Disinfection By-Products from Halogenation of Aqueous Solutions of Terpenoids

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ABSTRACT

We report the formation of trihalomethanes and other disinfection by-products from four polyfunctional terpenoids during simulated chlorination of natural waters. Complex suites of products were identified by closed loop stripping analysis (CLSA)/gas chromatography-mass spectrometry (GC-MS) from halogenation of β-carotene and retinol. β-Ionone appeared to be a key intermediate in the halogenation of β-carotene and retinol, reacting further under the reaction conditions to produce *trans*-β-ionone-5,6-epoxide and β-cyclocitral. Halogenation of the four terpenoids also produced trihalomethanes (THMs), most likely through haloform reaction on methyl ketone groups within many of the intermediates. Since halogenation of retinol produced a significant quantity of THMs at a slow reaction rate, retinol-based structures may possibly contribute to the slow reacting phase of THM formation in natural waters. Two polyhydroxyphenol model compounds were halogenated for comparison. The only products identified by CLSA/GC-MS from halogenation of 4',5,7-trihydroxyflavanone and ellagic acid were THMs. 4',5,7-Trihydroxyflavanone rapidly produced THMs, with an extremely high molar yield (94 %) at pH 7. Terpenoids of the β-ionone and retinol type should be considered to be significant THM precursors, while 4',5,7-trihydroxyflavanone has been shown to be an extremely significant THM precursor, potentially present within natural organic matter in water treatment processes and distribution systems.

KEYWORDS

Terpenoids, Disinfection by-products, Trihalomethanes, Chlorine, Polyhydroxyphenols, Drinking water

1. Introduction

Terpenoids are produced by a wide variety of plants, animals and microorganisms for functional, defence and communication purposes. In plants, essential oils, latexes and resinous exudates are often composed mainly of terpenoids and terpenoids constitute important components of many wood extractives. Terpenoids, also referred to as isoprenoids, are defined as materials with molecular structures containing carbon backbones made up of isoprene (2-methylbuta-1,3-diene) units and include degradation products of terpenoids in which carbon atoms may have been lost through chemical and biochemical processes.

While terpenoids occur widely in nature, only very recently have they been considered as precursors of natural organic matter (NOM). Terpenoids were earlier proposed to be incorporated into soil humus based on infrared spectral comparisons (Stepen and Korsunova, 1988). In 2003, Leenheer *et al.* (2003) provided cross polarization magic angle spinning (CPMAS) ^{13}C NMR spectroscopic evidence of terpenoid precursor contribution to six dissolved organic matter (DOM) fractions derived from each of a river, a lake and its infiltrated groundwater (three samples) and a separate groundwater source. Electrospray ionization mass spectrometry on the DOM sample from the groundwater source also supported the hypothesis of degraded terpenoid contribution to the sample. In further work, Leenheer *et al.* (2007) found that terpenoids constituted a large part of the DOM in recharge waters of the Santa Ana River Basin. McIntyre *et al.* (2005) proposed terpenoid input into a groundwater-derived hydrophobic acid sample, based on CPMAS ^{13}C NMR spectroscopic evidence, consistent with the overlying vegetation (*Eucalyptus*) being rich in terpenoids. Terpenoids were also identified by gas chromatographic-mass spectrometric (GC-MS) analysis of the ester-based fractions of humic acid isolated from a volcanic soil (Fiorentino *et al.*, 2006). Badin *et al.* (2008) observed triterpanes upon GC-MS analysis of dichloromethane extracts of fractions of natural and anthropogenic OM from urban storm water sediments.

There have been many literature reports of direct identification of a variety of terpenoids, both polar and nonpolar, in water sources. The types of terpenoids identified primarily depend upon the plant, animal and microorganism input to the water source. For example, in 1974, Grob and Grob (1974) reported that terpenoids, in low ng L^{-1} concentrations, including hydrocarbons, aldehydes, ketones and alcohols, were found in three surface water sources studied. Three naturally occurring terpenoids, reported to be resulting from normal biological processes taking place in the river, were identified in Delaware River water during summer and/or winter sampling by dichloromethane extraction, liquid chromatographic cleanup and GC-MS analysis (Sheldon and Hites, 1978). These terpenoids were 6,10,14-trimethyl-2-pentadecanone (probably resulting from oxidative degradation of phytol), α-terpineol and chlorophyll (which was observed in the spectra as phytadienes produced in the injection port by pyrolysis of the phytol ester part of chlorophyll) (Sheldon and Hites, 1978). A number of terpenoids, attributed to phytoplankton, and possibly conifer, sources, were found in Berlin's surface water sources (Chorus *et al.*, 1992). Monoterpenoids (*e.g.*, indomyrmectin, limonene), sesquiterpenoids (*e.g.*, farnesol) and their derivatives (*e.g.*, di-epi-α-cedrene epoxide) were tentatively identified, through dichloromethane extraction and GC-MS analysis, as natural organic matter (NOM) constituents of the dissolved phase from surface runoff from individual irrigated agricultural fields, while a triterpenoid phytosterol from plant waxes was tentatively identified as being sorbed to suspended particles in the runoff samples (Pedersen *et al.*, 2002). Geosmin, β-cyclocitral, β-ionone, geranylacetone, limonene and 2-methylisoborneol (MIB) have been detected in Australian water sources (Jones and Korth, 1995).

Biological removal of terpenoids has been reported to be both effective and slow, depending on the structure of the terpenoid (e.g. Grob and Grob, 1974; Jüttner, 1995). Despite the potential for biodegradation / biological removal of some terpenoids, terpenoids have been detected in distributed drinking water. For example, Grob and Grob (1974) detected terpenoids in the drinking water derived from the three surface water sources, and terpenoids (including limonene, eucalyptol and 2

unidentified terpenes) have been identified in drinking water from Córdoba, Spain (Aramendía *et al.*, 1998). We have observed terpenoids, such as farnesol acetate, geranyl acetate and β-cyclocitral derivatives, in various chlorinated surface waters from towns in southern Western Australia (e.g. Heitz, 1997) These compounds are oxidative degradation products of carotenoids, such as β-carotene, and are believed to be indicators of considerable microbial activity in the water body (Jüttner, 1992). The terpenoids, geosmin and 2-methylisoborneol (MIB), are responsible for the majority of unpleasant odours in potable water, with cyanobacteria and actinomycetes being their major source (Jüttner, 1995; Ho *et al.*, 2004).

Chlorination of terpenoids in aqueous solutions to simulate disinfection of drinking waters has not been well-studied to date. Reactions of halogens, such as chlorine and bromine, with aquatic organic matter are numerous and include oxidation, substitution and addition reactions, and the haloform reaction from enolizable carbonyl compounds. For reaction of halogens with terpenoids, the terpenoids must contain reactive functional groups, such as benzenes and phenols, alkenes, methyl ketones, β-diketones and primary and secondary alcohols. In fact, the haloform reaction was classically used as a degradative reaction to elucidate the structure of mono-, di- and sesqui-terpenoids through reaction of halogens with methylketone intermediates, derived from initial oxidation of the terpenoids, allowing identification of carboxylic acid derivatives of the original terpenoids (Fuson and Bull, 1934).

Geosmin and MIB were found to be fairly resistant to removal by chlorine and chlorine dioxide treatment, because these terpenoids each contain only one functional group, a tertiary alcohol, which is not susceptible to oxidation (Lalezary *et al.*, 1986). α-Terpineol, which contains an alkene and a tertiary alcohol group, when chlorinated in water, was reported to give mainly monochloro derivatives (chlorohydrins) at pH 2, with an epoxide becoming a major product at pH 10 (Kopperman *et al.*, 1976). Larson and Marley (1988) separately treated camphene, limonene, α-pinene and β-pinene with a 10 molar excess of aqueous hypochlorite solution at pH 2, to simulate

acid bleaching of paper pulp, and at pH 8, to simulate drinking water treatment. At pH 8, the major products were reported to be ring-opened, oxygenated products, with small amounts of mono- and di-chloro derivatives (Larson and Marley, 1988). Mono- and di-chlorocamphenes were produced from camphene and hypochlorous acid in aqueous acetone (Buchbauer *et al.*, 1984). Hoehn *et al.* (1980) reported a correlation between the concentration of chlorophyll-a measured in a reservoir and the concentration of THMs, and found that high yields of THMs were produced from both algal biomass and algal extracellular products, possible sources of terpenoids.

The objective of the present study was to investigate the role of some polyfunctional terpenoids in the formation of DBPs, particularly THMs, during halogenation reactions that occur upon chlorination of natural waters. The terpenoid model compounds, β-carotene, retinol, β-ionone and geranyl acetate (Figure 1), were chosen as representatives of the carotenoid family and their oxidative degradation products, terpenoids which we have identified in local drinking waters. Closed loop stripping analysis followed by GC-MS was conducted for qualitative identification of halogenation products from the model compounds. Solid-phase microextraction followed by GC-MS was utilized for quantitative analysis of the THMs formed from halogenation of the model compounds. The reactivities of the terpenoid model compounds for THM formation were compared with those of a series of polyhydroxyphenols, containing functionalities which are well-known to form high concentrations of THMs (Rook, 1976; Boyce and Hornig, 1983; Gallard and von Gunten, 2002). These polyhydroxyphenols, 4',5,7-trihydroxyflavanone and ellagic acid (Figure 1), are found free and/or combined in plants. Reaction pathways and mechanisms for the formation of a series of intermediates from the terpenoids are also proposed.

2. Materials and methods

2.1. Standard solutions of model compounds

Standard solutions (1 g L^{-1}) of 4',5,7-trihydroxyflavanone (naringenin; Sigma), ellagic acid (Fluka), β-carotene (Roche), β-ionone (Aldrich), *all-trans*-retinol (Aldrich; referred to subsequently as

retinol) and geranyl acetate (Aldrich) in methanol (HPLC grade), and β-carotene and ellagic acid (for the THM quantification study) in a 50:50 mixture of methanol and redistilled dichloromethane (DCM), were prepared.

2.2 Aqueous halogenation of model compounds for closed loop stripping analysis

Solutions of bromide ion (0.5 mg L^{-1} (6 μM) as bromide; reagent used was KBr), phosphate buffer (pH 7, 100 mL of stock buffer solution: KH_2PO_4+Na_2HPO_4), sodium hypochlorite (10 mg L^{-1} (280 μM) as Cl_2), followed by the model compound (1.0 mg L^{-1}; 1.9-5.2 μM), were prepared in redistilled Milli-Q water (2 L). The reactions were allowed to proceed at room temperature (25 °C) in darkness with constant stirring. After 0.5, 1, 6 and 24 hours, subsamples (200 mL) of the mixture were quenched with an aliquot (1 mL) of aqueous sodium thiosulfate solution (8 g L^{-1} as $S_2O_3^{2-}$). The thiosulfate quenching reagent was added at a molar ratio of thiosulfate to initial Cl_2 concentration of 1.3:1. All model compound reactions were carried out in duplicate and with procedural blanks.

2.3 Closed loop stripping analysis followed by gas chromatography-mass spectrometry for identification of purgeable products from the reaction mixture

A similar set-up to the closed loop stripping procedure developed by Grob (1976) was used for the isolation and concentration of volatile analytes from the reaction mixtures. An aliquot (10 μL) of surrogate standard solution (1,2,3,5-tetramethylbenzene (Aldrich; 95%) in redistilled DCM; 55.3 ng μL^{-1}) was added directly into each quenched reaction mixture (100 mL). Closed loop stripping analysis (CLSA) was carried out using a sample purge temperature of 60 ^{o}C (water bath), purge gas (air) at a flow of approximately 425 mL min^{-1} and a Grob tube (carbon trap) containing activated carbon (1.5 mg) at a temperature of 65 ^{o}C. Analytes were eluted from the Grob tube with DCM (total 30 μL). In separate extractions, aliquots of DCM (2×9 μL, 2×6 μL) were placed onto the carbon filter and extracted as described by Heitz (2002). The solvent extracts were combined in a

156 microvial (100 μL) containing an aliquot (10 μL) of internal standard (1-chlorohexadecane (Merck-
157 Schuchardt) in hexane (AR HPLC grade; 7.0 ng μL^{-1}). After all four individual extractions, an
158 additional aliquot (20 μL) of DCM was added to the extracts. The reaction products in the extract
159 were separated and identified by gas chromatography-mass spectrometry (GC-MS) using a Hewlett-
160 Packard (HP) 6890 GC interfaced to a HP 5973 mass selective detector, operating in full scan mode.
161 The sample extract aliquot (1 μL) was delivered directly into the column *via* a HP on-column
162 injector. The column was a ZB-5 (Phenomenex; 60 m x 0.25 mm i.d.; phase thickness 0.25 μm;),
163 helium was the carrier gas, and the temperature program was: 30 ^{o}C (6 min) to 230 ^{o}C at a rate of 5
164 ^{o}C min^{-1}, followed by 15 ^{o}C min^{-1} to 310 ^{o}C (10 min).

165 *2.4 Halogenation of aqueous solutions of model compounds for quantification of THM formation*
166 Reaction mixtures at pH 7 were prepared as described for CLSA and allowed to react for 168 hours.
167 To ensure there was a free chlorine residual after 168 hours, an initial sodium hypochlorite
168 concentration of 13 mg L^{-1} (180 μM; as Cl_2) was used for 4',5,7-trihydroxyflavanone and ellagic
169 acid, and 6 mg L^{-1} (85 μM; as Cl_2) was used for retinol, β-carotene, β-ionone and geranyl acetate.
170 Reactions were also conducted at pH 9 using a borax/HCl buffer. After 168 hours, the residual free
171 chlorine was measured and each mixture was quenched with a calculated aliquot of sodium
172 thiosulfate solution (8 g L^{-1} as $S_2O_3^{2-}$) such that the molar ratio of thiosulfate to final chlorine
173 concentration was 1:1. The free chlorine concentration was then measured to ensure no residual
174 remained. The reactions were carried out in duplicate, with procedural blanks. In order to examine
175 the THM formation potential of 4',5,7-trihydroxyflavanone and retinol, additional samples from
176 these reaction mixtures were quenched at times 1, 24, 48, 96, 120, 144 and 168 hrs, at both pH 7 and
177 9.

178 *2.5 Solid-phase microextraction / GC-MS analysis of THMs*

After quenching of the reaction mixture, THMs were recovered from the samples *via* manual headspace solid-phase microextraction (SPME). An aliquot (30 μL) of a surrogate standard solution (1,2-dibromopropane (Aldrich) in methanol (HPLC grade): 50 mg L^{-1}; 250 μM) was added directly into the sample (30 mL) contained in a 40 mL sample vial. A magnetic stirrer bar and sodium sulfate (5 g) were then added and the vial was capped. Headspace SPME using a 100 μm polydimethylsiloxane (PDMS) fibre (15 minutes) was followed immediately by GC-MS analysis. The THMs and the surrogate standard were separated using a HP 5890 gas chromatograph interfaced to a HP 5971 mass selective detector. Selected ions (m/z) were 83, 85, 96, 121, 123, 127, 129, 131, 173 and 175. The SPME fiber was injected manually *via* a split-splitless injector at 240 ^{o}C. The column was a ZB-5 (Phenomenex; 30 m x 0.25 mm i.d.; phase thickness 1 μm), helium was the carrier gas, and the temperature program was: 0 ^{o}C (liquid CO_2, 3 min) to 120 ^{o}C at a rate of 8 ^{o}C min^{-1}, followed by 15 ^{o}C min^{-1} until 305 ^{o}C (5 min).

3. Results and discussion

3.1 Closed loop stripping analysis of products from halogenation of terpenoid and polyhydroxyphenol model compounds

Halogenation was conducted on aqueous solutions of a range of terpenoid and polyhydroxyphenol model compounds at a concentration of 1 mg L^{-1} (1.9-5.2 μM), with the addition of bromide ion (0.5 mg L^{-1} (6 μM) as bromide) and sodium hypochlorite (free chlorine; 10 mg L^{-1} (280 μM)) at pH 6.9 at room temperature (25 °C). Closed loop stripping analysis (CLSA) was performed on quenched subsamples of the reaction mixtures at reaction times of 0.5, 1, 6 and 24 hours, but this paper focuses on the final reaction time (24 hours). Quenching was conducted with thiosulfate solution, which is suitable for analysis of THMs and some other DBPs, although it may cause some decomposition of other DBPs, e.g. some haloacetonitriles (Clesceri, 1998). CLSA allowed analysis

of volatile reaction products which could be purged from the reaction mixture; nonvolatile products can not be detected by this method. Chloroform was not analysed, since it co-eluted with the solvent (DCM). These experiments were primarily for qualitative purposes to identify reaction products; however, surrogate and internal standards were added for semiquantitative purposes. The reaction and analysis were carried out in duplicate for each model compound and typical chromatograms are presented here. The structures and names of the five model compounds, and geranyl acetate which was used in the subsequent quantitative study, are presented in Figure 1. The presence and absence of six halogenation and oxidation products in the chromatograms from reaction times of 24 hours for the five model compounds are indicated in Table 1.

The concentrations used in these experiments were chosen with specific reference to our challenging local water treatment conditions and to allow detection and identification of reaction products from the less reactive model compounds. In water supplies in Western Australia, the dissolved organic carbon (DOC) concentration is often particularly high (5 – 40 mg L^{-1}) and the concentrations of bromide ion commonly range from 0.2 – 0.5 mg L^{-1}. Treatment of these waters varies from disinfection with chlorine only to conventional alum coagulation after magnetic ion exchange resin (MIEX®) treatment, such that the DOC concentration in treated water being subjected to chlorination can range from 1-5 mg L^{-1}. While terpenoids or polyhydroxyphenols would form only a fraction of this total DOC in the water, the concentration of the model compounds in this study (1 mg L^{-1}) was chosen to model this overall DOC concentration in order to ensure sufficient concentrations of products to allow detection by CLSA. To ensure adequate disinfection throughout the distribution system, final chlorination doses can be up to 12 mg L^{-1}, sometimes resulting in total THM concentrations close to the Australian Drinking Water Guideline value of 250 μg L^{-1}. The concentrations of free chlorine and bromide used in these experiments were chosen to maximise the concentrations of products formed, while still representing concentrations which are sometimes present in the local drinking water systems.

227 The only products identified by CLSA/GC-MS from the polyhydroxyphenol model compounds
228 (4',5,7-trihydroxyflavanone and ellagic acid) were three trihalomethanes (bromodichloromethane,
229 dibromochloromethane and bromoform) (Table 1). Only a few, very minor, other peaks were
230 observed in these chromatograms. 1,3-Dihydroxybenzenes are well-known for their prolific THM
231 production upon halogenation (e.g. Rook, 1976; Boyce and Hornig, 1983). Phenols are also known
232 to produce THMs, albeit in lower yields (e.g. Gallard and von Gunten, 2002). 1,3-Dihydroxybenzene
233 and phenolic moieties are present in the structures of 4',5,7-trihydroxyflavanone and ellagic acid,
234 accounting for the formation of THMs from these model compounds.

235 In contrast, more complex suites of products were identified by CLSA/GC-MS from halogenation
236 of the terpenoid model compounds, β-carotene and retinol. To illustrate this, the total ion
237 chromatogram (TIC) of the mixture obtained from halogenation of β-carotene after 24 hours is
238 presented in Figure 2, including the identified reaction products. Of the identified reaction products
239 from β-carotene and retinol, 1,8-cineole, β-cyclocitral and a variety of trimethylcyclohexanones and
240 trimethylcyclohexenones have been found in various chlorinated surface waters from towns in
241 southern Western Australia (e.g. Heitz, 1997), suggesting the possible presence of β-carotene-type
242 terpenoid precursors in these surface waters.

243 Three other products, β-cyclocitral, β-ionone and *trans*-β-ionone-5,6-epoxide (Figure 3), were of
244 particular interest because of their relative abundances over the 24 hour reaction period (0.5, 1 and 6
245 hours: results not shown). The relative abundances of β-cyclocitral and β-ionone were highest after
246 0.5 hour, and decreased gradually to be present in low abundance after 24 hours. The moderately low
247 relative abundance of *trans*-β-ionone-5,6-epoxide was constant over the 24 hour reaction period.
248 These trends indicated that β-cyclocitral and β-ionone may have been rapidly formed intermediate
249 products, which were then slowly converted into other products over time, in the halogenation of β-
250 carotene. *Trans*-β-ionone-5,6-epoxide is a likely product of further reaction of β-ionone (Figure 3).

Its relatively constant abundance over the reaction period indicates that, while it appears to have been formed from β-ionone, it must itself be converted into other products. No possible products from β-cyclocitral, including THMs, were found to increase in abundance over the reaction period, suggesting that THMs were not degradation products of β-cyclocitral and that the degradation products of β-cyclocitral could not be detected by this analytical method.

Halogenation of β-ionone itself produced a less complex mixture of products. A very high, relatively constant, abundance of *trans*-β-ionone-5,6-epoxide was produced, confirming the reaction pathway of β-carotene to β-ionone to the epoxide. β-Cyclocitral was also produced from β-ionone, with its relative abundance showing the same decreasing trend as in the β-carotene reaction. The only other identified products were bromodichloromethane and bromoform which were formed in very low, gradually increasing, abundance after 6 and 24 hours.

The structures of β-carotene and retinol are very similar (Figure 1), with retinol being much more water soluble than β-carotene, since β-carotene can be enzymatically cleaved into two molecules of retinol. Not surprisingly, halogenation of retinol yielded a similar suite of identified products, in different abundances, to halogenation of β-carotene, except that no 3,3-dimethylcyclohexanone was formed. β-Cyclocitral was initially formed in moderately high abundance, but decreased to a low abundance after 24 hours, again with no degradation products evident. β-Ionone was initially present but its abundance decreased over time until it was all consumed after 6 hours. Concurrently, one of the likely products from β-ionone, *trans*-β-ionone-5,6-epoxide, was again present in relatively constant, moderately low abundance over the 24 hour period.

β-Ionone appears to be the key intermediate in the halogenation of β-carotene and retinol, reacting further under the reaction conditions to produce *trans*-β-ionone-5,6-epoxide and, to a much lesser extent, β-cyclocitral. Another product, 2,2,6-trimethylcyclohexanone, identified in the reaction

mixtures from β-carotene and retinol, still contains the structural features of the ring system of β-carotene, retinol and β-ionone. Reaction pathways, including reaction mechanisms, for formation of these products are proposed in Figure 3. Electrophilic addition of hypochlorous or hypobromous acid (represented as HOX in Figure 3) to the double bond of β-carotene or retinol, followed by nucleophilic attack of water on the intermediate carbocation, produces the halohydrin with Markovnikov-type regiochemistry. Internal S_N2 attack of the nucleophilic hydroxide group results in an epoxide intermediate. Epoxide formation from the chlorohydrin was also observed at pH 10, and to a lesser extent at lower pH, in the aqueous chlorination of α-terpineol (Kopperman *et al.*, 1976). Nucleophilic attack of water on the least hindered carbon of the epoxide produces the vicinal diol, which is subject to oxidative cleavage under the reaction conditions (March, 1992) to produce the key intermediate, β-ionone. Similar reactions could produce β-cyclocitral and *trans*-β-ionone-5,6-epoxide from β-ionone (as depicted in Figure 3). β-Cyclocitral could also be produced more directly from β-carotene, retinol or from another longer chain intermediate, rather than from β-ionone. Since 2,2,6-trimethylcyclohexanone was not observed in the β-ionone product mixture, it is likely to arise from reaction of β-carotene or retinol or from another longer chain intermediate, through acid-catalysed hydration of the cyclic double bond, followed by another similar series of halogenation reactions (Figure 3).

β-Ionone, *trans*-β-ionone-5,6-epoxide and many other possible reaction intermediates contain a methyl ketone functional group (Figure 3), a moiety which is well-known to produce THMs (e.g. Morris and Baum, 1978) and a carboxylic acid *via* the haloform reaction. THMs were produced from halogenation of these terpenoids (Table 1). Of the three overall reaction pathways depicted in Figure 3, the pathway from β-ionone was confirmed to produce THMs, the pathway including β-cyclocitral did not appear to produce THMs, while THM formation from the pathway including 2,2,6-trimethylcyclohexanone remains unclear. Having established that halogenation of β-carotene-based

298 terpenoids can produce THMs, it was important to investigate the quantity of THMs produced, and
299 the rate of their formation over 7 days, to determine if these terpenoid structures could be significant
300 THM precursors within NOM in water treatment processes and distribution systems.

301 *3.2 Quantification of trihalomethanes produced from halogenation of terpenoid and*
302 *polyhydroxyphenol model compounds*

303 Each of the six terpenoid and polyhydroxyphenol model compounds (Figure 1), at a concentration of
304 1 mg L^{-1} (1.9-5.2 μM), were subjected to halogenation, with the addition of bromide ion (0.5 mg L^{-1}
305 (6 μM) as bromide) and sodium hypochlorite (free chlorine; 13 mg L^{-1} (180 μM) for
306 polyhydroxyphenols and 6 mg L^{-1} (85 μM) for terpenoids), at pH 7 and 9. The concentrations of
307 individual and total THMs were determined over a 7 day period for 4',5,7-trihydroxyflavanone and
308 retinol, and after 7 days for the other four model compounds, after quenching of the free chlorine
309 equivalent residual with the exact concentration of sodium thiosulfate solution required. A time
310 period of 7 days was chosen to be consistent with the methods standardised by Clesceri (1998), other
311 work in our laboratory and local water distribution times. Retinol was chosen for detailed study of
312 the rate of THM formation from the terpenoids, since it appeared to produce the most THMs in the
313 semi-quantitative study. These quantitative analyses were carried out in duplicate for each model
314 compound and typical formation curves are presented here. Total THM formation curves for 4',5,7-
315 trihydroxyflavanone and retinol at pH 7 and at pH 9 are presented in Figures 4 a) and 4 b),
316 respectively. The chlorine dose, 7 day oxidant demand, molar concentrations of individual and total
317 THMs, and specific and conventional yields of total THMs for all halogenation experiments at pH 7
318 and pH 9 are presented in Tables 2 and 3, respectively.

319 As expected for a substrate containing phenolic and 1,3-dihydroxybenzene moieties, 4',5,7-
320 trihydroxyflavanone produced THMs in high abundance (Figure 4). THM formation from 4',5,7-
321 trihydroxyflavanone appeared to include a period of very rapid production, followed by a period of
322 slower production, corresponding most likely to the formation of THMs from the 1,3-

dihydroxybenzene and phenolic moieties, respectively, within this model compound. In kinetic studies, Gallard and von Gunten (2002) found that THM precursors in NOM could be divided into fast and slow reacting fractions. The rapid production of THMs from 4',5,7-trihydroxyflavanone is consistent with their hypothesis that *meta*-dioxygenated (resorcinol-type) sites could be, at least in part, responsible for the fast reacting THM precursors in NOM.

Halogenation of retinol produced a lower, but still very significant, quantity of THMs with an apparently slower reaction rate. These results are consistent with our semi-quantitative CLSA study (Section 3.1) where THMs were formed from retinol, but various other intermediates, some of which were methyl ketones, and therefore recognised THM precursors, were also identified over the first 24 hours. This was in contrast to the nearly exclusive production of THMs from 4',5,7-trihydroxyflavanone. The reactions to convert retinol (and the other related terpenoid structures) into the methyl ketone intermediates appeared to be slow reactions and methyl ketones are reported to react slowly to produce THMs (e.g. Gallard and von Gunten, 2002). Thus, it is proposed that halogenation of retinol-type terpenoids may possibly contribute to the slow reacting phase of THM formation in natural waters.

Increasing the pH from 7 to 9 resulted in an increase in total THM formation from both 4',5,7-trihydroxyflavanone and retinol, with increased rates of THM production in the initial fast phase (Figure 4). In kinetic studies of the halogenation of phenols, apparent second-order rate constants for reaction of chlorine and bromine with the phenols were found to be at a maximum around pH 8-9 (e.g. Acero *et al.*, 2005), with the rate constants for the reactions with bromine being much faster than for the reactions with chlorine (Acero *et al.*, 2005). This pH range of maximum halogenation appears to correspond to the presence of the highest proportion of phenoxide ions (Acero *et al.*, 2005) and of the major electrophilic species HOBr (pK_a 8.6), with the weaker electrophile OCl^- (pK_a HOCl 7.5). The increase in total THM formation for 4',5,7-trihydroxyflavanone at pH 9 in the current study is consistent with the most favoured reaction pathway between HOBr and deprotonated

348 forms of 4',5,7-trihydroxyflavanone at pH 9, with weak competition from the much less
349 electrophilic OCl^- species. At pH 9, retinol is unlikely to be deprotonated, but the basic conditions
350 would also promote the haloform reaction on methyl ketone intermediates, leading to increased
351 THM formation (Fuson and Bull, 1934).

352 Comparison of the molar concentrations of total THMs produced after 7 days from the six model
353 compounds (Tables 2 and 3) shows that 4',5,7-trihydroxyflavanone produced 3.4 and 2.6 times the
354 molar concentration of THMs of any other model compound. In the only previous study of THM
355 formation from 4',5,7-trihydroxyflavanone (naringenin), under similar conditions of chlorination but
356 without the addition of bromide ion, it was found that 270-298 μg THM / mg C, presumably all as
357 chloroform, was formed after 24 hours (Matsuo *et al.*, 1989). In the current study, the 7 day total
358 THM mass concentration was 526 $\mu g\ L^{-1}$, corresponding to 790 μg THM / mg C, where the THMs
359 were comprised of the four chlorinated and brominated THMs. The current results are consistent
360 with more THMs being formed in the presence of the more reactive halogenating agent HOBr over a
361 longer reaction period than in the Matsuo *et al.* (1989) study. β-Ionone, ellagic acid, and retinol
362 produced the next highest molar concentrations of THMs at pH 7, with significant concentrations
363 formed from these model compounds. Ellagic acid is a cyclic ester dimer of gallic acid which we
364 have found in another, unpublished, study to be a moderate producer of THMs. At pH 9, the
365 terpenoids, β-ionone and retinol, produced higher molar concentrations of THMs than ellagic acid.
366 At pH 7, only very low molar concentrations of THMs were formed from β-carotene and geranyl
367 acetate, although the concentrations increased at pH 9. For the terpenoid model compounds,
368 increasing the pH from 7 to 9 would again promote THM formation through base catalysis of the
369 haloform reaction of methyl ketone intermediates.

370 The specific yields of total THMs, calculated as μmol total THMs / mol carbon (C) of the model
371 compound, are presented in Tables 2 and 3. The yields of total THMs (Tables 2 and 3) represent the

372 conventional yield calculation of the conversion of the model compound to total THMs, calculated
373 as the sum of the moles of total THMs produced divided by the initial moles of model compound
374 present, as a percentage. This molar yield parameter does not take into account the number of
375 carbons which are likely THM precursor sites within the model compound, since this number is not
376 definitively known. The specific yields of total THMs and the yields of total THMs generally
377 followed the same trends as the molar concentrations of total THMs formed after 7 days at both pH
378 7 and 9. Retinol and β-ionone, however, produced essentially the same yields of total THMs
379 (approximately 20 % at pH 7 and 33 % at pH 9), possibly indicating the very similar reaction
380 pathways to THMs from these model compounds. The pH did not affect the specific yields of total
381 THMs (21-22 %) or the yields of total THMs (30 %) from ellagic acid, as was found with the molar
382 concentrations of total THMs produced (989-1000 nM). Extremely high yields of total THMs were
383 observed for 4',5,7-trihydroxyflavanone, 94 and 127 %, at pH 7 and 9, respectively. Reaction yields
384 greater than 100% are possible if more than one carbon in the model compound reacts to form
385 THMs. The greater than 100 % conversion at pH 9 is consistent with the hypothesis that THMs are
386 being formed from more than one carbon within the 4',5,7-trihydroxyflavanone structure, i.e. from
387 the 1,3-dihydroxybenzene and phenolic moieties within the structure. Indeed, this may also occur at
388 pH 7, with lower conversions from more than one carbon site, resulting in a total conversion of 94
389 %. As comparison, Rook (1977) found that the yield of $CHCl_3$ from chlorination of highly reactive
390 resorcinol (*meta*-dihydroxybenzene) for 2 hours at 15 ºC was 85 % at pH 7 and 100% at pH 9.
391 Others (e.g. Boyce and Hornig, 1983) have found similar yields for resorcinol. Chlorination of the β-
392 keto acid, 3-ketoglutaric acid (acetone dicarboxylic acid), with two carbons reactive to THM
393 formation, was found to produce chloroform in yields ≥ 90% over time periods of 0.75 – 24 hours
394 (Larson and Rockwell, 1979; Hasegawa *et al.*, 1983).

The oxidant demands of the polyhydroxyphenol model compounds (μM Cl_2 equivalents; Tables 2 and 3) were higher than those of the terpenoid model compounds. Ellagic acid had similar oxidant demands to 4',5,7-trihydroxyflavanone, with much lower yields of total THMs, indicating that oxidant was consumed in more non-THM forming reactions with ellagic acid. Similarly, oxidant appeared to be consumed in more non-THM forming reactions with β-carotene and geranyl acetate, as compared to retinol and β-ionone. The oxidant demands were lower at pH 9 than at pH 7, while the yields of total THMs were higher, indicating more effective conversion to THMs at the higher pH from all model compounds. This trend of lower oxidant demand with higher THM formation at higher pH (pH 6.8-10.7) has also been observed for chlorination of syringaldehyde after 6 and 24 hours, although in a similar experiment, with a different mole ratio of chlorine to syringaldehyde and after a reaction time of 60 hours, the oxidant demand did not change with pH (Morris and Baum, 1978). Reckhow and Singer (1985) also observed the trend of lower oxidant consumption with higher chloroform formation at higher pH (12 *vs.* 7) for chlorination of syringaldehyde, while both oxidant consumption and chloroform formation increased with increasing pH (7 *vs.* 12) for chlorination of pyruvic acid.

4. Conclusions

This is the first study to demonstrate the formation of THMs from β-carotene-type terpenoids. Halogenation of β-ionone and retinol and, to a lesser extent, β-carotene and geranyl acetate produced THMs, most likely through haloform reactions on methyl ketone groups in the complex suites of reaction intermediates. Since halogenation of retinol produced a significant quantity of THMs at a slow reaction rate, retinol-based structures may possibly contribute to the slow reacting phase of THM formation in natural waters. β-Ionone appeared to be a key intermediate in the halogenation of β-carotene and retinol, reacting further under the reaction conditions to produce *trans*-β-ionone-5,6-epoxide and β-cyclocitral.

The only identified products from halogenation of 4',5,7-trihydroxyflavanone and ellagic acid were THMs. 4',5,7-Trihydroxyflavanone rapidly produced THMs at pH 7, with an extremely high molar yield of total THMs (94 %), significantly higher than any other model compound studied. At pH 7, ellagic acid produced a similar 7 day molar concentration of total THMs to β-ionone, with a slightly higher specific and conventional yield of total THMs than β-ionone.

Terpenoids of the β-ionone and retinol type could therefore be significant THM precursors, while 4',5,7-trihydroxyflavanone has been shown to be an extremely significant THM precursor, potentially present within NOM in water treatment processes and distribution systems. Since β-ionone can be derived from microbial activity and 4',5,7-trihydroxyflavanone is a moiety in condensed tannins, management strategies for source waters containing high levels of microbial activity or high levels of tannin input should consider the role of these respective moieties in DBP formation from these source waters.

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