

Comparison of reagents for off-line thermochemolysis of natural organic matter

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Abstract

A range of tetramethylammonium salts with different base strengths was compared for off-line thermochemolysis of an aquatic natural organic matter sample. Tetramethylammonium acetate (TMAAc) in methanol produced a different suite of compounds to the more alkaline tetramethylammonium hydroxide (TMAH) in methanol and tetramethylammonium carbonate (TMACO₃) in water. TMAAc treatment produced methyl 3-methylmercaptopropanoate, but no sulfur compounds were produced from TMAH or TMACO₃. Only TMAAc in methanol produced methyl esters. A mechanism for this formation of methyl esters, involving transesterification with methoxide ion derived from the solvent, was proposed. Off-line TMAAc in methanol appears to avoid decarboxylation, provide more information than on-line TMAAc and preserve more structural information than off-line TMAH in methanol or TMACO₃ in water.

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1. Introduction

Pyrolysis with *in situ* methylation (pyrolysis-methylation) with the reagent tetramethylammonium hydroxide (TMAH) was developed for the structural characterisation of polymers by Challinor (1989). TMAH is not only a methylating agent, it is also a strong base, and the highly basic conditions are likely to induce a variety of additional reactions. Subsequent research (de Leeuw and Baas, 1993; Martin et al., 1994; Hatcher and Clifford, 1994; Kralert et al., 1995) has established that the process does in fact consist of thermally-assisted chemolysis, termed “thermochemolysis” or “thermally-assisted hydrolysis and

methylation”, rather than pyrolysis followed by *in situ* methylation of the pyrolysis products. McKinney and co-workers (1995) then reported the development of an “off-line” thermochemolysis method using TMAH, which was found to produce nearly identical products to the pyrolysis-methylation technique. We have favoured the use of the off-line technique (Heitz et al., 2001; Joll et al., *in press*), since internal standards can be added and by-products of TMAH can easily be removed prior to analysis by gas chromatography-mass spectrometry (GC-MS). This improves chromatographic resolution and minimises deterioration of injection systems and columns from attack by aggressive reagents and by-products. A preparative scale, off-line TMAH thermochemolysis method has also been developed (Grasset and Amblès, 1998).

The TMAH thermochemolysis methods have been used for structural characterisation of a wide variety of

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polymeric organic materials, including synthetic resins, lipids, waxes, natural resins, wood products, soil, sediments, proteins and carbohydrates [reviewed recently by Challinor (2001)]. One of the main advantages of the TMAH thermochemolytic methods over pyrolytic methods is that the TMAH methods are commonly considered to result in methylation of polar carboxylic acids (e.g., de Leeuw and Baas, 1993; Martin et al., 1994; Hatcher and Clifford, 1994; Kralert et al., 1995), which have poor chromatographic behaviour or undergo decarboxylation in conventional pyrolysis. This allows identification of carboxylic acids as their methyl esters. However, our recent studies on model compound carboxylic acids showed that some *ortho*- and/or *para*-substituted aromatic carboxylic acids undergo decarboxylation during off-line TMAH thermochemolysis (Joll et al., in press). There have been previous reports of decarboxylation during model compound reactions (Saiz-Jimenez et al., 1993; Saiz-Jimenez, 1994; Galletti and Bocchini, 1995; Tanczos et al., 1999) and our mechanism for decarboxylation can also account for these results. Off-line TMAH thermochemolysis of model compound lignin dimers, not containing any carboxyl groups, was reported to produce significant amounts of “oxidised products”, including 4-methoxy- and 3,4-dimethoxy-benzoic acids as their methyl esters (Hatcher and Minard, 1995). This work showed that benzoic acids could be produced by TMAH thermochemolysis reactions on aromatic compounds without carboxyl groups. However, subsequent partial decarboxylation of these two benzoic acids in the reaction conditions was not investigated, and the respective decarboxylation products, methoxybenzene and 1,2-dimethoxybenzene, may still have been present, since they would have eluted during the unpublished early portion of the product chromatogram. Interpretation of product mixtures from TMAH thermochemolysis of polymeric organic matter must therefore be made with caution and, in particular, the origins of hydroxybenzene and methoxybenzene products must be considered to include hydroxybenzoic acids and methoxybenzoic acids. In TMAH thermochemolysis, methyl ethers are formed from alcohols and phenols, though conversion is sometimes incomplete (e.g., Challinor, 1989).

The use of tetramethylammonium acetate (TMAAc) as a reagent in thermochemolysis was first reported by Hardell and Nilvebrant (1999). They found that, in on-line thermochemolysis, TMAAc was a selective reagent for the methylation of free acids without altering any esters present, as these did not react under the mildly alkaline reaction conditions. This was in contrast to TMAH, which is strongly alkaline and resulted in hydrolysis of esters and methylation of the resultant acids, as well as methylation of any free acids present. The susceptibility to decarboxylation of some *ortho*- and/or *para*-substituted aromatic carboxylic acids (Joll

et al., in press) and the isomerization observed with some unsaturated fatty acids (Hardell and Nilvebrant, 1999) increases the complexity of interpretation of TMAH thermochemolysis product mixtures. More recently, Asperger et al. (2001) used the selectivity of on-line TMAAc thermochemolysis to measure the abundances of free fatty acids, without releasing the ester-bound acids, in fresh and aged lanolin samples. Grasset and Amblès (1998) adopted TMAH and tetraethylammonium acetate (TEAAc) thermochemolysis in a preparative off-line mode to study the free lipid, humic acid and humin fractions from a peat sample.

TMAAc therefore seems to have appeal as an alternative thermochemolysis reagent to TMAH, due to its mildness and selectivity. In this paper, we report the results of a comparative study of the treatment of an aquatic natural organic matter (NOM) sample with a range of tetramethylammonium salts with different base strengths in off-line thermochemolysis experiments. Four thermochemolysis reagents, with anions of decreasing basicity, were selected: TMAH, tetramethylammonium carbonate (TMACO₃), TMAAc and tetramethylammonium bromide (TMABr). Their order is based upon the increasing strength of their conjugate acids (pK_a values: H₂O: 14; H₂CO₃: 6.37; CH₃CO₂H: 4.75; HBr: -9). The use of TMACO₃ and TMABr as thermochemolysis reagents has not previously been reported. In this study, TMABr provided an example of a weakly alkaline thermochemolysis reagent of the tetramethylammonium type.

2. Experimental methods

2.1. Chemicals

The thermochemolysis reagents were obtained as follows: tetramethylammonium acetate (95%) and tetramethylammonium carbonate (99.99% as 25% w/w solution in water) from Aldrich; tetramethylammonium hydroxide pentahydrate (97%) from Sigma; tetramethylammonium bromide (98.5%) from BDH. Methanol (HPLC grade) was obtained from Mallinckrodt and the dichloromethane (AR grade) was purified by fractional distillation. Magnesium sulfate (APS) was heated at 600 °C prior to use. Hydrochloric acid (31.5–33.0%) was obtained from BDH. Purified laboratory water was prepared by passing deionised or distilled water through a Barnstead Milli-Q system.

2.2. Sample collection and treatment

A raw water sample (80 l; dissolved organic carbon concentration: 24 mg l⁻¹) was obtained on 14/09/99 from a shallow groundwater bore (W80) located in the Wanneroo borefield, Perth, Western Australia. The

aquatic NOM in the sample was desalted and concentrated by tangential flow ultrafiltration using a SEPA[®] CF Membrane Cell (Osmonics Inc.) with a BQ01 membrane of nominal molecular weight (NMW) cut off 5000 Da. The water remaining in the retentate was removed by freeze-drying using a Labconco Freezone 4.5 Freeze Dry System. The freeze-dried aquatic NOM sample (NMW > 5000 Da) was used for the thermochemolysis experiments. Total sulfur was determined on a GBC ELTRA CS-2000 Carbon Sulfur Determinator.

2.3. Off-line thermochemolysis procedure

The procedure used was similar to that developed by McKinney et al. (1995). Briefly, the freeze-dried NOM sample (5 mg) was weighed into a pre-annealed Pyrex tube (7×125 mm) that had been sealed at one end. The thermochemolytic reagent was added either as a methanolic solution (TMAH, TMAAc, TMABr) or as an aqueous solution (TMACO₃) (300 µl, 25% w/v). The walls of the tube were washed with methanol (~10 µl) and the tube was subjected to ultrasound (30 min) to ensure thorough mixing. The volume was then reduced to one-third under a gentle flow of nitrogen and the tube was flushed with nitrogen before being sealed under vacuum. The reaction mixture was heated (250 °C, 90 min), allowed to cool to room temperature, before cooling further (–170 °C) and the tube was opened. The reaction products were extracted twice with dichloromethane (0.5 ml) using ultrasound (30 min). The combined dichloromethane extracts were washed with dilute aqueous hydrochloric acid solution (2×1 ml, 1 M), followed by water (1 ml; Milli-Q), and then dried (MgSO₄). The volume of dichloromethane was reduced to 100 µl using a flow of nitrogen gas, prior to analysis by gas chromatography-mass spectrometry.

2.4. Gas chromatography-mass spectrometry

The thermochemolysis extracts were analysed using capillary gas chromatography-mass spectrometry (GC-MS) using a Hewlett Packard (HP) 6890 capillary gas chromatograph interfaced to a HP 5973 quadrupole scanning mass spectrometer. Separation of compounds was achieved using a Phenomenex ZB-1 fused silica capillary column (60 m×0.25 mm i.d.; 0.25 µm film thickness). Helium was employed as the carrier gas at a linear flow velocity of 24 cm s⁻¹. The sample (1 µl) was injected on to the column using a HP 6890 autosampler and cool on-column injection. The GC oven was programmed from 35 °C (held for 5 min) to 230 °C at 5 °C min⁻¹ and then to 310 °C (held for 10 min) at 15 °C min⁻¹. The mass spectrometer was operated in the full scan mode (mass range *m/z* 33–600), with ionisation energy 70 eV. Mass spectrometric detection commenced after a 12 min solvent delay period. Computer matching

of mass spectra with those in the Wiley 275 mass spectral database was used for initial identification of a product. Detailed comparison of the mass spectrum of the product with those generated from the database was then carried out to confirm the product identification. Identification of all three trimethoxybenzene isomers was confirmed by comparison of retention times with those of authentic standards as previously determined (Heitz, 2002).

3. Results and discussion

3.1. Overall comparison of four thermochemolytic reagents

An aquatic NOM sample of nominal molecular weight (NMW) > 5000 Da was isolated from a water sample collected from a local groundwater bore using tangential flow ultrafiltration followed by freeze-drying. Off-line thermochemolysis (250 °C, 90 min), followed by GC-MS analysis, was conducted on this aquatic NOM sample with the four thermochemolytic reagents. In order of decreasing basicity of the anion, these were tetramethylammonium hydroxide (TMAH) in methanol, tetramethylammonium carbonate (TMACO₃) in water, tetramethylammonium acetate (TMAAc) in methanol and tetramethylammonium bromide (TMABr) in methanol. The high viscosity of the residue made it difficult to completely remove all of the solvent prior to heating these mixtures. The distribution of products from the mixtures was found to be virtually identical, whether or not attempts were made to evaporate the residue to dryness, so a procedure where the solvent was only partially evaporated from the reaction mixture was adopted. The heating time was longer than commonly reported literature heating times: e.g., 250 °C for 30 min (Hatcher and Minard, 1995) or 300 °C for 10 min (McKinney et al., 1995). We have found that longer heating times, up to 90 min, yield a similar product mixture where products are formed in higher abundance. These results will be the subject of a future paper. Each experiment here was performed in duplicate or triplicate, and the results were found to be qualitatively repeatable.

Typical total ion gas chromatograms from thermochemolysis of the aquatic NOM sample using the four thermochemolytic reagents, TMAH, TMACO₃, TMAAc and TMABr, are presented in Fig. 1. A solvent delay period of 12 min was employed to avoid detection of both solvent and any residual by-product, trimethylamine, remaining in the mixture after an acidic extraction. The major peaks are numbered in the chromatograms, with the corresponding compounds listed in Table 1. The three most abundant ions in the mass spectrum of each compound, including

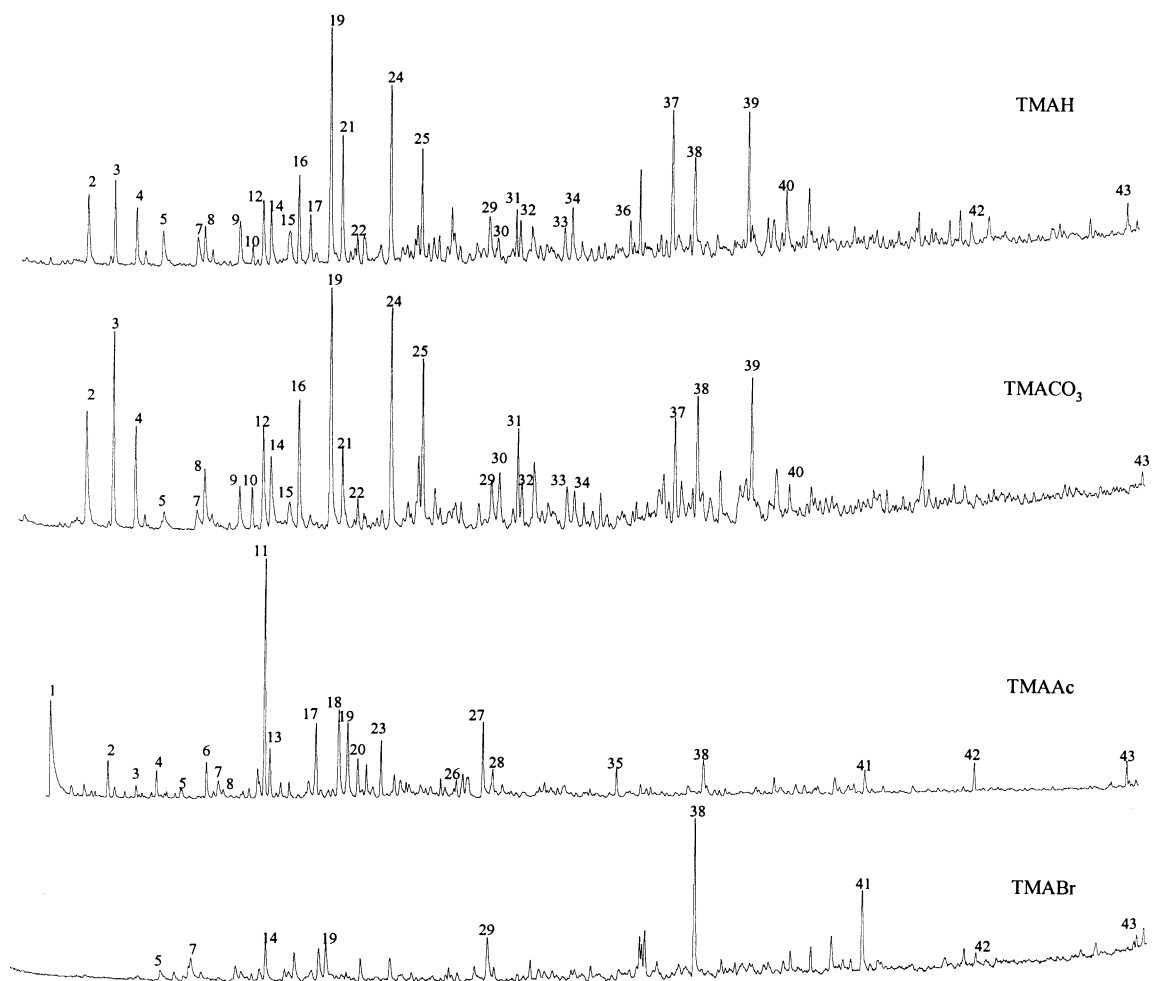


Fig. 1. Total ion chromatograms showing products from off-line thermochemolysis of the aquatic NOM sample using the four thermochemolytic reagents.

identification of the base peak, are also presented in Table 1.

Inspection of the total ion chromatograms in Fig. 1 suggests that broadly similar products, in similar abundances, were produced from thermochemolysis using the two most alkaline reagents, TMAH in methanol and TMACO_3 in water. Mass spectral identification of the major products in both chromatograms confirmed this and the compounds are listed in Table 1. The most abundant peaks in the chromatograms were methoxybenzene (3; Fig. 1), 2-methoxyphenol (19), 2-hydroxy-3-methoxyphenol (21), 1,2-dimethoxybenzene (24) and 1,3-dimethoxybenzene (25), 1,3,5-trimethoxybenzene (37) and 2,4,6-trimethoxytoluene (39). As expected, TMAAc in methanol was an ineffective thermochemolytic reagent and produced very few products. This is presumably because it is insufficiently alkaline to cause any significant cleavage of the macromolecular organic matter at the sub-pyrolysis temperature of 250 °C.

Treatment of the sample with the reagent of intermediate alkalinity, TMAAc in methanol, produced a different suite of compounds to those produced by TMAH and TMACO_3 , in slightly lower, but still comparable, overall abundances. Some of the most abundant peaks in the product were methyl 3-methylmercapto-propanoate (11; Fig. 1), methyl 2-methyl-4,4-dimethoxybutanoate (17), 2-methoxyphenol (19) and methyl 3-methylbenzoate (27), with two abundant peaks remaining unidentified.

3.2. Sulfur-containing products

The products obtained from treatment of the sample of aquatic NOM with TMAAc included some sulfur-containing compounds, with methyl 3-methylmercapto-propanoate (11; Fig. 1) being the most abundant of all of the products. Other sulfur-containing compounds, such as 2-(methylmercapto)benzothiazole (41), were

Table 1
Major products from TMAH, TMACO₃, TMAAc and TMABr thermochemolysis of the aquatic NOM sample

Peak no	Compound	Molecular weight	Abundant ions (base peak in bold)
1	<i>N,N</i> -Dimethylacetamide	87	15, 44 , 87
2	2-Methyl-2-cyclopentenone	96	53, 67 , 96
3	Methoxybenzene	108	65, 78, 108
4	2,5-Dimethyl-2-cyclopentenone	110	67, 95, 110
5	3-Methyl-2-cyclopentenone	96	53, 67, 96
6	Methyl 3-acetopropionate	130	43 , 99, 115
7	Phenol	94	39, 66, 94
8	2,5-Dimethyl-1,4-hexadiene	110	67, 95 , 110
9	1-Methoxy-2-methylbenzene	122	91, 107, 122
10	1-Methoxy-3-methylbenzene	122	77, 91, 122
11	Methyl 3-methylmercaptopropanoate	134	61 , 74, 134
12	2,3-Dimethyl-2-cyclopentenone	110	39, 67 , 110
13	Dimethyl butanedioate	146	55, 59, 115
14	2-Ethylhexanol	130	41, 43, 57
15	3,5-Dimethylcyclopentenolone	126	69, 111, 126
16	2,3,4-Trimethyl-2-cyclopentenone	124	81, 109 , 124
17	Methyl 2-methyl-4,4-dimethoxybutanoate	176	41, 59 , 129
18	Unknown A		42, 58, 127
19	2-Methoxyphenol	124	81, 109 , 124
20	Methyl benzoate	136	77, 105 , 136
21	2-Hydroxy-3-methoxyphenol	140	97, 125, 140
22	2,4-Dimethylanisole	136	91, 121, 136
23	Unknown B		54, 110, 139
24	1,2-Dimethoxybenzene	138	95, 123, 138
25	1,3-Dimethoxybenzene	138	78, 109, 138
26	Methyl 2-methylbenzoate	150	91, 119 , 150
27	Methyl 3-methylbenzoate	150	91, 119 , 150
28	Methyl 4-methylbenzoate	150	91, 119 , 150
29	4- <i>tert</i> -Butylcyclohexanol	156	57 , 67, 81
30	2,3-Dimethoxytoluene	152	109, 137, 152
31	2,5-Dimethoxytoluene	152	109, 137 , 152
32	2,4-Dimethoxytoluene	152	121, 137, 152
33	3-Methoxyacetophenone	150	77, 135 , 150
34	1,2,3-Trimethoxybenzene	168	110, 153, 168
35	Methyl 3-methoxybenzoate	166	107, 135 , 166
36	1,2,4-Trimethoxybenzene	168	125, 153 , 168
37	1,3,5-Trimethoxybenzene	168	69, 139, 168
38	2,4,7,9-Tetramethyl-5-dicyne-4,7-diol	226	43 , 109, 151
39	2,4,6-Trimethoxytoluene	182	151, 167, 182
40	3,4,5-Trimethoxybenzaldehyde	196	125, 181, 196
41	(2-Methylmercapto)benzothiazole	181	108, 148, 181
42	Methyl tetradecanoate	242	74 , 87, 143
43	Methyl hexadecanoate	270	74 , 87, 143

also present. The structures of these two sulfur-containing products are presented in Fig. 2. The environment from which this NOM sample was obtained is a shallow, unconfined aquifer system, with sediments consisting of coarse sand interdispersed with limestone. Water from this freshwater aquifer contains very high concentrations of dissolved organic carbon, up to 50 mg l⁻¹, total dissolved salts up to 400 mg l⁻¹ and sulfide up to 2 mg

l⁻¹. Organically-bound sulfur is of particular interest in this environment and we have previously reported the first identification of methyl 3-methylmercaptopropanoate from TMAH thermochemolysis, after treatment of aquatic NOM samples isolated similarly from groundwater bores adjacent to the bore used in the present study (Heitz et al., 2001; Heitz, 2002). In these previous studies, methyl 3-methylmercaptopropanoate was

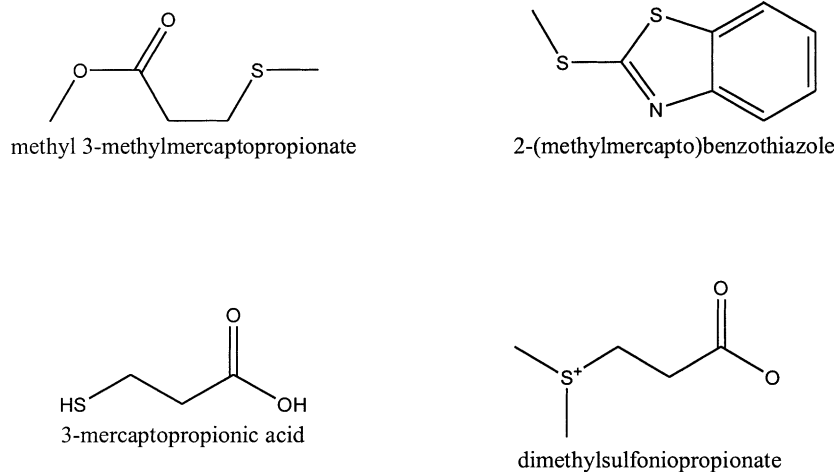


Fig. 2. Structures of two sulfur-containing products and possible precursor compounds of one of these products.

produced in relatively small amounts and was accompanied by small amounts of methyl 3-methylmercaptobutanoate. The mass spectrum of methyl 3-methylmercaptopropanoate shows a characteristic ion at m/z 61, representing the fragment ion $\text{CH}_3\text{SCH}_2^+$. Preliminary studies (Heitz et al., 2001) suggested that methyl 3-methylmercaptopropanoate originated from an ester-bound thiol rather than a methylsulfide compound. It was proposed that the origin of the compound released by TMAH treatment may be 3-mercaptopropanoic acid (3-MPA; Fig. 2) which was bound to NOM via an ester linkage; 3-MPA is a degradation product of dimethylsulfoniopropionate (Fig. 2), which is produced by marine organisms to regulate salinity changes in marine and estuarine environments (MacCrehan and Shea, 1995, and references therein). It was proposed that 3-MPA might be produced via this mechanism in sediments associated with wetlands in hydraulic contact with this groundwater aquifer, since these environments are subject to substantial annual salinity changes. Methyl 3-methylmercaptopropanoate was therefore proposed as a marker compound to identify NOM derived from this type of environment (Heitz et al., 2001).

The release of this marker compound is particularly favoured by the use of TMAAc in methanol as the thermochemolytic reagent. No sulfur-containing compounds were identified in the chromatograms from thermochemolysis with TMAH or TMACO_3 , even though the aquatic NOM sample contained a significant amount of sulfur (1.23% w/w). This may indicate a gentle but efficient cleavage of a 3-mercaptopropanoate ester linkage to the macromolecular NOM with TMAAc in methanol, which is in contrast to the reported stability of ester linkages to TMAAc treatment (Hardell and Nilvebrant, 1999; Asperger et al., 2001). Although TMAH is a more alkaline reagent than

TMAAc and should therefore have a greater capacity for ester cleavage, treatment with TMAH did not result in production of this marker compound from this sample and only produced the marker compound in low abundances from our earlier samples (Heitz et al., 2001; Heitz, 2002). One possible explanation is that 3-MPA was released from the macromolecular NOM after thermochemolysis with TMAH, but was unstable in the highly alkaline conditions and underwent further reaction, e.g., decarboxylation. The likely product of decarboxylation, ethyl methyl sulphide, would elute during the solvent delay period and would not be observed in the chromatogram.

3.3. Ester formation from benzoic acids

One of the more abundant peaks in the chromatogram from thermochemolysis with TMAAc in methanol was the methyl ester, methyl 3-methylbenzoate (27; Fig. 1), and many other methyl esters [e.g., methyl benzoate (20), methyl 2-methylbenzoate (26), methyl 4-methylbenzoate (28), methyl 3-methoxybenzoate (35)] featured in the product mixture. The production of esters sets apart the TMAAc in methanol product mixture, since no esters were produced by the other three thermochemolytic reagents. A comparison of the abundances of four of these esters produced by three different treatments, TMAH in methanol, TMACO_3 in water and TMAAc in methanol, is presented in Fig. 3. Since TMAH and TMACO_3 are more alkaline than TMAAc, they should have a greater capacity for cleavage of ester linkages in NOM, with release of carboxylic acids which might be expected to be methylated to give methyl esters under the reaction conditions. The most likely explanation for the absence of the methyl esters of benzoic acid and the three monomethylbenzoic acid isomers from the TMAH and

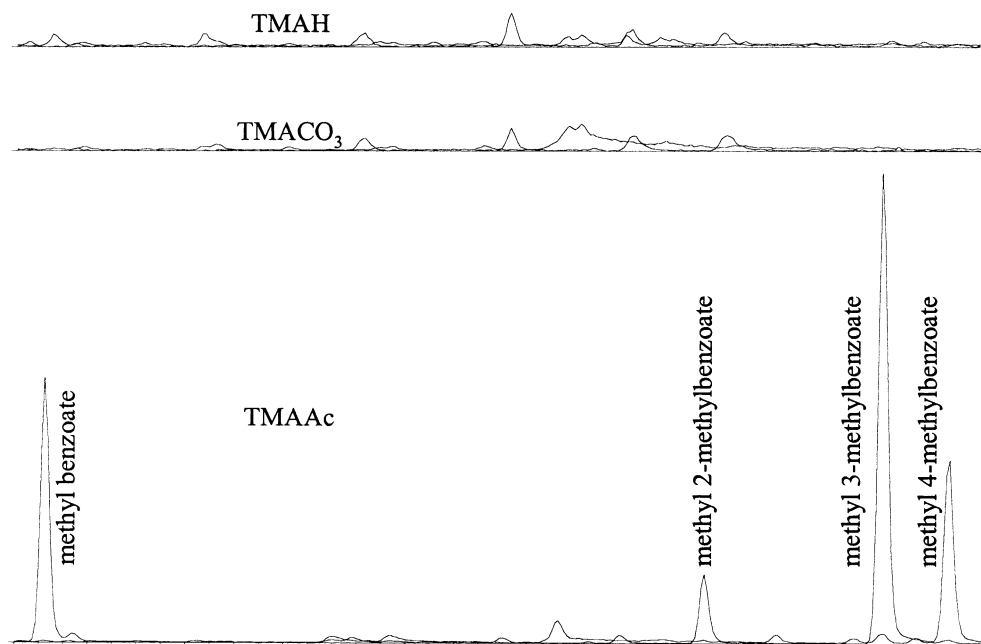


Fig. 3. Partial ion chromatograms (m/z 105, 119) showing relative abundances of methyl benzoates from off-line thermochemolysis of the aquatic NOM sample using three of the thermochemolytic reagents.

TMACO₃ product mixtures is that the acids themselves were in fact released, but underwent further reaction, such as decarboxylation to produce benzene and toluene, respectively, which would also elute during the solvent delay period and would not be observed in the chromatograms. Model aromatic carboxylic acids with *ortho* and/or *para* substituents which contain an unshared pair of electrons on the atom connected to the aromatic ring and which are activating to electrophilic aromatic substitution (e.g. phenoxide, hydroxy, methoxy, amino) were found to undergo decarboxylation during off-line TMAH thermochemolysis (Joll et al., *in press*), with the extent of decarboxylation dependent on the mole ratio of TMAH to model compound. No methylbenzoic acids were included in the model compound studies and, while benzoic acid did produce methyl benzoate, any decarboxylation to benzene would not have been observed. The present results indicate that decarboxylation in off-line TMAH thermochemolysis may also be possible with benzoic acid and the methylbenzoic acids, if the mole ratio of TMAH to acid is sufficiently high. Since this is the first reported use of TMACO₃ in water as a thermochemolytic reagent, decarboxylation with this reagent has not been studied. However, its alkaline nature and the similarity in its product distribution to that from the use of TMAH indicate that decarboxylation may also occur with TMACO₃ in water. Much of the structural information about the carboxylic acids derived from macromolecular NOM in thermochemolysis experiments is

lost with the use of the more alkaline reagents, TMAH in methanol and TMACO₃ in water. TMAAc in methanol therefore appears to be a useful reagent for releasing the acid portion of some esters in macromolecular NOM and for preserving structural information about the acid moiety through formation of the methyl ester.

These observations from off-line thermochemolysis experiments contrast with the findings of Hardell and Nilvebrant (1999) and Asperger et al. (2001), who found that, in on-line TMAAc thermochemolysis, esters were not cleaved under the reaction conditions. A different mechanism must have occurred in our off-line TMAAc in methanol experiments, where the acid portion of some esters within macromolecular NOM appears to have been released and the corresponding methyl esters formed.

The mechanism that we propose to explain this observation is as follows: a portion of the acetate ion deprotonates some of the methanol remaining in the reaction mixture to form acetic acid and methoxide ion at the elevated temperature of thermochemolysis, as illustrated in Fig. 4. In other words, some tetramethylammonium methoxide is generated in situ. This methoxide ion is then available for transesterification of ester linkages within macromolecular NOM to form the methyl ester directly, without formation of the carboxylic acid, as depicted in Fig. 5. Since formation of the carboxylic acid (or carboxylate anion) is avoided, no decarboxylation occurs. With TMAH in methanol,

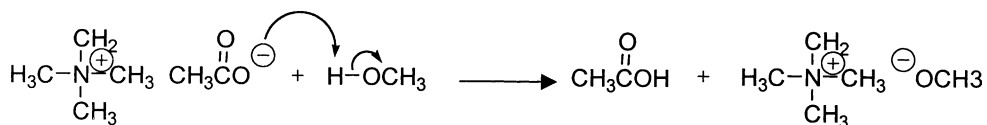


Fig. 4. Proposed mechanism of formation of in situ tetramethylammonium methoxide.

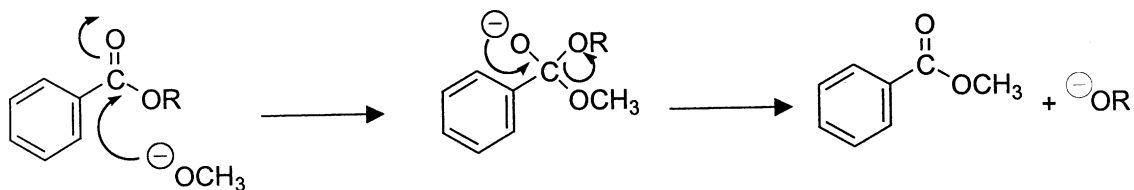


Fig. 5. Proposed mechanism of transesterification of ester linkages within macromolecular NOM with methoxide ion to produce methyl esters.

attack of hydroxide ion on ester linkages generates carboxylic acids (or carboxylate anions) and decarboxylation can occur, rather than methylation to form the methyl esters. A small amount of methoxide ion may also have been generated in situ with TMAH in methanol and, in this case, the same transesterification process would have occurred. However, if this happened, the resulting methyl esters could have been subjected to hydrolysis by hydroxide ion, producing the carboxylic acids (or carboxylate anions), with subsequent decarboxylation, supported by the absence of methyl esters in the reaction mixture. With TMAAc in methanol and, in this case, the same transesterification process would have occurred. However, if this happened, the resulting methyl esters could have been subjected to hydrolysis by hydroxide ion, producing the carboxylic acids (or carboxylate anions), with subsequent decarboxylation, supported by the absence of methyl esters in the reaction mixture. With TMAAc in methanol and, in this case, the same transesterification process would have occurred. However, if this happened, the resulting methyl esters could have been subjected to hydrolysis by hydroxide ion, producing the carboxylic acids (or carboxylate anions), with subsequent decarboxylation, supported by the absence of methyl esters in the reaction mixture. With TMAAc in methanol and, in this case, the same transesterification process would have occurred. However, if this happened, the resulting methyl esters could have been subjected to hydrolysis by hydroxide ion, producing the carboxylic acids (or carboxylate anions), with subsequent decarboxylation, supported by the absence of methyl esters in the reaction mixture.

In two previous studies of on-line TMAAc thermochemolysis (Hardell and Nilvebrant, 1999; Asperger et al., 2001), water and methanol, respectively, were used as the solvents. Deprotonation of the solvent does not appear to have occurred, since no methyl esters were observed. This is most likely due to the rapid evaporation of the solvent in the open system, aided by the application of heat in both studies, and to the short reaction time in the filament pyrolyser. The preparative off-line tetraethylammonium acetate (TEAAc) thermochemolysis work of Grasset and Amblès (1998) involved

the use of ethanol as the solvent which was not reported to be removed by evaporation prior to thermochemolysis. Many ethyl esters were formed, which the authors attributed to the presence of free fatty acids in their humin and humic acid samples. It is possible, however, that these esters were rather formed by transesterification of ester linkages within the organic matter with ethoxide ion derived from the solvent.

TMAAc in methanol, with probable in situ formation of tetramethylammonium methoxide, may prove to be a particularly useful off-line thermochemolytic reagent, since methoxide ion may act as a transesterification reagent, directly releasing the acid portion of esters from macromolecular NOM as their methyl esters and avoiding the decarboxylation that occurs with other reagents. Off-line TMAAc in methanol appears to provide more information about the NOM sample than on-line TMAAc and appears to preserve more structural information about the sample than TMAH in methanol or TMAAc in water. Our future work in this area will focus on obtaining evidence for the proposed methoxide ion transesterification mechanism.

3.4. Production and methylation of phenolic groups

Tetramethylammonium reagents have traditionally been used in thermochemolysis since they are capable of the methylation of existing or produced carboxylic acids and phenolic groups, rendering the products more amenable to gas chromatographic analysis as the methyl esters and methyl ethers, respectively. We have shown here and earlier (Joll et al., in press) that the pathway of carboxylic acid production and transformation is more complicated than this, with decarboxylation (TMAH) and transesterification (TMAAc in methanol) mechanisms probably involved.

In TMAH thermochemolysis, methylation of phenolic groups is observed, although these reactions are

sometimes incomplete (e.g. Challinor, 1989). The thermochemolysis reagents used in the present study are likely to show different efficiencies of cleavage of bound phenols and/or of methylation of phenoxides, as a result of their different alkalinities; hence, the capacity of TMAH in methanol, TMACO_3 in water and TMAAc in methanol for cleavage of bound phenols and methylation of phenolic groups was examined.

3.4.1. Methylation of phenol to form methoxybenzene (anisole)

Methylation of phenol released in thermochemolysis produces methoxybenzene (anisole). On the other hand, phenol is one precursor for anisole, but not necessarily the only precursor, in TMAH thermochemolysis (and, probably, TMACO_3 in water thermochemolysis), since decarboxylation of a number of hydroxy- or methoxy-

substituted benzoic acids could also produce anisole. The relative proportions of phenol and anisole from thermochemolysis using the reagents TMAH in methanol, TMACO_3 in water and TMAAc in methanol are shown in Fig. 6. The most weakly alkaline reagent, TMAAc in methanol, produced the highest ratio of phenol to anisole, reflecting the weaker capacity of the reagent to methylate phenoxide. Decarboxylation of any carboxylic acids is unlikely to be a source of anisole with this reagent. Both TMAH in methanol and TMACO_3 in water produced lower ratios of phenol to anisole than TMAAc in methanol. This may be due to the more alkaline nature of the TMAH and TMACO_3 reagents resulting in more deprotonation of phenol and thus in their being more efficient in methylation than TMAAc. However, the possible input of anisole from decarboxylation of methoxybenzoic acids with the use of TMAH, and possibly TMACO_3 , must also be considered.

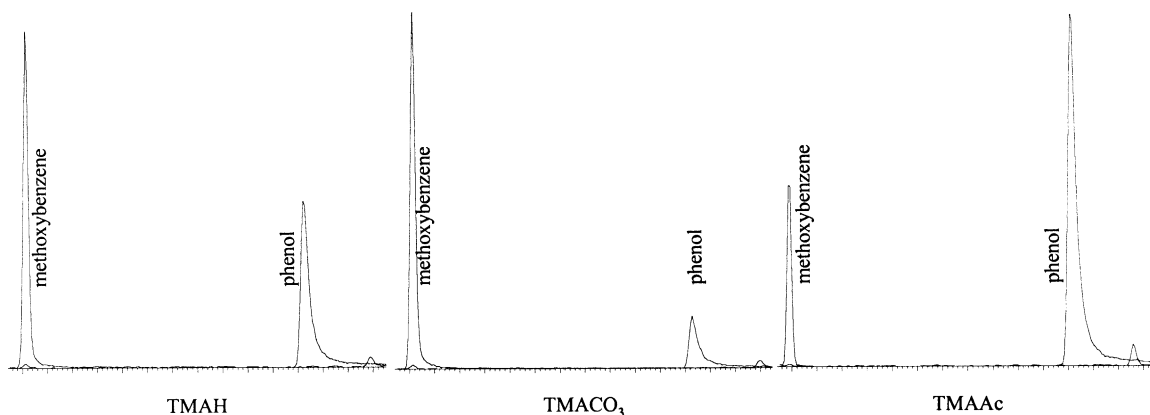


Fig. 6. Partial ion chromatograms (m/z 94, 108) showing relative abundances of phenol and anisole from off-line thermochemolysis of the aquatic NOM sample using three of the thermochemolytic reagents.

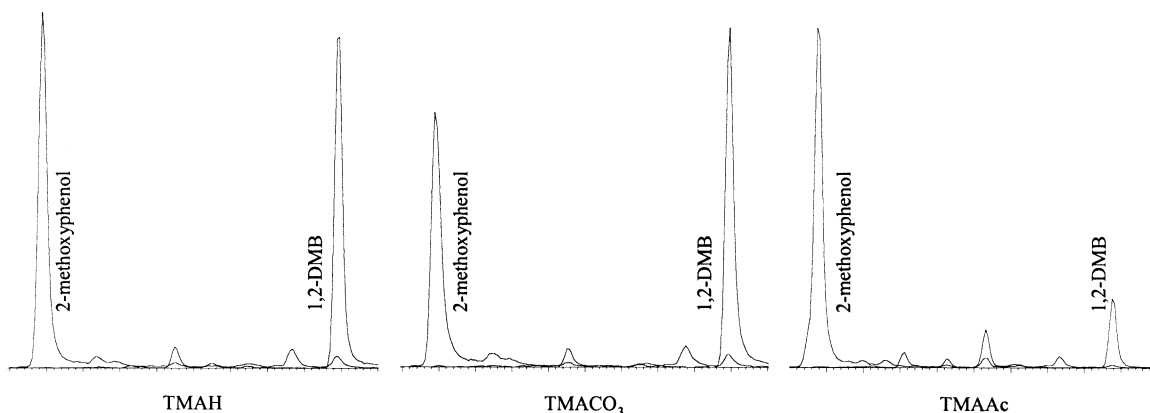


Fig. 7. Partial ion chromatograms (m/z 124, 138) showing relative abundances of 2-methoxyphenol and 1,2-dimethoxybenzene (1,2-DMB) from off-line thermochemolysis of the aquatic NOM sample using three of the thermochemolytic reagents.

3.4.2. Methylation of 2-methoxyphenol to form 1,2-dimethoxybenzene (1,2-DMB)

Applying the same rationale, methylation of any 2-methoxyphenol released or produced in thermochemolysis will produce 1,2-dimethoxybenzene (1,2-DMB) and 2-methoxyphenol is one possible precursor for 1,2-DMB. A comparison of the ratios of 2-methoxyphenol to its methylated product, 1,2-DMB, using the reagents TMAH in methanol, TMACO_3 in water and TMAAc in methanol is shown in Fig. 7. Again, the most weakly alkaline reagent, TMAAc in methanol, produced the highest ratio of 2-methoxyphenol to 1,2-DMB, reflecting the weaker capacity of the reagent to methylate the phenoxide, with decarboxylation as a source of 1,2-DMB unlikely with this reagent. Similarly, both TMAH in methanol and TMACO_3 in water produced lower ratios of 2-methoxyphenol to 1,2-DMB than TMAAc in methanol. The more alkaline nature of the TMAH and TMACO_3 reagents may have resulted in more deprotonation of 2-methoxyphenol and more efficient methylation, but some 1,2-DMB may also have been produced from decarboxylation mechanisms, increasing its relative proportion in the product mixtures.

3.4.3. Methylation of hydroxymethoxytoluenes (HMTs) to form dimethoxytoluenes (DMTs)

The trend of increased methylation with increasing alkalinity of the thermochemolysis reagent is reiterated when the relative abundances of another group of compounds, the hydroxymethoxytoluenes (HMTs), and their methylated analogues, the dimethoxytoluenes (DMTs), are compared. Methylation of any HMTs released or produced in thermochemolysis will produce DMTs and HMTs are one possible precursor for DMTs. A comparison of the ratios of HMTs to their methylated products, DMTs, using the reagents TMAH in methanol, TMACO_3 in water and TMAAc in

methanol is shown in Fig. 8. Treatment of the aquatic NOM sample with TMAAc in methanol resulted in the production of several co-eluting HMTs and two DMTs, with the HMTs in greater relative abundance. This observation may again demonstrate the weaker capacity of the reagent to methylate the phenoxides, with decarboxylation as a source of the DMTs unlikely with this reagent. Both TMAH in methanol and TMACO_3 in water produced lower ratios of HMTs to DMTs than TMAAc in methanol. The same HMTs were observed, but all six isomers of DMTs were present and the two DMT isomers produced with TMAAc in methanol were in relatively low abundance. Since TMAAc in methanol appears to avoid any decarboxylation, the two DMT isomers produced with this reagent can be assumed to be derived from the HMTs by methylation. The three or four remaining DMT isomers from treatment with TMAH in methanol and TMACO_3 in water must either be derived from complete methylation of a HMT or a dihydroxytoluene, such that no unmethylated product was present, or by some other reaction pathway, e.g., decarboxylation of hydroxy- or methoxy-substituted benzoic acids. We have previously observed 2,4,6-trimethoxytoluene in the TMAH thermochemolytic product mixture from 2,4,6-trihydroxybenzoic acid and proposed that it arose from C-methylation of the triphenolate (or trienolate) anion after decarboxylation of the carboxylic acid group (Joll et al., in press). The higher abundance DMT peaks from thermochemolysis with TMAH in methanol and TMACO_3 in water may arise through a similar mechanism from hydroxy-substituted benzoic acids.

3.5. Relative abundances of dimethoxybenzenes (DMBs) and trimethoxybenzenes (TMBs)

A comparison of the ratios of the dimethoxybenzenes (DMBs) and the trimethoxybenzenes (TMBs) from use

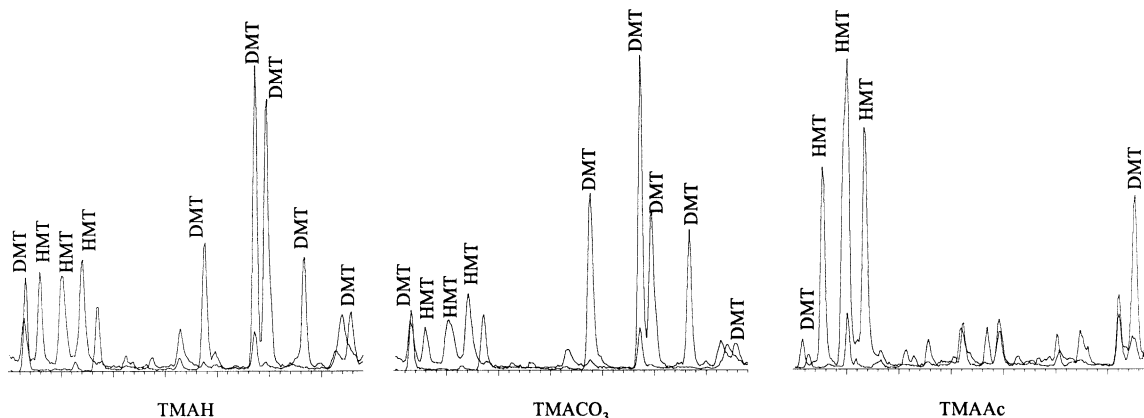


Fig. 8. Partial ion chromatograms (m/z 138, 152) showing relative abundances of hydroxymethoxytoluenes (HMTs) and dimethoxytoluenes (DMTs) from off-line thermochemolysis of the aquatic NOM sample using three of the thermochemolytic reagents.

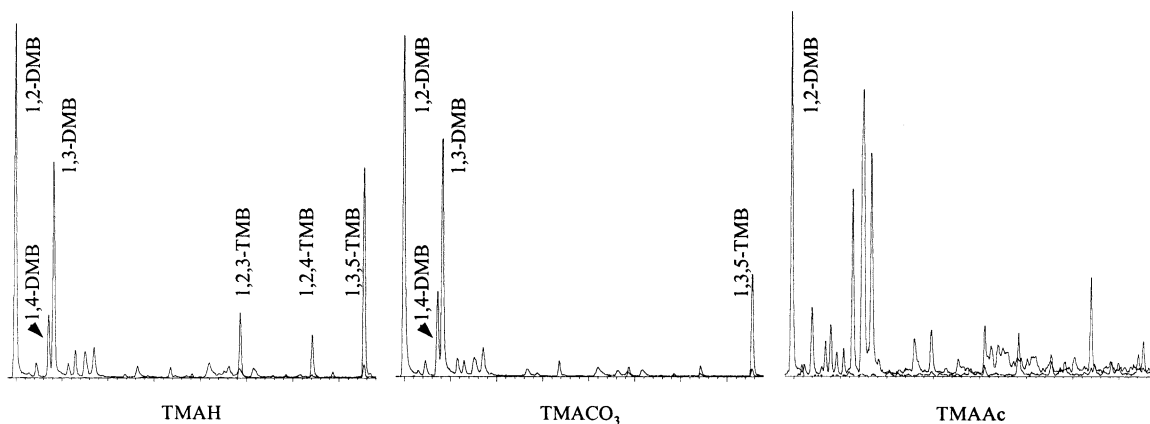


Fig. 9. Partial ion chromatograms (m/z 138, 168) showing relative abundances of dimethoxybenzenes (DMBs) and trimethoxybenzenes (TMBs) from off-line thermochemolysis of the aquatic NOM sample using three of the thermochemolytic reagents.

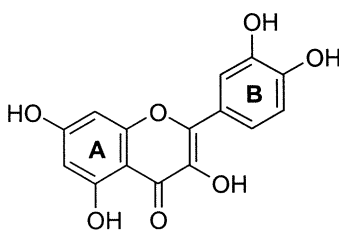


Fig. 10. Structure of quercetin, showing A and B rings.

of the reagents TMAH in methanol, TMACO_3 in water and TMAAc in methanol is shown in Fig. 9. TMAAc in methanol produced only 1,2-DMB and no TMBs. We have observed the production of 1,2-DMB from TMAH in methanol treatment of the plant pigment quercetin (Fig. 10; Huynh, 1999) and of tannins leached from the bark of *E. camaldulensis* (Heitz, 2002), possibly from the flavanol B ring through a decarboxylation-type mechanism, but only in low relative abundance. In the present TMAAc in methanol experiment, 1,2-DMB is more likely to be derived from methylation of 2-methoxyphenol released from the macromolecular NOM, e.g., as the alcohol moiety of an ester.

TMAH in methanol produced all possible isomers of the DMBs and the TMBs, with 1,2-DMB and 1,3,5-TMB being the most abundant. TMACO_3 in water produced a DMB distribution similar to that of TMAH, but produced only 1,3,5-TMB. The production of 1,3- and 1,4-DMBs by these more alkaline reagents may be a result of cleavage of more resistant linkages or the involvement of decarboxylation mechanisms; e.g., in TMAH thermochemolytic model compound studies, 2,4- and 2,6-dimethoxybenzoic acids produced some 1,3-DMB (Joll et al., in press). However, the esters of these acids, or the corresponding hydroxy acids, were not apparent from any of the three reagents.

The three TMB isomers have previously been observed in off-line TMAH thermochemolysis product mixtures from treatment of tannins leached from the bark of *E. camaldulensis* and NOM samples derived from groundwater and surface water (Heitz, 2002). The origin of TMBs has previously been attributed to lignin, tannin and carbohydrate precursors (Pulchan et al., 1997) and to cutan (McKinney et al., 1996), but Heitz (2002) provided evidence that cutan did not contribute significantly to a similar groundwater sample. Heitz (2002) proposed that eucalypt tannins, particularly condensed tannins, were potential precursors of 1,3,5-TMB and 1,2,3-TMB. In subsequent work, Joll et al. (in press) found that TMAH thermochemolysis of gallic acid (3,4,5-trihydroxybenzoic acid) yielded 1,2,3-TMB and that 2,4,6-trihydroxybenzoic acid and quercetin yielded 1,3,5-TMB, as major products, most likely through decarboxylation mechanisms. Any tannins containing a gallic acid-type subunit, e.g., gallotannins, ellagitannins and some condensed tannins are thus potential precursors of 1,2,3-TMB. Any tannins containing a 2,4,6-trihydroxybenzoic acid-type subunit or even a 1,3,5-trihydroxybenzene substitution pattern, e.g., condensed tannins, are potential precursors of 1,3,5-TMB. The formation of only 1,3,5-TMB from treatment with TMACO_3 in water may be explained by the lower alkalinity of this reagent than TMAH, resulting in the occurrence of less efficient cleavage and decarboxylation reactions.

4. Conclusions

Similar distributions and abundances of products were observed from thermochemolysis of the aquatic NOM sample with the most alkaline reagents, TMAH in methanol and TMACO_3 in water. TMABr in methanol was an ineffective thermochemolytic reagent and produced very few products from the sample. Treatment of

the sample with the reagent of intermediate alkalinity, TMAAc in methanol, produced a different suite of compounds from those produced by TMAH and TMACO_3 , in slightly lower, but still comparable, overall abundance.

The most abundant component from TMAAc treatment was methyl 3-methylmercaptopropanoate, with no sulfur-containing compounds being identified in the chromatograms from thermochemolysis with TMAH or TMACO_3 . TMAAc in methanol may have resulted in a gentle, but efficient, cleavage of a 3-mercaptopropanoate ester linkage to the macromolecular NOM, in contrast to the highly alkaline reagent TMAH, where decarboxylation of the 3-MPA may have occurred.

A highlight of the product mixture from treatment with TMAAc in methanol was the presence of many methyl esters, since no esters were produced by the other three thermochemolytic reagents. Decarboxylation in off-line TMAH thermochemolysis may occur with, for example, benzoic acid and the methylbenzoic acids, resulting in the corresponding methyl esters being absent from the product mixture. A mechanism for the formation of methyl esters in off-line TMAAc in methanol thermochemolysis, involving deprotonation of a portion of the methanol remaining in the reaction mixture by the acetate ion to form some acetic acid and methoxide ion, with methoxide ion then attacking ester linkages in macromolecular NOM to lead to direct formation of methyl esters through transesterification, was proposed. TMAAc in methanol, with probable in situ formation of tetramethylammonium methoxide, may prove to be a particularly useful off-line thermochemolytic reagent, avoiding the decarboxylation that is evident with other reagents. Off-line TMAAc in methanol appears to provide more information about the NOM sample than on-line TMAAc and preserve more structural information about the sample than TMAH or TMACO_3 .

In general, the mildly alkaline reagent, TMAAc in methanol, appeared to show higher ratios of phenolic compounds to their corresponding methyl ethers than TMAH in methanol and TMACO_3 in water, possibly reflecting the weaker capacity of TMAAc in methanol to methylate the phenoxides. TMAH in methanol and TMACO_3 in water produced all six isomers of dimethoxytoluenes (DMTs) and three or four of these isomers may arise from hydroxy-substituted benzoic acids through a decarboxylation mechanism. The 1,2-dimethoxybenzene (DMB) formed from thermochemolysis with TMAAc in methanol may have been derived from methylation of 2-methoxyphenol released from the macromolecular NOM, e.g., as the alcohol moiety of an ester. TMAH in methanol produced all possible isomers of the trimethoxybenzenes (TMBs), while TMACO_3 in water produced only 1,3,5-TMB. Tannins containing a gallic acid-type subunit were proposed as potential

precursors of 1,2,3-TMB and tannins containing a 2,4,6-trihydroxybenzoic acid-type subunit or even a 1,3,5-trihydroxybenzene substitution pattern were proposed as potential precursors of 1,3,5-TMB.

Acknowledgements

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References

- Asperger, A., Engewald, W., Fabian, G., 2001. Thermally assisted hydrolysis and methylation—a simple and rapid online derivatization method for the gas chromatographic analysis of natural waxes. *Journal of Analytical and Applied Pyrolysis* 61, 91–109.
- Challinor, J.M., 1989. A pyrolysis-derivatization-gas chromatography technique for the structural elucidation of some synthetic polymers. *Journal of Analytical and Applied Pyrolysis* 16, 323–333.
- Challinor, J.M., 2001. Review: the development and applications of thermally assisted hydrolysis and methylation reactions. *Journal of Analytical and Applied Pyrolysis* 61, 3–34.
- de Leeuw, J.W., Baas, M., 1993. The behaviour of esters in the presence of tetramethylammonium salts at elevated temperatures—flash pyrolysis or flash chemolysis. *Journal of Analytical and Applied Pyrolysis* 26, 175–184.
- Galletti, G.C., Bocchini, P., 1995. Behaviour of catechin and ellagic acid subjected to thermally-assisted hydrolysis-methylation/gas chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry* 9, 250–254.
- Grasset, L., Amblès, A., 1998. Structural study of soil humic acids and humin using a new preparative thermochemolysis technique. *Journal of Analytical and Applied Pyrolysis* 47, 1–12.
- Hardell, H.-L., Nilvebrant, N.-O., 1999. A rapid method to discriminate between free and esterified fatty acids by pyrolytic methylation using tetramethylammonium acetate or hydroxide. *Journal of Analytical and Applied Pyrolysis* 52, 1–14.
- Hatcher, P.G., Clifford, D.J., 1994. Flash pyrolysis and in situ methylation of humic acids from soil. *Organic Geochemistry* 21, 1081–1092.
- Hatcher, P.G., Minard, R.D., 1995. Comment on the origin of benzenecarboxylic acids in pyrolysis methylation studies. *Organic Geochemistry* 23, 991–994.
- Heitz, A., Joll, C., Alexander, R., Kagi, R.I., 2001. Characterisation of aquatic natural organic matter in some Western Australian drinking water sources. In: Swift, R.S., Spark, K.M. (Eds.), *Understanding and Managing Organic Matter in Soils, Sediments and Waters*. Proc Int. Humic Substances Society, Adelaide, Australia, pp. 429–436.
- Heitz, A., 2002. Malodorous dimethylpolysulfides in Perth drinking water. PhD thesis, Department of Applied Chemistry, Curtin University of Technology.

- Huynh, T., 1999. Mechanistic Studies of TMAH Thermochemolysis for Characterisation of Macromolecular Organic Matter Using Model Compounds. Honours dissertation, School of Applied Chemistry, Curtin University of Technology.
- Joll, C.A., Huynh, T., Heitz, A. Off-line tetramethylammonium hydroxide thermochemolysis of model compound aliphatic and aromatic carboxylic acids: decarboxylation of some *ortho*- and/or *para*-substituted aromatic carboxylic acids. *Journal of Analytical and Applied Pyrolysis* (in press).
- Kralert, P.G., Alexander, R., Kagi, R.I., 1995. An investigation of polar constituents in kerogen and coal using pyrolysis-gas chromatography-mass spectrometry with in situ methylation. *Organic Geochemistry* 23, 627–639.
- MacCrehan, W., Shea, D., 1995. Temporal relationship of thiols to inorganic sulfur compounds in anoxic Chesapeake Bay sediment porewater. In Vairavamurthy, M.A., Schoonen, M.A.A. (Eds.), *Geochemical Transformations of Sedimentary Sulfur*. ACS Series 162.
- Martin, F., Gonzalez-Vila, F.J., Del Rio, J.C., Verdejo, T., 1994. Pyrolysis derivatization of humic substances. I. Pyrolysis of fulvic acids in the presence of tetramethylammonium hydroxide. *Journal of Analytical and Applied Pyrolysis* 28, 71–80.
- McKinney, D.E., Carson, D.M., Clifford, D.J., Minard, R.D., Hatcher, P.G., 1995. Off-line thermochemolysis versus flash pyrolysis for the in situ methylation of lignin: is pyrolysis necessary? *Journal of Analytical and Applied Pyrolysis* 34, 41–46.
- McKinney, D.E., Bortiatynski, J., Carson, D.M., Clifford, D.J., de Leeuw, J.W., Hatcher, P.G., 1996. Tetramethylammonium hydroxide (TMAH) thermochemolysis of the aliphatic biopolymer cutan: insights into the chemical structure. *Organic Geochemistry* 24, 641–650.
- Pulchan, J., Abrajano, T.A., Helleur, R., 1997. Characterisation of TMAH thermochemolysis products of near-shore marine sediments using GC-MS and GC-CSIA-MS. *Journal of Analytical and Applied Pyrolysis* 42, 135–150.
- Saiz-Jimenez, C., 1994. Pyrolysis/methylation of soil fulvic acids: benzenecarboxylic acids revisited. *Environmental Science and Technology* 28, 197–200.
- Saiz-Jimenez, C., Hermosin, B., Ortega-Calvo, J.J., 1993. Pyrolysis/methylation—a method for structural elucidation of the chemical nature of aquatic humic substances. *Water Research* 27, 1693–1696.
- Tanczos, I., Rendl, K., Schmidt, H., 1999. The behavior of aldehydes—produced as primary pyrolysis products—in the thermochemolysis with tetramethylammonium hydroxide. *Journal of Analytical and Applied Pyrolysis* 49, 319–327.