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# Secondary ozonides of substituted cyclohexenes: A new class of pollutants characterized by collision-induced dissociation mass spectrometry using negative chemical ionization

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#### Abstract

Recent studies indicate that secondary ozonides of cyclic alkenes are formed in atmospheric reactions and may be relatively stable. The secondary ozonides (SOZs) of cyclohexene (1), 1-methylcyclohexene (2), 4-isopropyl-1-methylcyclohexene (3) and 4-isopropenyl-1-methylcyclohexene (limonene) (4) have been characterized by rapid gas chromatography electron ionization (EI), negative and positive chemical ionization (CI: ammonia, isobutane and methane) and collision-induced dissociation (CID) mass spectrometry. Both EI and positive CI spectra were found unsuitable for reproducible analysis. However, negative CI showed stable  $(M-H)^-$  ions with minor fragmentation. CID of the  $(M-H)^-$  ions resulted in simple and reproducible fragmentation patterns for all four SOZs with loss of m/z 18, 44 and 60, tentatively assigned as  $H_2O$ ,  $CO_2$  and  $C_2H_4O_2$  or  $CO_3$ , respectively. Thus, negative CI-MS–MS in combination with rapid gas chromatography is the preferred method for identification of secondary ozonides of cyclohexenes.

Keywords: Indoor air; Mass spectrometry; Secondary ozonides; Substituted cyclohexenes

# 1. Introduction

Although substantial research has been carried out on indoor air pollutants and their potential health impact, there are still many unknowns (Carslaw and Wolkoff, 2006). Most research has focussed on single compounds present indoors and relatively little attention has been paid to the significance of the reactions between them, which are likely to occur (Weschler et al., 2006; Wolkoff et al., 2006). There is an increasing concern about the health impact of oxidation products from reaction between ozone and chemically reactive volatile organic compounds (VOCs) that are emitted from a number of building materials and consumer products (e.g. Nazaroff and Weschler, 2004). There are indications that some of the causative species have hitherto not

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been identified, because of their instability. Thus, a number of relatively unstable oxidation products have been identified using "soft" sampling and chromatographic techniques for identification (Li et al., 2002: Docherty et al., 2004). Recently, the secondary endo-ozonide of limonene has been identified by use of pressurized liquid extraction and coldon-column gas chromatography as an abundant oxidation product (Nørgaard et al., 2006), trapped from the gas phase ozonolysis of limonene, a common VOC in indoor environments (e.g. Järnström et al., 2006). Higher concentration of alkenes can build up in indoor environments (Singer et al., 2006), as opposed to outdoors, and result in the formation of secondary ozonides (SOZs), e.g. from ozonolysis of terpenes. This has been substantiated in recent studies about cyclic alkenes (Aschmann et al., 2003; Nøjgaard et al., 2007). Such SOZs are expected to degrade at elevated temperature (cf. Tobias et al., 2000; Nørgaard et al., 2006), thus warranting a versatile analytical method for

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Fig. 1. Secondary ozonides (SOZs) of cyclohexene (1), 1-methylcyclohexene (2), 1-methyl-4-isopropylcyclohexene (3), limonene (4) and their corresponding cyclohexenes (5–8).

future indoor campaigns, because they are of concern regarding sensory irritation of the eyes and upper airways, including inflammation in the lower airways (Cvitas et al., 2005; Wolkoff et al., 2006).

The purpose of this study was to identify the best analytical conditions for SOZs of cyclic alkenes (see Fig. 1) by combined gas chromatography and mass spectrometry as an alternative to spectroscopic methods (FT-IR and NMR), which have previously been used for SOZ identification (Griesbaum et al., 1996; Neeb et al., 1996; Winterhalter et al., 2000). Characterization of the mass spectral fragmentation patterns has been carried out for some SOZs of alkyl and alkenyl substituted cyclohexenes under various conditions of ionization including electron ionization (EI) and positive chemical ionization (CI) using ammonia, isobutane and methane as reagent gas and negative CI using isobutane and methane.

# 2. Experimental

# 2.1. Chemicals

Cyclohexene (5) (puriss > 99.5%), 1-methylcyclohexene (6) (puriss > 99%), 4-isopropyl-1-methylcyclohexene (7) (purum > 97%), R-(+)-limonene (8) (purum > 96%), triphenyl phosphine (TPP) (puriss > 98.5%) and triphenyl phosphine oxide (TPPO) (purum > 98%) were purchased from Fluka. The purity of all alkenes was checked by GC–MS analysis prior to ozonolysis. Dichloromethane (DCM) (>99%) and methanol (99.8%) were obtained from Merck. Pentane (98%) was purchased from Aldrich. O<sub>2</sub> (>99.9999%) from Hydro Gas (Norway) was used to generate O<sub>3</sub>. Methane (quality 5.5), isobutane (quality 3.5) and ammonia (quality 4.8) for CI-MS experiments were obtained from AGA (Denmark).

#### 2.2. Instrumentation

Three different mass spectrometers (I–III) were used in this study. MS I: Perkin Elmer GC–MS (GC: Autosystem XL/MS: TurboMass); MS II: Varian GC–MS/MS (GC: CP-3800/MS: 1200L). Both instruments were equipped with auto sampler and on-column injector, a medium polar deactivated pre-column 2.5 m × 0.53 mm i.d. (Varian) and a 10 m × 0.53 mm i.d. Varian VF-5ms Rapid Low Bleed/ MS (0.25  $\mu$ m film thickness) column. GC oven program was: 50 °C hold 1 min, ramp 1: 5 °C min<sup>-1</sup> to 80 °C, ramp 2: 25 °C min<sup>-1</sup> to 250 °C hold 2 min for SOZ analysis and 50 °C hold 1 min, ramp 1: 5 °C min<sup>-1</sup> to 80 °C, ramp 2: 45 °C min<sup>-1</sup> to 250 °C hold 10 min for analysis of TPPO. Helium was used as carrier gas; the pressure was adjusted to be equal to a column flow of 1.5 ml min<sup>-1</sup>.

MS III: Kratos Profile double focusing magnetic sector instrument equipped with a HP 5890 GC and on-column injector for manual injection. The chromatographic system consisted of a medium polar retention gap (Varian deacti- $2.5 \text{ m} \times 0.53 \text{ mm}$  i.d.) pre-column and vated а  $60 \text{ m} \times 0.32 \text{ mm}$  i.d. Chrompack CP-Sil 19 CB low bleed/ MS (0.25 µm film thickness) column. The GC oven program was: 30 °C hold 2 min, ramp: 4 °C min<sup>-1</sup> to 250 °C hold 5 min. Helium was used as carrier gas; column flow: 1.5 ml min<sup>-1</sup>. Injection volumes were 0.5 µl. The MS transfer lines and MS source temperatures were kept at 120 °C. Ionization energy of 70 eV was applied in EI mode. For CI experiments ammonia, isobutane and methane were used as reagent gases at pressures between 1 and 5 torr and ionization energy of 30 eV (MS I and III) and 150 eV (MS II). Typical scan ranges were m/z 35–400 in EI mode and m/z60-400 in CI mode. Precursor and product ion scans were carried out on MS II. For CI-MS-MS experiments only methane and isobutane could be used as reagents gasses. Argon was used as collision gas at pressures of 1–2 mtorr and the collision energy (CE) was usually ramped (2-24 eV) during most MS-MS experiments.

# 2.3. Synthesis and purification of secondary ozonides of cyclic alkenes

The SOZs were synthesized by cryo-ozonolysis of the alkenes (500  $\mu$ l) dissolved in 100 ml pentane in a 200 ml impinger until a pale blue colour appeared (Griesbaum et al., 1996). O<sub>3</sub> was generated photochemically in pure O<sub>2</sub> with a mercury lamp (185 and 254 nm) in a thermostatic lamp housing controlled by a high performance variable power supply (Clausen et al., 2001).

The reaction mixture was purged with  $N_2$  to remove excess  $O_3$  and to reduce the volume to about 2 ml. Subsequently, the SOZs were purified by the use of preparative normal phase LC-UV. The system consisted of a Perkin Elmer series 410 LC-pump, a Waters model 440 UV-detector and a 50 cm stainless steel column (i.d. 3.9 mm) packed with silica gel (Merck type 9385, 230–400 Mesh). The reaction mixture (2 ml) was, by loop injection, introduced into



Fig. 2. EI mass spectra of secondary ozonides (1-4). A proposed fragmentation pathway is shown for SOZ 2 in Fig. 3.

the chromatographic system. First, excess alkene was eluted with 95% pentane and 5% DCM), subsequently, the SOZ was eluded with 100% DCM followed by other oxidation products. The purified SOZs, in DCM, were stored at 5 °C until use.

# 2.4. Reaction with triphenyl phosphine

0.5 M TPP in DCM (50 µl) was added to a purified solution of SOZ (approx. 250 µl). The solutions were stored at room temperature for at least 48 h before analysis. The oxidation of TPP to TPPO by SOZs is well known (e.g. Griesbaum et al., 1996; Fleming et al., 1997). The samples were analyzed by rapid GC–MS and the identity of TPPO was confirmed by comparison with an authentic standard.

# 3. Results and discussion

# 3.1. EI-MS

Multiple EI experiments were performed on the three different instruments and slightly different spectra were achieved. Such differences have been reported for analysis of alicyclic epoxides when using different mass spectrometers (Budzikiewicz et al., 1967). EI mass spectral data for the SOZs 1-4 are presented in Fig. 2. SOZ 1 and SOZ 2 show molecular ions  $M^+$  at m/z 130 and 144, respectively. A molecular ion of low abundance could in some experiments be observed at m/z 186 in the case of SOZ 3, while the molecular ion of SOZ 4 was absent. SOZ 1 and SOZ 2 show loss of molecular oxygen  $(O_2)$  directly from the molecular ion, which was confirmed by MS-MS experiments. O<sub>2</sub> loss from the molecular ion has also been reported in a previous study of the fragmentation patterns of some secondary ozonides (Castonguay et al., 1969). The subsequent fragmentations after the loss of O2 are similar to those reported for the 1,2-epoxides of cyclohexenes 5 and 6, respectively (Budzikiewicz et al., 1967; Strong et al., 1969), see Fig. 3 for proposed fragmentation. SOZ 3 and SOZ 4 show minor losses of 32 Da and 34 Da, respectively, which are tentatively assigned as O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub>; the latter has previously been confirmed for SOZ 4 by accurate mass determination of ion m/z 150 (Nørgaard et al., 2006). The overall fragmentation pathways for SOZ 3 and SOZ 4 after the loss of O<sub>2</sub> or H<sub>2</sub>O<sub>2</sub> appear to mimic the mass spectra of their corresponding epoxides; this is shown, for example, by comparison of the mass spectrum of limonene epoxides (NIST Mass spectral database v2.0). Loss of O<sub>2</sub> from SOZs



Fig. 3. Proposed EI fragmentation pathway for the SOZ of 1-methylcyclohexene (according to Budzikiewicz et al., 1967; Strong et al., 1969).



Fig. 4. CID mass spectra of secondary ozonides in negative chemical ionization (methane).

1–3 and loss of both  $O_2$  and  $H_2O_2$  from SOZ 4 have been reported in a previous study of SOZs by use of atmospheric

sampling Townsend discharge mass spectrometry (ASTDI-MS) (Nøjgaard et al., 2007).

#### 3.2. CI-MS, positive mode

 $(M+H)^+$  ions were observed in CI-MS in positive mode for all SOZs.  $(M+H)^+$  ions were observed by use of methane or isobutane as reagent gas. Ammonia as reagent gas produced  $(M+NH_4)^+$  ions. The relative abundance (r.a.) of the  $(M+H)^+$  and  $(M+NH_4)^+$  ions ranged from 6% to 55% for all reagent gasses.

The appearance of the  $(M+NH_4)^+$  ions and the absence of  $(M+H)^+$  ions with ammonia as reagent gas indicates that the proton affinities (PA) of the SOZs are above 787 kJ/mol, which is the energy required for formation of the  $(M+NH_4)^+$  adduct ion (Keough and DeStefano, 1981; Westmore and Alauddin, 1986) and below 854 kJ/ mol (the PA for ammonia).

All SOZs showed a high degree of fragmentation in all experiments, and their CI spectra were complex and difficult to reproduce on the different instruments; thus indicating the instability of the  $(M+H)^+$  ions. Common fragment ions in the positive CI spectra of all SOZs correspond to fragment losses with the mass of 16, 17, 18, 28, 32, 34, 36 and 44 Da, which tentatively are assigned as 'O, 'OH, H<sub>2</sub>O, CO, O<sub>2</sub>, H<sub>2</sub>O<sub>2</sub>, 2H<sub>2</sub>O and CO<sub>2</sub>, respectively. All SOZs eliminate 'OH, H<sub>2</sub>O<sub>2</sub> with methane as reagent gas, while elimination of 'O, H<sub>2</sub>O<sub>2</sub> and CO<sub>2</sub> was observed with isobutane as reagent gas. In the case of NH<sub>3</sub> all the SOZs eliminate 'O; in addition, SOZs 2–4 eliminate two fragment ions at 33 and 60 Da less than  $(M+NH_4)^+$ .

The positive CI-MS–MS experiments with methane and isobutane as reagent gas confirmed some of the losses mentioned above. In general, collision-induced dissociation (CID) of the  $(M+H)^+$  ions resulted in extensive fragmentation for all four SOZs even at a CE as low as 2 eV. For example, the most abundant peaks for SOZs 1–3 originate from the elimination of 'OH and H<sub>2</sub>O, while SOZ 4 eliminates 'OH, H<sub>2</sub>O and H<sub>2</sub>O<sub>2</sub>. Although the positive CI spectra show unique losses, they cannot be used for characterization due to their large variability between instruments.

# 3.3. CI-MS, negative mode

All SOZs show base peak  $(M-H)^-$  ions and negligible fragmentation is observed by use of methane and isobutane as reagent gasses. SOZ 1 shows consistently elimination of 18 Da (~1% r.a.), assigned as H<sub>2</sub>O loss, while SOZs 2–4 all show an abundant  $(M-15)^-$  ion, see Fig. 4. These  $(M-15)^-$  ions were examined by various MS–MS experiments, however, no precursor ions could be identified; probably, these ions originate from the loss of a methyl group during the ionization process. The fact that no  $(M-15)^-$  ion was observed for SOZ 1 adds further support, since this SOZ contains no methyl group.

The  $(M-H)^-$  ions of all SOZs appear to be relatively stable, and significant fragmentation was first observed at CE of at least 8 eV. Results from such negative CI-MS– MS experiments are presented in Fig. 4. All SOZs eliminate the following fragments: 18, 44 and 60, which are tentatively assigned as  $H_2O$ ,  $CO_2$ , and  $C_2H_4O_2$  or  $CO_3$ , respectively. In addition, SOZ 1 shows loss of 28 and 32 tentatively assigned as CO and  $O_2$ , respectively.

The high stability of the  $(M-H)^-$  ions of all four SOZs indicate the presence of an acidic hydrogen atom, presumably the ring bound H. This stability indicates that the negative charge is delocalized in the trioxolane ring.

# 4. Conclusions

GC–EI-MS will in some cases be insufficient for identification of the SOZs, because of the low abundance or absence of their molecular ions for SOZs 3–4. This may explain why SOZs have not been detected previously in atmospheric samples. In addition, EI is further invalidated by substantial variation of the fragmentation patterns that depends on the instrument used. Positive chemical ionization results in extensive fragmentation even when using a soft reagent gas such as ammonia. Thus, positive CI provides much information; however, interpretation is hampered by poor reproducibility. The simple and predictable fragmentation patterns in negative CI-MS–MS mode makes this method ideal for identification of the examined SOZs.

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