphenyl)-3-methoxyprop-1-ene
G10	*cis*-1-(3,4-Dimethoxyphenyl)-methoxyprop-1-ene
G11	*trans*-1-(3,4-Dimethoxyphenyl)-methoxyprop-1-ene
G12	3-(3,4-Dimethoxyphenyl)-propanoic acid methyl ester
G13	*trans*-1-(3,4-Dimethoxyphenyl)-3-methoxyprop-1-ene
G14	*threo*/*erythro* 1-(3,4-Dimethoxyphenyl)-1,2,3-trimethoxypropane
G15	*threo*/*erythro* 1-(3,4-Dimethoxyphenyl)-1,2,3-trimethoxypropane
G16	*cis*-1-(3,4-Dimethoxyphenyl)-1,3-dimethoxyprop-1-ene
G17	*cis*-3-(3,4-Dimethoxyphenyl)-3-propenoic acid methyl ester
G18	*trans*-3-(3,4-Dimethoxyphenyl)-3-propenoic acid methyl ester
G19	*trans*-1-(3,4-Dimethoxyphenyl)-1,3-dimethoxyprop-1-ene
G20	3,4-Dimethoxybenzenemethanol methyl ether
G21	1-(3,4-Dimethoxyphenyl)-1-propene
G22	1-(3,4-Dimethoxyphenyl)-2-propanone
G23	1-(3,4-Dimethoxyphenyl)-2-methoxypropane
G24	3,4-Dimethoxybenzeneacetic acid methyl ester
Syringyl structures	
S1	1,2,3-Trimethoxybenzene
S2	3,4,5-Trimethoxytoluene
S4	3,4,5-Trimethoxybenzaldehyde
S5	3,4,5-Trimethoxyacetophenone
S6	3,4,5-Trimethoxybenzoic acid methyl ester
S7	*cis*-1-(3,4,5-Trimethoxyphenyl)-2-methoxyethylene
S8	*trans*-1-(3,4,5-Trimethoxyphenyl)-2-methoxyethylene
S9	*cis*-1-(3,4,5-Trimethoxyphenyl)-3-methoxyprop-1-ene
S10	*cis*-1-(3,4,5-Trimethoxyphenyl)-methoxyprop-1-ene
S11	*trans*-1-(3,4,5-Trimethoxyphenyl)-methoxyprop-1-ene
S12	3-(3,4,5-Trimethoxypehnyl)-propanoic acid methyl ester
S13	*trans*-1-(3,4,5-Trimethoxyphenyl)-3-methoxyprop-1-ene
S14	*threo*/*erythro* 1-(3,4,5-Trimethoxyphenyl)-1,2,3-trimethoxypropane
S15	*threo*/*erythro* 1-(3,4,5-Trimethoxyphenyl)-1,2,3-trimethoxypropane
S16	*cis*-1-(3,4,5-Trimethoxyphenyl)-1,3-dimethoxyprop-1-ene
S22	1-(3,4,5-Trimethoxyphenyl)-2-propanone
S24	3,4,5-Trimethoxybenzeneacetic acid methyl ester

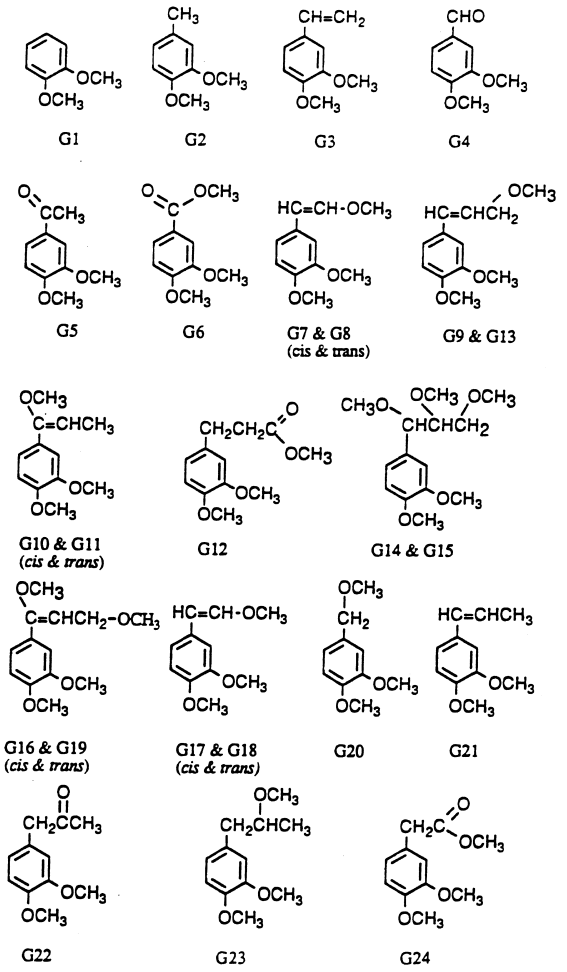

Fig. 3. Structures of the compounds released after TMAH/thermochemolysis of lignin. Only the structures with guaiacyl skeleton (noted as G prefixes) are shown. Similar structures with syringyl skeletons are also found (noted as S prefixes).

The peak area ratios of the vanillyl aldehyde to vanillic acid $(Ad/Al)_v$ have been shown to be an excellent indicator of wood decomposition in CuO oxidation studies [34,35]. A similar ratio of 3,4-dimethoxybenzoic acid methyl ester (G6) and 3,4-dimethoxybenzaldehyde (G4) $(Ad/Al)_v$, was used to examine the decomposition of these wood samples. The concentration of 3,4-dimethoxybenzoic acid methyl ester increases as lignin polymers are attacked by microbial enzymes which selectively oxidizes the α-carbon in the side-chain [34]. The values of the vanillyl and syringyl acid/aldehyde ratios for the selected fresh and degraded woods are reported in Table 3. In all the cases, the $(Ad/Al)_v$ and $(Ad/Al)_s$ ratios increased with increasing decomposition as microbial oxidation takes place in the lignin macromolecule.

The ratio of syringyl units to vanillyl units (S/V) in woods has often been used as a means of distinguishing angiosperm from gymnosperm woods [2]. The fresh birch sample shows an S/V ratio of 2:1, indicating that the syringyl units are the dominant lignin phenols produced. The fresh pine sample contains no syringyl phenols, consistent with known lignin composition. With increasing white-rot fungal degradation the S/V ratio for the degraded birch sample decreases, suggesting a preferential removal of syringyl structures over vanillyl structures. The same trend has been reported in studies employing the CuO oxidation method [35].

For lignin, the TMAH thermochemolysis procedure shows great promise in being able to quantitatively assess the amount and level of degradation of lignin in wood samples. Data comparable and complementary to the CuO oxidation procedure are obtained. The one advantage presented by the technique is its ease of implementation. Under the conditions employed, methylation of chemolytically-produced compounds occurs in a one-step process and provides compounds which are immediately available for GC, unlike the CuO procedure which requires subsequent extraction and derivatization prior to GC.

Table 3
Main lignin parameters for some fresh and degraded woods

	Λ	$(Ad/Al)_v$	$(Ad/Al)_s$	S/V
Fresh pine	4.60	0.52	–	–
Degraded pine (soft-rot)	17.19	9.60	–	–
Fresh birch	8.82	0.96	0.54	2.10
Degraded birch (white-rot)	5.24	4.40	3.03	1.63

3.1.2. Polyesters

Aliphatic biopolymers such as cutin from the leaf cuticles of different plants are primarily composed of ether and ester-linked polymers of long-chain fatty acids and alcohols. In the case of esters, the TMAH thermochemolysis method induces a saponification/transesterification reaction at high temperature and releases the methyl esters of fatty acids and methyl ethers of alcohols. Figs. 4 and 5 show the chromatograms of the products released after TMAH/thermochemolysis of tomato cutin and *Agave americana* cuticle, respectively.

The main compounds released upon TMAH/thermochemolysis of tomato cutin were the methyl esters of *p*-coumaric acid, ω-methoxyhexadecanoic acid, and 10,16-dimethoxyhexadecanoic acid. The 10,16-dihydroxyhexadecanoic acid is a major component in tomato cutin, as has already been reported by other authors [36]. Minor amounts of 8,16- and 9,16-dimethoxyhexadecanoic acid methyl esters were also found coeluting with the 10,16-dimethoxyhexadecanoic acid methyl ester. The relative composition of the dimethoxyhexadecanoic acid methyl esters are shown in Table 4. A compound was tentatively identified as 9,10,16-trimethoxyhexadecanoic acid methyl ester. In contrast to the pyrolysis/TMAH [9], very minor amounts of partially methylated hydroxyl groups, were released.

In the *Agave americana* cuticle, the main components were the 9,10,18-trimethoxyoctadecanoic acid methyl ester and the 10,16-dimethoxyhexadecanoic acid methyl ester. Minor amounts of 8,16- and 9,16-dimethoxyhexadecanoic acid methyl esters were also detected, in agreement with previous studies [36]. The relative composition of the dimethoxyhexadecanoic acid methyl esters are shown in Table 4. Also, a compound was identified as 9,10,12,18-tetramethoxyoctadecanoic acid methyl ester. However, the previously reported 9,10-epoxy, 18-hydroxyoctadecanoic [37] could not be identified here. A series of fatty alcohol methyl ethers and fatty acid methyl esters up to C_{32}, with strong even carbon predominance were also released.

In general, the analysis of polyesters releases the same products as in the saponification, but do not need additional derivatization steps because the products are already released as the methylated analogs.

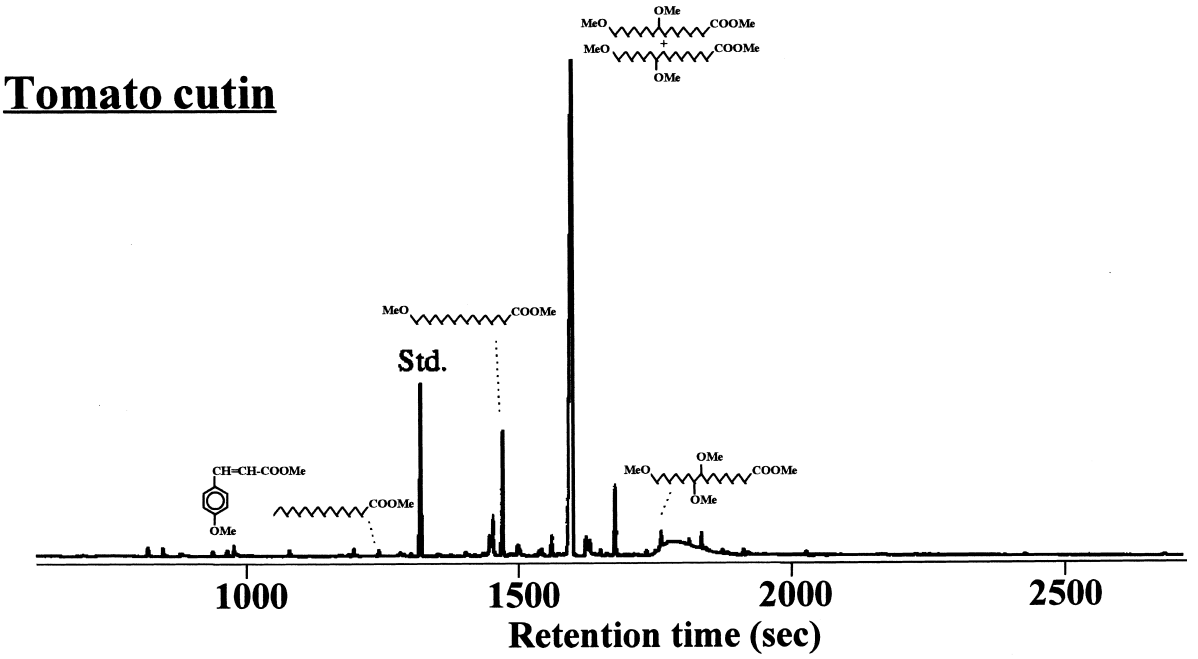

Fig. 4. Gas chromatogram of the TMAH/thermochemolysis products of tomato cutin. The structure of the main compounds are shown on the chromatographic peaks. The standard (std.) is eicosane.

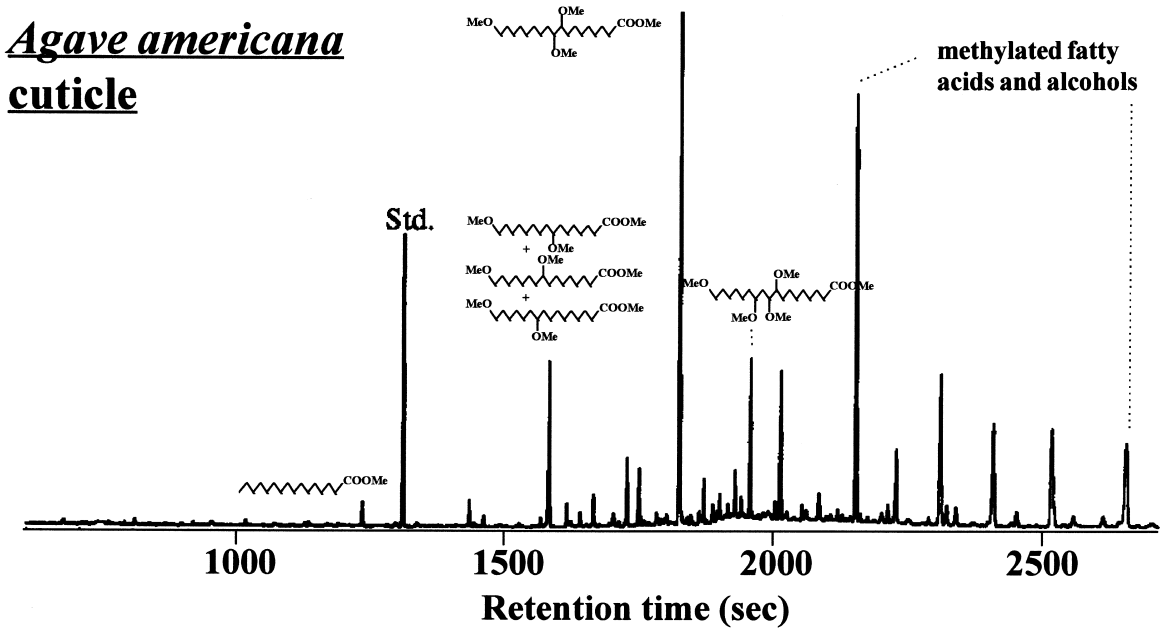

Fig. 5. Gas chromatogram of the TMAH/thermochemolysis products of *Agave americana* cuticle. The structure of the main compounds are shown on the chromatographic peaks.

3.2. Select geopolymers in modern environments

3.2.1. Humic substances

The chromatogram of the TMAH thermochemolysis products released from humic acids extracted from a Minnesota peat is shown in Fig. 6. The concentrations of the identified lignin-phenol derivatives and other compounds are listed in Table 5. The main products released are lignin-derived phenol derivatives, long-chain fatty acid methyl esters, α,ω-dicarboxylic acid dimethyl esters, ω-methoxyfatty acid methyl esters and long-chain alcohol methyl ethers. Analysis of a large set of humic and fulvic acids [38] shows that the long-chain lipids are released mainly from the HAs and are generally absent or present in very minor amounts in the FAs. Different compounds arising from proteins and polysaccharides were also detected in most of the samples, although in lesser and varying amounts.

Among the lignin-derived phenol derivatives, we note the presence of some benzenecarboxylic acids with *p*-coumaryl, guaiacyl and syringyl skeletons: 4-methoxybenzenecarboxylic acid methyl ester (P6), 3,4-dimethoxybenzenecarboxylic acid methyl ester, (G6) and 3,4,5-trimethoxybenzenecarboxylic acid methyl ester (S6), respectively. Some benzeneacetic acid methyl esters (P24, G24 and S24) as well as benzenepropenoic acid methyl esters, 4-methoxybenzenepropenoic acid methyl ester (P17) and 3,4-di-

Table 4
Relative composition of the different isomers of dimethoxyhexadecanoic acid methyl ester in the different cutin samples

Isomer	*Agave americana* cutin	Tomato cutin
8,16-Dimethoxy-hexadecanoic acid methyl ester	18.0	6.3
9,16-Dimethoxy-hexadecanoic acid methyl ester	10.3	7.6
10,16-Dimethoxy-hexadecanoic acid methyl ester	71.7	86.0

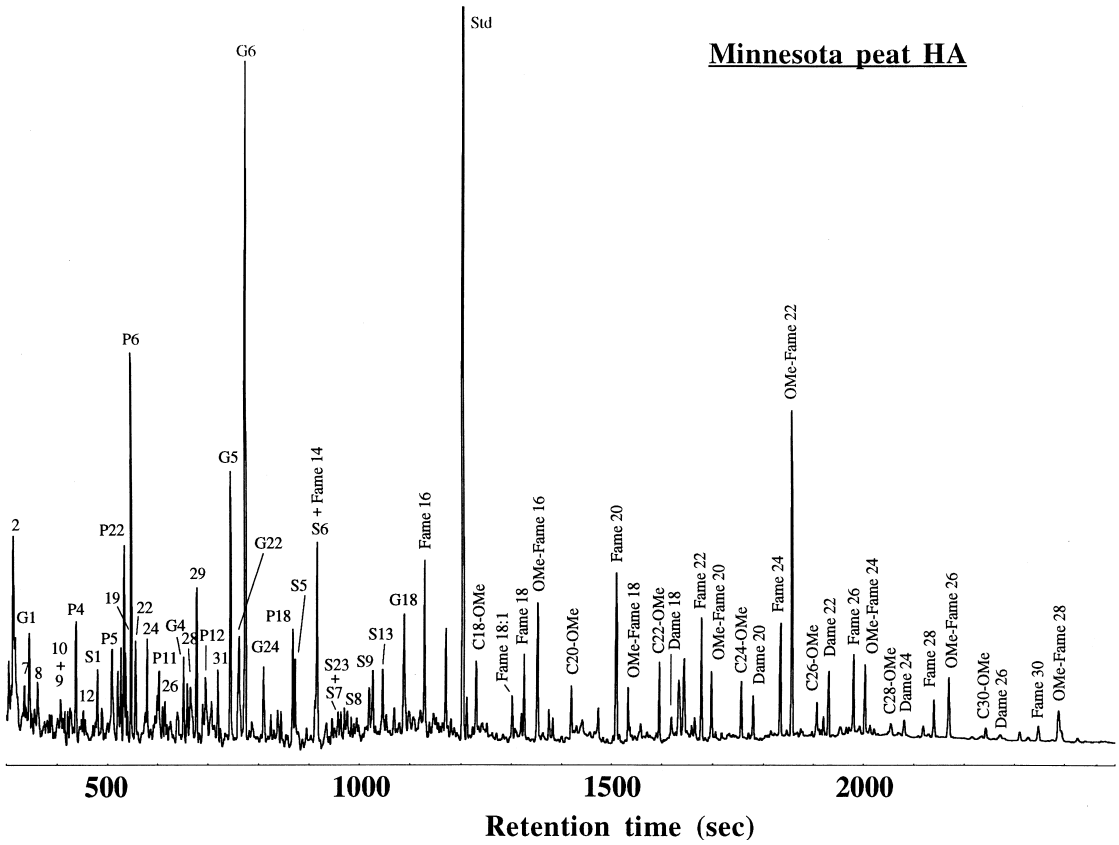

Fig. 6. Gas chromatogram of the products released after TMAH/thermochemolysis of the Minnesota peat humic acids. For peak identification refer to Table 5. Fame i and i:1=fatty acid methyl ester where i=number of carbons in the chain and i:1 refers to a single unsaturated bond; Ci–OMe=methoxy ether of a fatty alcohol having i number of carbons; OMe–Fame i=methoxy ether of a hydroxy fatty acid with i carbons.

methoxybenzenepropenoic acid methyl ester (G17 and G18) are also identified in the HA sample. Although aromatic acids have been previously reported as being building blocks of the humic structure in models based upon oxidative degradations [39], the presence of these compounds has been previously undetected by conventional pyrolysis due to decarboxylation processes. The use of thermochemolysis with TMAH avoids decarboxylation by protecting the carboxyl groups, and releases them as their methyl esters. Large quantities of aromatic acids are also released from different terrestrial and aquatic HAs and FAs upon pyrolysis/TMAH [10–13]. The aromatic acids released from the different HAs and FAs might represent final steps in the oxidation of the side-chain during microbial degradation of lignins and are probably pristine components of the humic structure, as also suggested by several authors [5,11,13,15]. Therefore, the use of TMAH/themochemolysis seem to corroborate the presence of lignin-derived aromatic acids in the structure of the humic materials.

Other phenolic compounds, not related to lignin-derived structures, such as 1,3,5-trimethoxybenzene and 2,4,6-trimethoxytoluene, are also detected. Similar compounds were found as main products from the TMAH/thermochemolysis of cutan [40], the highly aliphatic and resistant biopolymer present in leaf cuticles from certain plants, which might indicate the contribution of this biopolymer to the structure of the humic acids.

Prominent series of fatty acid methyl esters,

Table 5
Composition and amounts of the different lignin-derived monomers and fatty acid methyl esters in the Minnesota HA (as mg per 100 mg of TOC)

Lignin-derived compounds	
4-Methoxybenzaldehyde (P4)	0.21
4-Methoxyacetophenone (P5)	0.12
4-Methoxybenzoic acid methyl ester (P6)	0.60
trans-1-(4-Methoxyphenyl)-2-methoxyprop-1-ene (P11)	0.04
trans-3-(4-Methoxyphenyl)-3-propenoic acid methyl ester (P18)	0.20
1-(4-Methoxyphenyl)-2-propanone (P22)	0.30
4-Methoxybenzeneacetic acid methyl ester (P24)	0.10
1,2-Dimethoxybenzene (G1)	0.15
3,4-Dimethoxybenzaldehyde (G4)	0.15
3,4-Dimethoxyacetophenone (G5	0.49
3,4-Dimethoxybenzoic acid methyl ester (G6)	1.28
3-(3,4-Dimethoxyphenyl)-propanoic acid methyl ester (G12)	0.13
trans-3-(3,4-Dimethoxyphenyl)-3-propenoic acid methyl ester (G18)	0.28
3,4-Dimethoxybenzenemethanol methyl ether (G20)	0.04
1-(3,4-Dimethoxyphenyl)-2-propanone (G22)	0.32
3,4-Dimethoxybenzeneacetic acid methyl ester (G24)	0.16
1,2,3-Trimethoxybenzene (S1)	0.11
3,4,5-Trimethoxyacetophenone (S5)	0.15
3,4,5-Trimethoxybenzoic acid methyl ester (S6)	0.40
cis-1-(3,4,5-Trimethoxyphenyl)-2-methoxyethylene (S7)	0.04
trans-1-(3,4,5-Trimethoxyphenyl)-2-methoxyethylene (S8)	0.04
cis-1-(3,4,5-Trimethoxyphenyl)-3-methoxyprop-1-ene (S9)	0.23
trans-1-(3,4,5-Trimethoxyphenyl)-3-methoxyprop-1-ene (S13)	0.15
1-(3,4,5-Trimethoxyphenyl)-2-propanone (S22)	0.05
1-(3,4,5-Trimethoxyphenyl)-2-methoxypropane (S23)	0.04
Fatty acid methyl esters and fatty alcohol methyl ethers	
Butanedioic acid dimethyl ester	0.28
Pentanedioic acid dimethyl ester	0.04
Octanedioic acid dimethyl ester	0.04
Nonanedioic acid dimethyl ester	0.08
Tetradecanoic acid methyl ester	0.12
Hexadecanoic acid methyl ester	0.19
Octadec-9-enoic acid methyl ester	0.06
Octadecanoic acid methyl ester	0.10
16-Methoxyhexadecanoic acid methyl ester	0.20
Eicosanol methyl ether	0.05
Hexadecane-1,16-dioic acid dimethyl ester	0.04
Eicosanoic acid methyl ester	0.24
18-Methoxyoctadecanoic acid methyl ester	0.06
Docosanol methyl ether	0.08
Octadecane-1,18-dioic acid dimethyl ester	0.04
Docosanoic acid methyl ester	0.14
20-Methoxy-eicosanoic acid methyl ester	0.19
Tetracosanol methyl ether	0.07
Eicosane-1,20-dioic acid dimethyl ester	0.08
Tetracosanoic acid methyl ester	0.13
22-Methoxydocosanoic acid methyl ester	0.50
Docosane-1,22-dioic acid dimethyl ester	0.11
Hexacosanoic acid methyl ester	0.10
24-Methoxy-tetracosanoic acid methyl ester	0.11
Octacosanol methyl ether	0.02
Tetracosane-1,24-dioic acid dimethyl ester	0.04

Table 5. Continued

Octacosanoic acid methyl ester	0.06
26-Methoxyhexacosanoic acid methyl ester	0.12
Triacontanol methyl ether	0.03
Hexacosane-1,26-dioic acid dimethyl ester	0.03
Triacontanoic acid methyl ester	0.03
28-Methoxy-octacosanoic acid methyl ester	0.06
Calculation of some lignin parameters and total amount of fatty acids and fatty alcohols	
Λ	5.78
$(Ad/Al)_v$	8.53
S/V	0.40
C/V	0.16
Total amount of fatty acids and alcohols (per 100 mg of TOC)	3.44

methyl ethers of long chain alcohols, methylated *n*-ω-hydroxylated fatty acids and *n*-α,ω-dicarboxylic acids, with strong even carbon predominance, are released from the Minnesota peat HA (Fig. 5 Table 5). These series probably arise from higher plant cutin and perhaps suberin residues, and are likely to be chemically bound to the humic macromolecule through ester linkages.

3.2.2. Dissolved organic matter (DOM)

The chromatograms of the products from the TMAH-thermochemolysis of the DOM isolated from some selected samples are shown in Figs. 7–9 and the product identifications are listed in Table 6. Different lignin-derived phenol derivatives were identified among the products, and these originate from the cleavage of the β-O-4 bonds from incorporated lignin-derived structures. The reaction products are similar to those released upon pyrolysis/TMAH [25,26]. Methylated derivatives of the three lignin phenol families (the *p*-hydroxyphenyl, vanillyl and syringyl phenols) were present. Likewise, several di- and trimethoxybenzenes, which are not lignin-derived structures [1,4-dimethoxybenzene (peak 7),

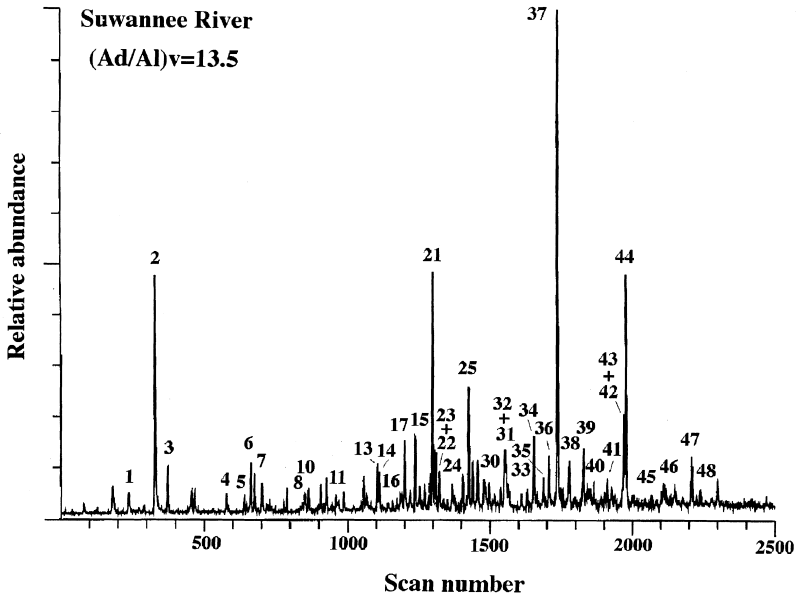

Fig. 7. Gas chromatogram of the TMAH/thermochemolysis products of DOM from the Suwannee River. For peak identifications refer to Table 6.

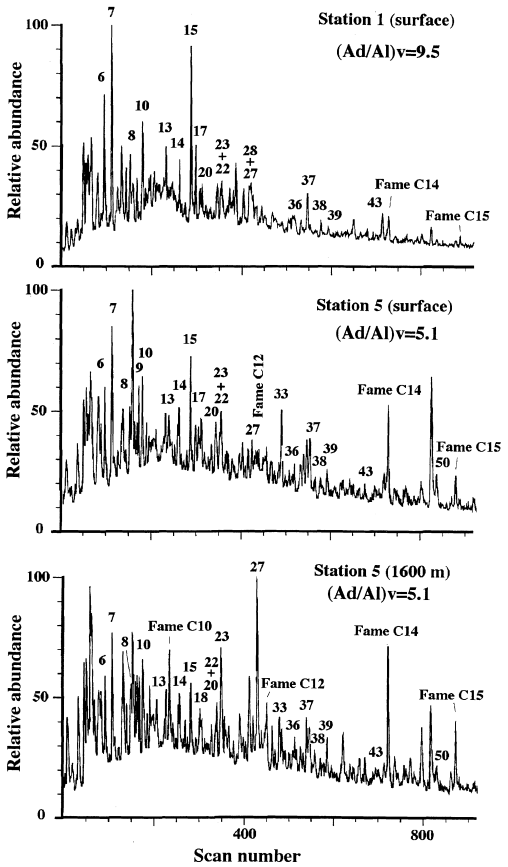

Fig. 8. Total ion chromatogram of the TMAH thermochemolysis products from the DOM of the Gulf of Mexico samples. For peak identifications refer to Table 6.

2,5-dimethoxytoluene (peak 10) and 1,2,4-trimethoxytoluene (peak 15)], were also identified. The origin of these compounds is unknown. Few peaks were identified as being derived from carbohydrates because the procedure appears to be more selective for lignin phenols, as also reported by Wetzel et al. [41]. Some fatty acid methyl esters were also present, with a strong even-over-odd carbon number predominance.

The Suwannee river DOM yielded relatively high amounts of lignin-derived phenols (Fig. 7). The major components identified are the benzoic acid derivatives of the vanillyl and syringyl structures, namely 3,4-dimethoxybenzoic acid methyl ester (37) and 3,4,5-trimethoxybenzoic acid methyl ester (44), respectively. This latter product can also be derived from hydrolyzable tannin derivatives. The 4-methoxybenzoic acid methyl ester (17) was also identified in minor amounts. Benzoic acid derivatives have also been identified as major compounds in the DOM from decomposing *Juncus effusus* upon pyrolysis/TMAH [41]. The presence of these phenols indicates that the Suwannee River DOM contains lignin-derived structures which have been oxidized at the α-carbon of the side chain. Several other aromatic acid derivatives, having the carboxyl group at the β- and γ-carbons of the side-chain, such as the benzeneacetic acid derivatives, 4-methoxybenzeneacetic acid methyl ester (24), 3,4-dimethoxybenzeneacetic acid methyl ester (39) and 3,4,5-trimethoxybenzeneacetic acid methyl ester (47), as well as the propanoic acid derivative 3,4-dimethoxybenzenepropanoic acid methyl ester (43), were also detected in the Suwannee River DOM, albeit in relatively minor amounts.

Another striking feature of the Suwannee River DOM was the presence of 1,3,5-trimethoxybenzene (21) and 2,4,6-trimethoxytoluene (25), in relatively high amounts. These compounds are not related to lignin and have been recently detected as major components in the TMAH thermochemolysis of cutan [40], the highly aliphatic and resistant biopolymer present in leaf cuticles from certain plants [30].

In the oceanic DOM samples from the Gulf of Mexico (Fig. 8), different lignin-derived phenol-derivatives, which indicate the presence of organic matter originating from vascular plants and therefore from terrestrial (upland and coastal marsh) ecosystems, were identified. These compounds were detected in the nearshore DOM (Station 1) as well as in both offshore DOM (Station 5), at the surface and at 1600-m depth. The results are consistent with the recent studies of Bianchi et al. [42] which indicate a significant terrestrial component for these DOM samples based on CuO oxidation studies. There is a predominance of di- and trimethoxybenzenes in these samples, the major compounds being the 1,2-dimethoxybenzene (6), 1,4-dimethoxybenzene (7), 2,5-dimethoxytoluene (10) and 1,2,4-trimethoxybenzene (15). The benzenecarboxylic acid derivatives are less abundant than in the Suwannee River DOM, and only the *p*-coumaryl and guaiacyl derivatives, 4-methoxybenzoic acid methyl ester (17) and 3,4-dimethoxybenzoic acid methyl ester (37), respective-

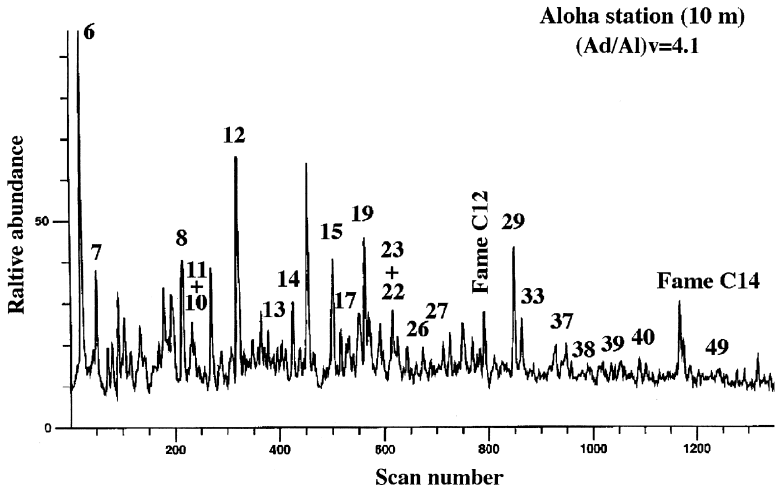

Fig. 9. Total ion chromatogram of the TMAH thermochemolysis products from the Aloha DOM sample. For peak identifications refer to Table 6. Chromatographic conditions for this sample were different from those depicted in Figs. 6 and 7.

Table 6
List of compounds identified in the DOM samples

1	Pentanoic acid methyl ester	26	Dimethoxybenzenemethanol, methyl ether (isomer of 32)
2	Butanedioic acid dimethyl ester	27	Benzenedicarboxylic acid, dimethyl ester (3 isomers)
3	Methylbutanedioic acid dimethyl ester	28	1-(3,4-Dimethoxyphenyl)-1-methoxyethane
4	Pentanedioic acid dimethyl ester	29	3-Chloro-4-methoxybenzoic acid, methyl ester
5	2-Methylpentanedioic acid dimethyl ester	30	1-(3,4-Dimethoxyphenyl)-1-propene
6	1,2-Dimethoxybenzene	31	3,4-Dimethoxybenzaldehyde
7	1,4-Dimethoxybenzene	32	3,4-Dimethoxybenzenemethanol, methyl ether
8	3,4-Dimethoxytoluene	33	1-(3,4-Dimethoxyphenyl)-2-methoxy-propane
9	3-Methoxy-4-acetyl-2-5(H)-furanone	34	Dimethoxybenzoic acid, methyl ester
10	2,5-Dimethoxytoluene	35	Dimethoxybenzenemethanol, methyl ether
11	4-Methoxybenzaldehyde	36	3,4-Dimethoxyacetophenone
12	2-Vinyl-5-methoxy-2,3-dihydrobenzo-furan	37	3,4-Dimethoxybenzoic acid, methyl ester
13	1,2,3-Trimethoxybenzene	38	1-(4-Methoxyphenyl)-1,2-dimethoxy-propane
14	3-Methoxybenzoic acid, methyl ester	39	3,4-Dimethoxybenzeneacetic acid, methyl ester
15	1,2,4-Trimethoxybenzene	40	*trans*-4-Methoxyacrylic acid, methyl ester
16	4-Methoxyacetophenone	41	3,4,5-Trimethoxyacetophenone
17	4-Methoxybenzoic acid, methyl ester	42	2-Methoxy-1,5-benzenedicarboxylic acid, methyl ester
18	Benzeneacrylic acid, methyl ester (*cis* or *trans*)	43	3-(3,4-Dimethoxyphenyl)propanoic acid, methyl ester
19	3,4-Dichlorobenzoic acid, methyl ester	44	3,4,5-Trimethoxybenzoic acid, methyl ester
20	1-(4-Methoxyphenyl)-methoxy-propane	45	1-(3,4-Dimethoxyphenyl)-1,2-dimethoxy-propane
21	1,3,5-Trimethoxybenzene	46	*cis*-1-(3,4-Dimethoxyphenyl)-1,3-di-methoxy-1-propene
22	3,4,5-Trimethoxytoluene	47	3,4,5-Trimethoxybenzeneacetic acid, methyl ester
23	1-(4-Methoxyphenyl)-methoxy-propane (isomer of 20)	48	*trans*-1-(3,4-Dimethoxyphenyl)-1,3-di-methoxy-1-propene
24	4-Methoxybenzeneacetic acid, methyl ester	49	2-Methoxy-1,5-benzenedicarboxylic acid, dimethyl ester
25	2,4,6-Trimethoxytoluene	50	1-(3,4-Dimethoxyphenyl)-2-methoxy-propanoic acid, methyl ester

ly, were found in detectable amounts. In general, among the lignin-derived compounds, there is a predominance of *p*-coumaryl and guaiacyl structures in these samples. Among the syringyl structures, only 1,2,3-trimethoxybenzene (13) and 3,4,5-trimethoxytoluene (22) were found in trace amounts. These could originate either from lignin or tannins.

The DOM isolated from the Aloha station in the central Pacific (Fig. 9) is representative of open oceanic regimes and is the least affected by direct river inputs. The presence of possible lignin derivatives in this sample is an indication of the input of organic matter derived from terrestrial sources, and, thus, this would indicate the possibility of terrestrial markers in the Aloha DOM. There is a predominance of dimethoxybenzenes with a vanillyl structure (1,2-dimethoxybenzene, 6, and 3,4-dimethoxytoluene, 8). Possible syringyl derivatives (1,2,3-trimethoxybenzene, 13, and 3,4,5-trimethoxytoluene, 22) were also detected but in minor amounts. As in the case of the Gulf of Mexico samples, benzenecarboxylic acid derivatives are less abundant, and only the *p*-coumaryl and vanillyl structures (4-methoxybenzoic acid methyl ester, 17, and 3,4-dimethoxybenzoic acid methyl ester, 32, respectively) were found, with the absence of the syringyl-derived units.

Ratios of vanillic acid/vanillin $(Ad/Al)_v$, which are indicative of the amount of oxidative degradation of the lignin component [35,43] were calculated for each DOM sample. It has already been shown [28] that a strong linear correlation exists between the $(Ad/Al)_v$ ratios calculated with the CuO oxidation and the TMAH procedures, but also that the TMAH method is more sensitive an indicator of lignin degradation due to its larger dynamic range for $(Ad/Al)_v$. This ratio is very high (13.5) in the case of the Suwannee river DOM (Fig. 7), indicative of the high extent of oxidative degradation of the lignin component in this sample. In the case of coastal DOM samples of the Gulf of Mexico (Fig. 8), the values are lower than those of the Suwannee River and decrease from nearshore (9.5) to offshore (5.1). For the Aloha sample, representative of oceanic systems, this ratio is even lower (4.1). Bianchi et al. [42], using the CuO oxidation method, also observed that $(Ad/Al)_v$ ratios of particulate organic matter (POC) and DOM decreased with increasing distance from shore in the Gulf of Mexico. Although a limited set of samples representative of a wide variety of systems have been selected for this study, it is apparent that the acid/aldehyde ratio seems to decrease in comparing riverine (Suwannee River) to coastal (Gulf of Mexico) and to open oceanic (Aloha) sites. At the same time, there is an increase in the proportions of di- and trimethoxybenzenes compared to their respective benzenecarboxylic acid methyl esters. The decrease in the $(Ad/Al)_v$ ratios can be explained as resulting from the fact that the effectiveness of lignin degradation decreases with increasing distance from shore, perhaps due to increasing paucity of lignin-degrading microorganisms. Alternatively, decarboxylation of lignin moieties could also likely explain this trend and this could be attributed to either microbial, chemical and/or photochemical processes taking place in solution. The di- and trimethoxybenzenes and toluenes would be the end products after extensive degradation and subsequent decarboxylation of lignin-derived structures in the DOM.

It is likely that any lignin-derived material becoming a part of DOM would have to be subjected to extensive chemical alteration, because lignin polymers are not normally soluble in natural or oceanic waters. The lignin units normally detected by the CuO procedure appear to bear this out as the phenolic acid units predominate over the phenolic aldehydes [44]. It is also likely that any lignin moiety that is entrained within DOM would have a significant amount of carboxyl functionality which would afford it solubility.

Some of the phenol-derivatives that have been assigned to derive from lignin structures may also have an alternative origin. This is the case, for instance, of some of the *p*-methoxyphenyl derivatives, especially the 4-methoxybenzaldehyde (11) and 4-methoxybenzoic acid methyl ester (17), which could also arise from the amino acid, tyrosine, in proteins. However, the presence of phenol derivatives which have an attached methoxylated propanoid side chain (i.e. peaks 20, 23, 28, 33, 38, 45, 46, 48, 50) in the studied DOM samples, is significant, since these compounds can be unambiguously related to lignin structures present in the DOM. Also, it is clear that the TMAH thermochemolysis induces cleavage of select ethers and methylation of hydroxyl groups attached to the side chain. This means that

the side chain units are preserved during the thermochemolysis, in contrast to the CuO oxidation procedure which causes side chain oxidation. The presence of some of these compounds (peaks 23, 33, 38), not only in the sample from the Suwannee river but also in the samples taken from oceanic systems (Aloha station) and coastal oceans (Gulf of Mexico, even at 1600-m depth), is noteworthy, since it suggests the preservation of lignin-moieties in these particular DOM samples.

4. Conclusions

A large set of biogeomacromolecules, such as fresh and degraded gymnosperm and angiosperm woods, proteins, polyesters, the diagenetically resistant biopolymer cutan, humic substances and dissolved organic matter in natural waters, were examined by TMAH/thermochemolysis. Performed in a sealed glass ampoule, this procedure consists mainly of a thermally assisted chemolysis and subsequent methylation. It methylates carboxylic and hydroxyl groups, rendering the chemolytic products more amenable to GC separation. It also induces cleavages of the β-O-4 ether bond in lignin. This is significant because it shows the potential to characterize lignin-derived compounds similar to the CuO oxidation and the same ratios of lignin-derived phenols can be determined here. Unlike pyrolytic methods, TMAH avoids decarboxylation of benzenecarboxylic acids, and produces more products with intact or partially altered side chains therefore giving a better insight into the molecular structure of bio- and geo-polymers. Finally, because the procedure is easily performed in sealed glass tubes at 250°C, it can be easily implemented in any laboratory having gas chromatographic capabilities, in contrast to other chemolytic or pyrolytic procedures.

Perhaps the one major advantage TMAH thermochemolysis has over pyrolysis and pyrolysis/methylation approaches to structural analysis, is the ability to add internal standards and to evaluate the yields of products. While most pyrolytic procedures have been considered to yield low amounts of products from natural geopolymers, the application of TMAH thermochemolysis allows estimation of the amounts of products formed. This enables one to evaluate the relative significance of the products in structural assignments. In this paper we have presented some yields from TMAH thermochemolysis, and, in most cases, generally less than 10% of the geopolymer macromolecules are converted to identifiable products. This low yield can hardly be called a quantitative assessment of structural composition. However, it represents a first step in providing details of compounds cleaved from a structural backbone of the geopolymers, even though they may not be representative of the backbone itself. From our studies of lignin, we have been able to recognize that large amounts of dimers, not easily separated by GC, are produced and these have many of the structural similarities of the monomers. Thus, in the case of lignin and wood samples, the low yields are reflective of the fact that dimers and perhaps trimers are reducing the overall monomer yields, but that the monomers are reflective of the overall structural composition of the samples. In the case of humic acids or cutin polyesters, we are less certain of the degree to which monomers represent the polymers. Ongoing work is seeking answers to this question.

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