



A new agent for derivatizing carbonyl species used to investigate limonene ozonolysis



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HIGHLIGHTS

- Lower molecular weight agent for carbonyl derivatization.
- Aqueous collection and derivatization of gas-phase dicarbonyls and limonene ozonolysis products.
- Multi-functional carbonyls detected from limonene ozonolysis

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ABSTRACT

A new method for derivatizing carbonyl compounds is presented. The conversion of a series of dicarbonyls to oximes in aqueous solution and from gas-phase sampling was achieved using *O*-*tert*-butylhydroxylamine hydrochloride (TBOX). Some advantages of using this derivatization agent include: aqueous reactions, lower molecular weight oximes, and shortened oxime-formation reaction time. Additionally, the TBOX derivatization technique was used to investigate the carbonyl reaction products from limonene ozonolysis. With ozone (O_3) as the limiting reagent, four carbonyl compounds were detected: 7-hydroxy-6-oxo-3-(prop-1-en-2-yl)heptanal; 3-isopropenyl-6-oxoheptanal (IPOH), 3-acetyl-6-oxoheptanal (3A6O) and one carbonyl of unknown structure. Using cyclohexane as a hydroxyl (OH^\bullet) radical scavenger, the relative yields (peak area) of the unknown carbonyl, IPOH, and 3A6O were reduced indicating the influence secondary OH radicals have on limonene ozonolysis products. The relative yield of the hydroxy-dicarbonyl based on the chromatogram was unchanged suggesting it is only made by the limonene + O_3 reaction. The detection of 3A6O using TBOX highlights the advantages of a smaller molecular weight derivatization agent for the detection of multi-carbonyl compounds. The use of TBOX derivatization if combined with other derivatization agents may address a recurring need to simply and accurately detect multi-functional oxygenated species in air.

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1. Introduction

Occupants are exposed to a number of chemicals (either by inhalation or through skin absorption) in the indoor environment by: emissions from building materials (e.g. vinyl and linoleum floorings, carpets, treated woods, office furniture, etc.), applications of paints, lacquers, cleaning agents, air fresheners, and formation of reaction products of indoor chemistry. These chemicals are mainly composed of volatile organic compounds (VOCs) and semi-VOCs that may act as a single irritant or combine in mixtures resulting in a synergistic effect. The key to

unraveling the complexity of indoor chemical exposures is to first identify those compounds which are suspected irritants and determine their expected concentrations indoors. The challenges in accurately characterizing the indoor environment are in the sheer number of compounds found indoors and the fact that most are not directly detectable by typical analytical methods due to either their instability during the analytical procedure or short lifespan.

In general, the specific health effects of many of the VOCs and semi-VOCs that have been identified indoors have been studied (Anderson et al., 2012, 2010, 2007; Banerjee and Annesi-Maesano, 2012; Billionnet et al., 2011; de Blas et al., 2012; Gallego et al., 2012; Mizukoshi et al., 2010; Nyrud et al., 2012; Peng et al., 2013; Wang et al., 2011; Yu and Kim, 2012; Zhou et al., 2011). However, the presence of these compounds does not fully explain the irritation

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levels that have been observed/reported in epidemiological literature (Annesi-Maesano et al., 2012; Dalton and Jaen, 2010; Fong et al., 2010; Hulin et al., 2012; Lan et al., 2011; Lin et al., 2012; Logue et al., 2012; Milner et al., 2011; Mohai et al., 2011; SCHER, 2007). It is anticipated that these elevated irritation levels may be explained by exposure to oxidation products such as: aldehydes, ketones, dicarbonyls, carboxylic acids, and organic nitrates that elude conventional analytical methods. This is why current research efforts into improved derivatization chemistry and better analytical instrumentation/methods have received considerable attention.

The detection of carbonyl compounds, which can be significant fractions of both gas-phase and particulate matter (PM), has been a focus in characterization of both indoor and outdoor environments (Kundu et al., 2012; Leungsakul et al., 2005a, 2005b; Pan et al., 2009; Pathak et al., 2012). Furthermore, toxicological studies of dicarbonyls have implicated these compounds in both respiratory inflammation and sensitization. Anderson et al. investigated the allergic responses of dicarbonyls (such as glyoxal, methyl glyoxal, glutaraldehyde, 4-oxopentanal, and diacetyl) in a series of *in vitro* exposure studies (Anderson et al., 2010). These studies showed that exposures to a variety of these dicarbonyls lead to increased expression of inflammatory cytokines such as IL-6, IL-8, and TNF- α . These results indicated that dicarbonyls stimulate the release of proinflammatory mediators from lung epithelial cells and suggest that these oxygenated products may be contributing to adverse health effects. A recent publication by (Wolkoff et al., 2013) suggested that indoor terpene oxidation conditions leading to 4-oxopentanal and/or 3-isopropenyl-6-oxoheptanal (IPOH) formation parameters should be more accurately characterized based on the carbonyls' derived human reference values for sensory irritation. There has been increasing interest in structure reactivity models for respiratory sensitization/irritation and some models identified associations between number of functional groups (carbonyl, alcohol, carboxylic acid, etc.) in a molecule, exposure to that molecule, and occupational asthma (Dworak et al., 2013; Enoch et al., 2012; Jarvis et al., 2005; Mekenyan et al., 2014; Pralong et al., 2013; Tarlo and Lemiere, 2014). Clearly these concepts between terpene oxidation and health effects are still developing, and data is still needed to meaningfully assess air quality.

For carbonyl compounds there have been numerous publications regarding detection and quantification using either dinitrophenyl hydrazine, methoxyamine, or *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxyamine (PFBHA) (Cirera-Domenech et al., 2013; Dai et al., 2012; Forester and Wells, 2009; Gomez Alvarez et al., 2012; Halket and Zaikin, 2005; Hudson et al., 2011; Kowalewski and Gierczak, 2011; Lopez-Aparicio and Hak, 2013; Rossignol et al., 2012). Current methodologies have included derivatization techniques to transform these compounds for gas chromatography/mass spectrometry (GC/MS) detection. Derivatization is required for these compounds as they thermally degrade during injection or during the GC oven ramp. Two common derivatization agents used for carbonyl compounds are dinitrophenyl hydrazine (DNPH) and PFBHA (Borras and Antonio Tortajada-Genaro, 2012; Bunkoed et al., 2012; Cirera-Domenech et al., 2013; Dai et al., 2012; Pacolay et al., 2008; Yu et al., 1998). Both of these methods have been successfully used to improve understanding of volatile organic compound oxidation mechanisms. There are some limitations using these methods such as 24-h derivatization reaction times, formation of oximes with 700 + molecular weights, and multiple sample processing steps. A derivatization agent that could reduce or eliminate these limitations would benefit both oxidation research as well as indoor/outdoor field sampling campaigns.

A new method has been developed for simultaneous detection of multiple carbonyl compounds in aqueous samples driven, in part, by the identified need to use lower molecular weight derivatization agents in the characterization of reaction products from terpene oxidation. Aqueous sample collection simplified trapping of gaseous species and added a point of practicality for potential incorporation into field sampling campaigns. This method was developed using a mixture of commercially available dicarbonyls and then utilized to investigate gas-phase limonene ozonolysis. The selection of *O*-*tert*-butylhydroxylamine hydrochloride (TBOX) was based on the successful oxime formation chemistry of PFBHA (Yu et al., 1998, 1995). This method provided the sensitivity, ease of use, and wide-ranging applicability needed for detection of carbonyl compounds at expected indoor air concentrations. Additionally, there has been a recent in-depth model (Carslaw, 2013) which explored both the ozone and OH \cdot radical initiated mechanisms of limonene oxidation chemistry. The modeling results highlighted the need to collect and identify multifunctional oxidized species resulting from terpene oxidation (Carslaw, 2013). The limonene ozonolysis data presented here was compared to this model.

2. Experimental methods

2.1. Chemicals and solvents

All compounds were used as received and had the following purities: from Sigma–Aldrich/Fluka (St. Louis, MO): *O*-*tert*-Butylhydroxylamine hydrochloride, (TBOX, 99%), limonene (99+%), toluene (HPLC grade, 99+%), cyclohexane (HPLC grade, 99+%), glyoxal (40 wt% in water), methyl glyoxal (40 wt% in water), 2,4-pentanedione (99+%), and glutaraldehyde (50 wt% in water); from TCI America (Portland, OR) 2,3-heptanedione (98%); from Fisher Scientific (Pittsburgh, PA): methanol (HPLC grade, 99.9%). Water (DI H₂O) was distilled, deionized to a resistivity of 18 M Ω cm, and filtered using a Milli-Q[®] filter system (Billerica, MA). Helium (UHP grade), the carrier gas, was supplied by Butler Gas (McKees Rocks, PA) and used as received.

Ozone (O₃) was produced by photolyzing air with a mercury pen lamp (Jelight, Irvine, CA) in a separate Teflon chamber. Aliquots of this O₃/air mixture were added to the Teflon reaction chamber using a gas-tight syringe. O₃ concentrations were measured using a Thermo Electron (Waltham, MA) UV photometric ozone analyzer Model 49C.

2.2. Methods and materials

Experiments were carried out at (297 \pm 3) K and 1 atm pressure. Compressed air from the National Institute for Occupational Safety and Health (NIOSH) facility was passed through anhydrous CaSO₄ (Drierite, Xenia, OH) and molecular sieves (Drierite) to remove both moisture and organic contaminants. This treated dry air from the NIOSH facility flowed through a mass flow controller and into a humidifying chamber and was subsequently mixed with dry air to the pre-determined relative humidity of 50%. The filler system was equipped with a heated syringe injection port facilitating the introduction of liquid reactants into the chamber. All reactant mixtures were generated by this system.

A 2.46 $\times 10^{-4}$ M solution of dicarbonyls (glyoxal, methyl glyoxal, 2,3-heptanedione, 2,4-pentanedione, and glutaraldehyde; Table S1 of Supporting Information) in methanol was used for preparation of liquid and gas-phase samples. This mixture was utilized as a stock solution for testing derivatization chemistry (both liquid and gas-phase collection) as well as oxime chromatography and mass spectrometry.

A three part approach was used to investigate the feasibility of using TBOX as a carbonyl derivatization agent: 1) Derivatization of five dicarbonyls in aqueous solution; 2) aqueous collection of five dicarbonyls from the gas phase and subsequent derivatization; and 3) aqueous collection of the gas-phase limonene ozonolysis reaction products and subsequent derivatization. Experimental details are described below.

Derivatization of the dicarbonyl compounds (5×10^{-6} M each of glyoxal, methyl glyoxal, 2,3-heptanedione, 2,4-pentanedione, and glutaraldehyde) was achieved by adding 100 μ L of aqueous 250 mM TBOX to 25 mL deionized H₂O in a 40 mL vial (reaction schematic in Fig. 1). The vial was then placed in a 40 °C water bath for 1 h. After removing the vial from the 40 °C water bath and cooling in a room temperature water bath, 1 mL of toluene was added to the vial. The vial was then shaken for 30 s and allowed to separate into a toluene layer and aqueous layer. The toluene layer was then removed with a pipette and placed in a 2 mL autosampler vial (Resett, Bellefonte, PA). Then 1 μ L of the extract was injected onto the gas chromatographic/mass spectrometric system (conditions described below).

To investigate the gas-phase collection of carbonyl species, the five dicarbonyl mix (glyoxal, methyl glyoxal, 2,4-pentanedione, 2,3-heptanedione, and glutaraldehyde) was injected into an 80 L air-filled Teflon chamber. The concentration of each dicarbonyl was 50 ppb (1.25×10^{12} molecule cm⁻³) which was chosen to simulate concentrations possible in indoor environments (Nazaroff and Weschler, 2004; Singer et al., 2006; Weschler, 2011). Then 60 L was sampled with a pump (URG 3000-02Q, Chapel Hill, NC) (4 L/min for 15 min) into a 60 mL Teflon impinger (Saville, Eden Prairie, MN) filled with 25 mL of deionized water. After collection the impinger contents were placed into a 40 mL glass vial, TBOX derivatized, extracted, and analyzed as described above.

After the dicarbonyl mixture experiments described above, an investigation of gas-phase limonene ozonolysis was initiated. In an 80 L volume of air, O₃ (50 ppb; 1.25×10^{12} molecule cm⁻³) was added to 1.7 ppm limonene (4.25×10^{13} molecule cm⁻³), and allowed to react for 30 min. After the reaction, a 60 L sample was collected into 25 mL of water using an impinger, TBOX derivatized, extracted, and analyzed. Additional experiments in part 3 included the addition of cyclohexane (280 + ppm ($\sim 6 \times 10^{20}$ molecule cm⁻³)) to the reaction mixture to scavenge OH radicals formed from Criegee intermediates of limonene ozonolysis (Atkinson and Aschmann, 1993; Carslaw, 2013; Criegee, 1975; Forester and Wells, 2011) to glean information on secondary product formation chemistry.

Experiments to measure gas-phase dicarbonyls (glyoxal, methyl glyoxal, 2,3-heptanedione, 2,4-pentanedione, and glutaraldehyde) or the gas-phase reaction products formed from the reaction of limonene with O₃ were conducted with a previously described apparatus (Wells, 2005). A brief description is provided here. Reactants were introduced and samples were withdrawn through a 6.4-mm Swagelok (Solon, OH) fitting attached to the Teflon-film chamber. The chamber was filled with 50% relative humidity air (described above).

All samples were analyzed using a Varian (Palo Alto, CA) 3800/Saturn 2000 GC/MS system operated in the electron impact (EI) mode. Compound separation was achieved by an Agilent (Santa Clara, CA) HP-5MS (0.25 mm I.D., 30 m long, 0.25 μ m film thickness)

column and the following GC oven parameters: 40 °C for 2 min, then 5 °C min⁻¹ to 200 °C, then 25 °C min⁻¹ to 280 °C and held for 5 min. One μ L of each sample was injected in the splitless mode, and the GC injector was returned to split mode 1 min after sample injection, with the following injector temperature parameters: 110 °C for 2 min then 200 °C min⁻¹ to 300 °C and held for 5 min. The Saturn 2000 ion trap mass spectrometer was tuned using perfluorotributylamine (FC-43). Full-scan EI ionization spectra were collected from m/z 40–650.

All solutions were added through the syringe injection port (described above). An additional port attached to the Teflon chamber enabled the injection of O₃. All sample collections (described above) were treated the same. Each experiment was conducted at least three times. The results from each of the three phases are described below.

The temperature and TBOX concentration parameters used for the experiments above were determined from modifying previous PFBHA derivatization work conducted in this laboratory (Forester and Wells, 2009; Ham et al., 2006; Ham and Wells, 2008). The toluene extracts were not blown to dryness because loss of the lower molecular weight oximes was observed.

3. Results

3.1. TBOX derivatization chemistry validation

Part #1 above resulted in the derivatization and detection of each dicarbonyl as doubly derivatized oximes with the exception of 2,3-heptanedione which was observed as a singly derivatized oxime (Fig. 1). Derivatization of nonsymmetric carbonyls using TBOX typically resulted in multiple chromatographic peaks due to stereoisomers of the oximes. The following retention times were observed: 15.8 min for doubly derivatized glyoxal (MW = 200), 16.7 min for doubly derivatized methyl glyoxal (MW = 214), 17.1 min for singly derivatized 2,3-heptanedione (MW = 199), 18.8 and 19.0 min for doubly derivatized 2,4-pentanedione (MW = 242), and 22.2, 22.5, 22.7 min for doubly derivatized glutaraldehyde (MW = 242, Table S1 in Supporting Information). Typically an M^{+/-} ion was observed for the derivatized oxime compounds. Retention times for the dicarbonyl oximes were assigned based on derivatization and analysis of solutions containing each dicarbonyl separately. Identification of multiple peaks of the same oxime compound is relatively simple since the mass spectra for each chromatographic peak of a particular oxime composition are almost identical. In most cases, the m/z = 57 ion relative intensity for the chromatographic peaks of the oximes was greater than 50% in the mass spectrum. This ion was attributable to the *t*-butyl group (C₄H₉⁺ fragment) and could be effectively used to generate selected ion chromatograms to identify derivatized carbonyl compounds in a mixture (see Supporting Information). Both the loss of the *t*-butoxyl radical (MW = 73; C₄H₉O[•]) and isobutene (MW = 56; C₄H₈) resulted in a M-73 and M-56 ions, respectively and were used to identify derivatized carbonyls. These results established that TBOX has the potential to be a useful and practical derivatization agent for carbonyls in water.

3.2. Gas-phase sampling of dicarbonyls

Similar to the vial experiment above, the 2,3-heptanedione was observed as the singly derivatized oxime and the others were detected as doubly derivatized oximes. The observation of these dicarbonyls using this sampling/derivatization method demonstrated that gas-phase carbonyls can be collected into water using an impinger with subsequent TBOX derivatization. The chromatogram for the 50 ppb gas-phase sample is shown in Fig. 2.

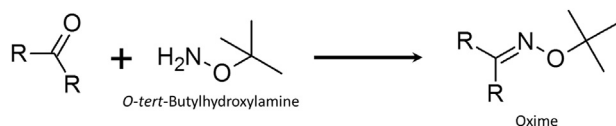


Fig. 1. Reaction schematic for carbonyl derivatization by *O*-tert-Butylhydroxylamine (TBOX) to form an oxime.

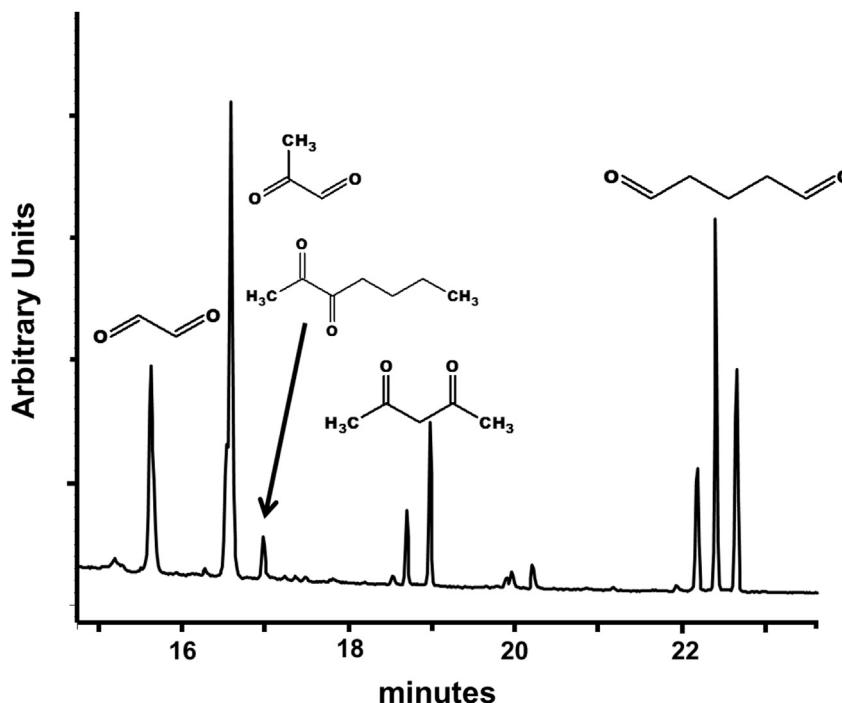


Fig. 2. Total ion chromatogram (TIC) of 50 ppb dicarbonyls derivatized as oximes resulting from TBOX derivatization. Chemical structures of dicarbonyls are shown above chromatographic peak of derivatized oxime.

3.3. Limonene + O₃ experiments

With the successful demonstration that gas-phase carbonyl compounds can be sampled into 25 mL of DI H₂O using an impinger, an experiment to simulate terpene oxidation (ozonolysis) in indoor environments was conducted by investigating the gas-phase reaction of limonene + ozone. Limonene has both exocyclic and endocyclic carbon–carbon double bonds providing sites for ozone addition. A benefit of investigating the limonene + O₃ system using a new derivatization method is that there is a significant body of scientific literature, from both the outdoor and indoor research community, in which the oxidation products have been identified and characterized (Atkinson, 2003; Atkinson and Aschmann, 1993; Herrmann et al., 2010; Kundu et al., 2012; Leungsakul et al., 2005a, 2005b). This body of scientific literature can be contrasted against the results shown here.

The reaction between 1.7 ppm of limonene and 50 ppb of ozone (limiting reagent) in either air or nitrogen yielded four products: 25.0, 25.3 min (OH radical influenced), 25.4, 25.8 min (not OH radical influenced), 28.7, 28.9, 29.1, 29.3 min 6-oxo-3-(prop-1-en-2-yl)heptanal, and 33.2, 33.4, 33.5, 33.6 min 3-acetyl-6-oxoheptanal. Scavenging secondary OH radicals by adding cyclohexane (280 + ppm) to the reaction mixture resulted in a reduction of chromatographic peak areas of all products except the 25.4 and 25.8 min product (Fig. 3). This reduction in reaction product peak area was consistent with the high yield of OH (64%) formed from ozonolysis of limonene and further suggests the importance of this secondary OH radical source in indoor chemistry (Atkinson, 2003; Atkinson and Aschmann, 1993; Herrmann et al., 2010). The following chronological chromatographic retention time results and mass spectra data were observed utilizing TBOX derivatization and the Varian 3800/Saturn 2000 GC/MS system. The reaction products' chromatographic peak areas were observed to increase only after introduction of O₃ to limonene/methanol/air mixtures. Derivatization experiments performed in the absence of limonene,

but in the presence of all other chemicals in the reaction chamber (O₃/air/methanol) did not result in any of the data reported below.

The four main products (provisionally identified as a carbonyl, a hydroxy carbonyl, a dicarbonyl, and a tricarbonyl) from limonene ozonolysis are listed below:

3.3.1. Retention time 25.0 and 25.3 min

This reaction product was observed from limonene ozonolysis and it appears to be dependent on OH radical reactions as the peak areas were significantly reduced when the OH radical was scavenged. Additionally, this appears to be a lower yield product (based on peak area). The main ions (% relative peak height) are 57 (100), 108 (33), 125 (90), 133 (36), 151 (30), 166 (27), 183 (27), 224, 239 both (~3). If the 239 is taken as the M^{+/-} ion of the derivatized molecule then the molecular weight of the dicarbonyl is 97 which is not likely for a compound composed of C, H, and O. Therefore, a singly derivatized carbonyl of molecular weight of 168 is proposed. Although several molecular structures are possible based on this data, no definitive product could be determined based on current reaction mechanisms (Fig. 3 and Supporting Information Figure S2).

3.3.2. Retention time 25.4 and 25.8 min

This reaction product is formed directly from limonene ozonolysis and its yield appears, based on peak area, to be unaffected by OH radical scavenging. The main ions (% relative peak height) are 57 (100), 107 (100), 126 (55), 139 (40), 167 (20), 182 (20), 199 (20), 255 (2). If 255 m/z is the M^{+/-} ion then a molecular weight of 184 is expected for the carbonyl compound. A possible hydroxy-dicarbonyl could be 7-hydroxy-6-oxo-3-(prop-1-en-2-yl)heptanal (Fig. 3 and Supporting Information Figure S3). This product was previously observed by (Norgaard et al., 2013).

3.3.3. Retention time 28.7, 28.9, 29.1, and 29.3 min

The four peaks at this retention time are attributed to 6-oxo-3-(prop-1-en-2-yl)heptanal (also known as 3-isopropenyl-6-

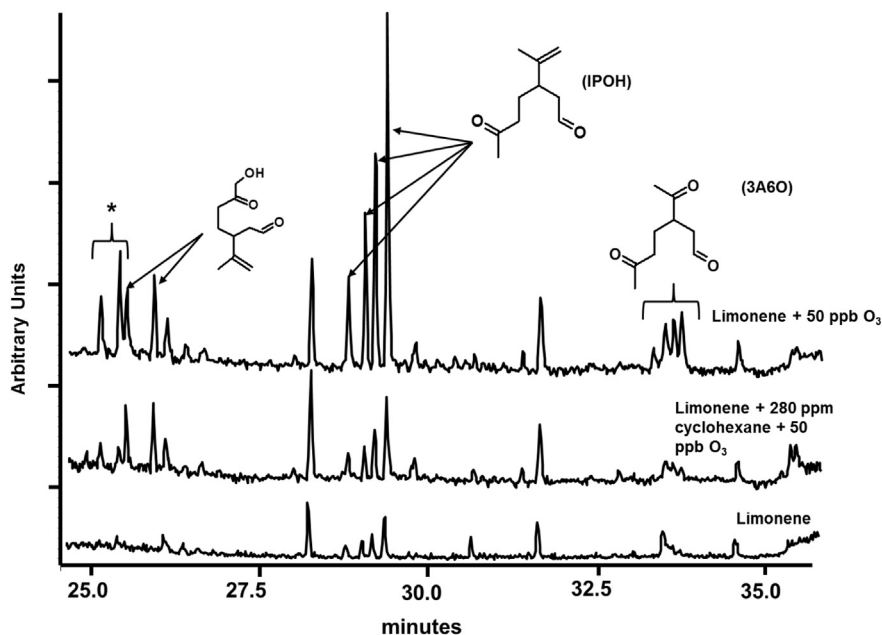


Fig. 3. Total ion chromatograms (TICs) 50 ppb O_3 , 1 ppm limonene, comparison of limonene only, limonene + O_3 , limonene + O_3 with cyclohexane scavenger with products identified from TBOX derivatization. Chromatograms are normalized. 25.4 and 25.8 min: 7-hydroxy-6-oxo-3-(prop-1-en-2-yl)heptanal; 28.7–29.3 min: 3-Isopropenyl-6-oxoheptanal (IPOH); 33.2–33.6 min: 3-acetyl-6-oxoheptanal (3A6O). *unidentified derivatized carbonyl.

oxoheptanal (IPOH)) and are the result of ozone adding to the endocyclic carbon–carbon double bond of limonene. This dicarbonyl has been detected or predicted and its formation mechanism has been presented in previous investigations of limonene oxidation (Jiang et al., 2013; Kundu et al., 2012; Pathak et al., 2012; Wolkoff et al., 2013). A table of ions with relative intensities and mass spectrum are presented in the Supporting Information (Table S2, Figure S4).

3.3.4. Retention time 33.2, 33.4, 33.5, and 33.6 min

The four peaks at this retention time are attributed to 3-acetyl-6-oxoheptanal, a tri-carbonyl compound. This compound has been implicated in particulate matter formation (Norgaard et al., 2013; Pathak et al., 2012) and its yield has been recently modeled by (Carslaw, 2013). While the peak areas of this compound are small it can be seen in Fig. 3 that its formation is influenced by secondary OH radicals, possibly OH addition to the carbon–carbon double

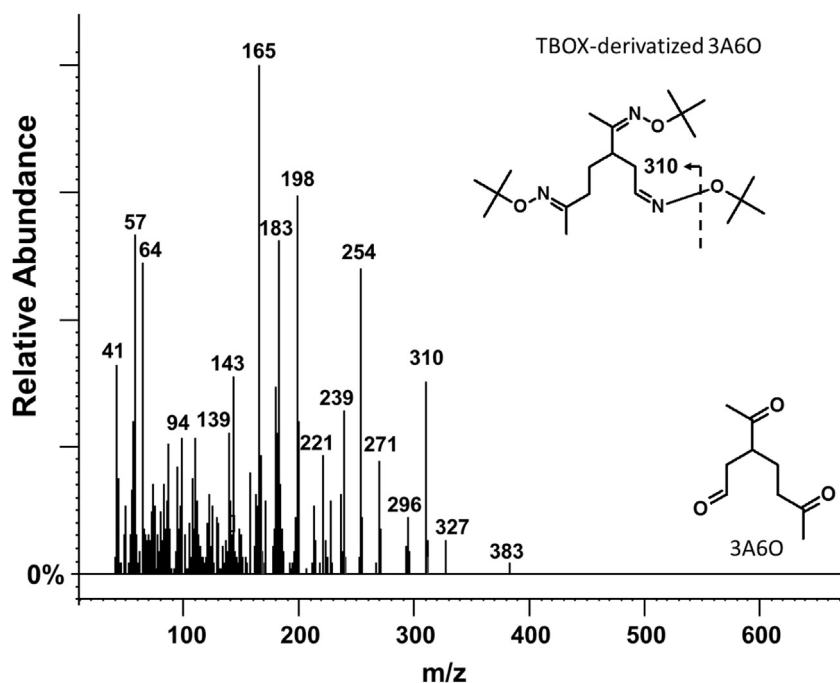


Fig. 4. Mass spectrum of observed oxime due to the limonene + O_3 reaction. Possible mass fragment assignments are indicated on the oxime structures and the proposed reaction product 3-acetyl-6-oxoheptanal (3A6O) is shown.

bond in IPOH. The mass spectrum of the derivatized tricarbnonyl is shown in Fig. 4.

4. Discussion

Detection methods that are sensitive yet easy to use for characterizing oxidized organic compounds will benefit both the outdoor and indoor research communities (Ford et al., 2007; Kowalewski and Gierczak, 2011; Li et al., 2009; Schummer et al., 2009; Yu et al., 1998). Research areas such as chemical reaction mechanism model development, secondary organic aerosol formation, and exposure science would all profit from improved detection methods. To accomplish these challenges chemical derivatization has been effective for stabilizing oxygenated compounds for routine analysis (Rossignol et al., 2013; Yu et al., 1998). While using a derivatization agent like PFBHA to derivatize carbonyl moieties has resulted in the identification of many oxygenated species, its mass may have prevented the identification of tri- and higher carbonyl species due to the limited mass range of typical mass spectrometers in use. For instance, Carslaw's recent modeling of limonene oxidation by OH radical and O₃ has identified the formation of several tricarbnonyl and multi-oxygenated species which would have masses in excess of 750 amu if PFBHA were used for derivatization (Carslaw, 2013; Norgaard et al., 2013). Additionally, compounds with formula weights above 700 amu may not be suitable for conventional capillary chromatography. Based on the results above using TBOX to derivatize carbonyl compounds to oximes appears to address many of these limitations. For instance TBOX adds 71 amu per derivatized carbonyl opposed to 195 amu using PFBHA (approximately 64% mass reduction per carbonyl). Additionally, the use of mild conditions (40° C for 1 h) as well as conducting the derivatization in water are other benefits. While there may be some limitations to using TBOX for carbonyl derivatization, this agent appears to be a practical alternative to PFBHA for both the laboratory and field investigations.

The vial experiments demonstrated the applicability of the TBOX method and oxime-forming chemistry for double derivatization of dicarbonyls. While the α , β -dicarbonyls glyoxal and methyl glyoxal were doubly derivatized, 2,3-heptanedione (another α , β -dicarbonyl) was singly derivatized. This single derivatization of an α , β -dicarbonyl may be the effect of steric hindrance due to a multi-carbon group attached to the one of the carbonyls. While it would be preferable to have both carbonyls derivatized in larger α , β -dicarbonyls, the fact that 2,3-heptanedione had a similar chromatographic retention time to the other "non-sterically hindered" doubly derivatized α , β -dicarbonyls could allow investigators to use chromatographic retention time and mass spectrometer data to propose plausible α , β -dicarbonyl chemical structures for unknown compounds.

The collection of gas-phase dicarbonyls into an impinger filled with water and subsequent TBOX derivatization further demonstrated the utility of this derivatization agent. Unfortunately, peak areas of 25–40% of the vial experiments for the same compounds were observed. Nevertheless, the convenience and inherent safety of using water instead of other solvents such as methanol, methylene chloride or benzene is a true benefit that can be exploited in indoor and outdoor field sampling campaigns. Additionally, these experiments highlighted the method's sensitivity even without concentrating the sample through an extract solvent blow-down step.

The series of dicarbonyl/TBOX experiments above helped to establish a method for collection of the oxidation products from the limonene + O₃ reaction system. There have been numerous investigations of this reaction by the indoor and outdoor research communities and a limonene + O₃ reaction mechanism model

proposing the formation of tricarbnonyl species has been published recently by Carslaw (Carslaw, 2013; Norgaard et al., 2013; Pathak et al., 2012). For the experiments conducted here, ozone (50 ppb) was the limiting reagent with limonene (~2 ppm) in excess. The addition of cyclohexane to scavenge OH radicals (in some experiments) provided additional insight into the limonene + O₃ reaction mechanism. Under the experimental conditions above only four carbonyl products were observed: a carbonyl, a hydroxy carbonyl, IPOH, and 3-acetyl-6-oxoheptanal (3A6O) (Fig. 3). The findings and implications of the results are discussed below.

The reaction product observed at retention times 25.4 and 25.8 min was not formed due to reaction with secondary OH radicals. Its peak areas did not change when cyclohexane was added to scavenge OH radicals formed from Criegee radical decomposition. Similar to 2,3-heptanedione this hydroxy dicarbonyl was singly derivatized. Even though this compound was not an α , β -dicarbonyl the OH moiety could hinder the derivatization of the second carbonyl.

It is well established that ozone will preferentially add to limonene's endocyclic carbon–carbon double bond to break open the ring and form IPOH as can be observed in Fig. 3 (Herrmann et al., 2010; Kundu et al., 2012; Larsen et al., 2001; Leungakul et al., 2005a, 2005b). However, it is interesting to note that secondary OH radicals play an important role in IPOH formation as observed by the significant decrease in IPOH's chromatographic peak areas when OH is scavenged. This observation suggests that IPOH has two routes to its formation and the OH channel may be more important than O₃ addition.

The identification of 3A6O was facilitated by the use of TBOX as a derivatization agent (Fig. 4). Had PFBHA been used, this tricarbnonyl compound would have had a formula weight of 755 amu which is above the 650 amu limit on the mass spectrometer used for these experiments. The proposed 3A6O structure is also consistent with limonene oxidation literature and its detected formula weight (383 *m/z*) strongly suggests a triply derivatized compound (Carslaw, 2013; Norgaard et al., 2013; Pathak et al., 2012). Furthermore, the reaction product likely does not have an α , β -dicarbonyl fragment based on the derivatization data of 2,3-heptanedione above. Similar to IPOH an OH dependency was also observed for 3A6O. This can be clearly observed in Fig. 3 as the addition of cyclohexane (OH scavenger) decreased the peak areas by approximately 70%. Therefore a likely route to its formation is the OH addition to the carbon–carbon double bond of IPOH and subsequent loss of the methylene radical. Both the chromatographic retention time as well as the observed relationship with secondary OH radicals further supports the 3A6O assignment.

Importantly this series of experiments provided additional insight into terpene oxidation. The IPOH formation from limonene ozonolysis was enhanced by OH radicals formed from the Criegee biradical degradation. While the dicarbonyl was still detected in the absence of OH radicals, this observation further highlights the importance of OH radical chemistry indoors and its subsequent impact on indoor chemistry and thus occupant exposure (Alvarez et al., 2013; Nazaroff and Weschler, 2004; Rossignol et al., 2013). The O₃ concentration used in these experiments is significantly lower than most previous investigations of the limonene + O₃ reaction systems. With O₃ as the limiting reagent a reduction in the number of reaction products is observed when contrasted to previously published work on this reaction system (Carslaw, 2013; Jiang et al., 2013; Kundu et al., 2012; Pathak et al., 2012).

5. Conclusion

The use of *O*-*tert*-butylhydroxylamine hydrochloride (TBOX) as a carbonyl derivatization agent appears to be a practical alternative

to PFBHA for detecting multi-carbonyl and/or multi-functional oxygenated organic compounds. The practicality and sensitivity of this method is anticipated to benefit investigators in both atmospheric and indoor chemistry fields. The investigation of the limonene + O₃ reaction in the gas-phase using this new derivatization agent lead to the identification of multi-functional carbonyl compounds and importantly the detection of the tri-carbonyl, 3A6O. Future work will include the incorporation of TBOX with other derivatization agents in order to identify many other oxygenated species such as alcohols and carboxylic acids simultaneously in a single sample.

Disclaimer

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry. Mention of any commercial product or trade name does not constitute endorsement by the Centers for Disease Control and Prevention/NIOSH.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.atmosenv.2014.10.015>.

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