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Secondary ozonides of endo-cyclic alkenes analyzed by atmospheric sampling Townsend discharge ionization mass spectrometry

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Abstract

Secondary ozonides (SOZ) of cyclohexene, 1-methylcyclohexene, 4-isopropyl-1-methylcyclohexene and D-limonene were cryo-synthesized by ozonolysis in pentane and purified on a silica gel column. The mass spectra obtained by atmospheric sampling Townsend discharge ionization (ASTDI) and collision activated dissociation (CAD) of the protonized SOZ showed characteristic losses evident of the ozonide structure. Oxygen was eliminated as, e.g., O and O_2, and loss of (HCHO + HCHO) or ($O + CO_2$) corresponded to the SOZ base-peak for the substituted cyclohexenes by ASTDI-MS. The CAD spectra of the protonized species by use of methane as chemical ionization gas, showed consecutive losses of three oxygen atoms. Elimination of hydroxy-methyl hydroperoxide (HMHP) was particular important for the protonized SOZ, unlike consecutive loss of (HCHO + HCHO) or ($O + CO_2$). In addition, the spectra of D-limonene were characterized by an unique loss of H_2O_2. These losses appear to be useful for identification of SOZ in gas-phase ozonolysis mixtures of endo-cyclic alkenes, which makes ASTDI an alternative to other on-line techniques for analysis of SOZ in ozonolysis mixtures.

Keywords: Townsend discharge ;ASTDI; Alkene; Secondary ozonide; SOZ

1. Introduction

On-line techniques are particular convenient for the study of reaction products from oxidation of volatile organic compounds (VOCs). The development of these techniques has advanced significantly over the past years, and they do not suffer from the same inherent disadvantages as off-line techniques do [cf. 1]. On-line techniques are particular convenient for analysis of thermally labile/intermediary species susceptible to degradation during traditional off-line analysis, e.g., thermal desorption of adsorbent tubes or conventional gas chromatography–mass spectrometry (GC–MS). A few studies of alkene oxidation by ozone (O_3), that is ozonolysis, report that secondary ozonides (SOZ) degrade under these analytical conditions, e.g., the SOZ of D-limonene [2] and 1-tetradecene [3]. SOZ have been characterized by infrared spectroscopy [4–6], GC–MS [2,7] and NMR [8,9]. On-line SOZ formation in real time has been studied during ozonolysis of cyclohexene by atmospheric pressure ionization mass spectrometry (API-MS) [10] and 1-tetradecene by thermal desorption particle beam mass spectrometry (TDPBMS) [3]. SOZ are formed in ozonolysis of alkenes, which produce a vibrationally excited primary ozonide (POZ), which quickly decomposes to yield a carbonyl and an excited carbonyl oxide species, a criegee intermediate (CI). The CI may react further to produce, e.g., OH radicals or become collisionally stabilized (SCI) [11]. One of the reaction pathways of SCI is formation of SOZ by reaction with aldehydes. In the case of endo-cyclic alkenes (i.e., the double bond is located inside the ring) the carbonyl oxide and carbonyl moities are tethered in the same molecule, which provides a unimolecular pathway to formation of SOZ [12]. Ozonolysis of endo-cyclic alkenes will at some critical carbon number switch from a pathway leading to OH radical formation, with high efficiency, to a pathway leading to SOZ formation potentially efficient at the formation of organic aerosols [11].

Development of analytical techniques for analysis of SOZ and other thermally labile oxidation products is important due to their suspected irritating properties in humans and animals [13,14]. Recent research in the field of secondary organic aerosols (SOA) points towards low-volatile SOZ as being

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responsible for new particle formation [3,15,16]; in addition, a few alkene ozonolysis studies have reported SOZ as main products of alkene ozonolysis, when using "soft" analytical techniques [2,3]. However, these products decomposed to aldehydes/ketones and carboxylic acids by thermal desorption and conventional GC–MS analysis. The development of "soft" on-line techniques is therefore important in order to avoid possible artifact formation of thermally labile oxidation products during the analysis.

Atmospheric sampling Townsend discharge ionization mass spectrometry (ASTDI-MS) is a "soft" on-line MS technique operated at a relatively low temperature, which allows differentiation of functional groups, including molar mass assignment of unknowns [17]. Here we report the ASTDI and collision activated dissociation (CAD) mass spectra of four SOZ of endocyclic alkenes.

2. Experimental

2.1. Chemicals

Dry clean air was prepared from compressed outdoor air corresponding to medical grade, which included charcoal filtering and two particle filters: one FF 12/3, which removes 99.9% of particles larger than 0.1 μm, and one sterile filter SRF 24/3. Filters were supplied by Donaldson Ultrafilter, Denmark. Cyclohexene (puriss > 99.5%), 1-methylcyclohexene (puriss > 99%), 4-isopropyl-1-methylcyclohexene (purum > 97%), 4-isopropenyl-1-methylcyclohexene (D-limonene) (purum > 96 %) and α-pinene (puriss > 99.5%) were purchased from Fluka. The quality of limonene was chosen deliberately, because it contains fewer impurities than the puriss quality, cf. [18]. The purity of all compounds 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_2 (>99.999%) and N_2 ($O_2 + H_2O < 2$ ppm) were supplied from Hydro Gas (Norway). Silica gel was from Merck (grade 9385, 230–400 mesh).

2.2. Instruments

The experimental set-up for ASTDI has been described in details elsewhere [17]. In ASTDI a deactivated heated capillary serves to transfer the analytes from the sample site to the Townsend discharge source without chromatographic separation. In this study the sample introduction was modified in order to separate the analyte from the solvent, thus minimizing its influence of the ionization matrix, and thereby the mass spectra.

The SOZ (1–2 μl) dissolved in DCM was injected into a 1 ml vial and placed in a GC oven (Varian CP 3800, CA). The headspace was sampled and pre-concentrated on a 50 cm porabond (Varian CP7351, CA) fused silica column (capillary hereafter) during 2 min at 6 ml min^{-1}, while the temperature increased from 30 to 100 °C at 35 °C min^{-1}. The porabond column was selected, because of its relative insensitivity to molecular oxygen and therefore applicable for air sampling at high temperatures. At 100 °C the vial was removed and the capillary was supplied with clean dry air, prior to elution of the analyte. The air supply is necessary to sustain the ionization matrix of NO^+ and $O_2^{\bullet+}$ during the on-line sampling of reaction mixtures. A supply of *dry* air prevents ionization of the analyte with water clusters, $(H_2O)_nH_3O^+$, and thereby formation of protonated analytes [17]. The capillary was connected to a needle valve, which adjusted the sample flow and thereby the ion source pressure, located inside the GC. A 0.53 mm uncoated/deactivated capillary (Varian ME-05-14-1, CA) (40 cm) connected the needle valve to Townsend discharge source (100 °C) through the GC and transfer line (150 °C). Following, the analyte eluted into the source during a temperature ramp of 5 °C min^{-1} up to 200 °C.

A Varian 1200 L triple quadrupole mass spectrometer was equipped with a Townsend discharge ion source (Varian, CA) operated in positive mode at a discharge voltage of 1600 V. The air matrix was ionized when the Townsend source pressure exceeded ca. 0.36 Torr, after which the ionization efficiency decreased. Moreover, the flow into the Townsend source and the resulting source pressure decreases as the GC oven (and thereby the capillary) temperature increases. In order to sustain ionization, the source pressure was adjusted to 0.4 Torr at the elution temperature of the SOZ. The ASTDI scan range was m/z 90–250 at a scan time of 0.4 s, and m/z 70–250 for cyclohexene and 1-methylcyclohexene. Twenty spectra were averaged. A signal to noise ratio of S/N = 3 was determined by on-line ASTDI sampling of 3 ppb toluene (m/z 92). In ASTDI(Me) the same ion source pressure was mixed with methane up to a source pressure of 5 Torr, which produced abundant quasimolecular ions [17]. CAD mass spectra were obtained from $[M + H]^+$ obtained in ASTDI(Me) using Ar gas at a pressure of 1 mTorr. The standard scan range in ASTDI(Me) was from m/z 10 to 250 at a scan time of 0.4 s.

2.3. Liquid-phase ozonolysis experiments

The secondary ozonides were synthesized by cryo-ozonolysis of 500 μl alkene dissolved in 100 ml pentane in a 200 ml impinger, until a pale blue colour appeared [8]. O_3 was generated photochemically in pure O_2 with a mercury lamp (185 and 254 nm) in a thermo-stated lamp housing controlled by a high performance variable power supply (for details, see [19]). Subsequently, the reaction mixture was purged with N_2 to remove excess O_3 and to reduce the final volume to ca. 2 ml. The ozonides were purified by the use of preparative normal phase liquid chromatography. 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. Solvents for the mobile phase were A: 100% pentane and B: 100% DCM. The reaction mixture (2 ml) was, by loop injection, introduced into the chromatographic system. First, excess alkene eluded (95% A and 5% B), and then the SOZ (100% B) followed by other oxidation products. The separated ozonides, in DCM, were stored at 5 °C prior to analysis.

Table 1
Comparison of ASTDI spectra of secondary ozonides

Ozonide of	M^+	$[M-14]^{+a}$	$[M-O]^+$	$[M-OH]^+$	$[M-HCHO]^+$	$[M-O_2]^+$	$[M-H_2O_2]^+$	$[M-CO_2]^+$	$[M-60]^+$
Cyclohexene	100		14	3		6			34
1-Methylcyclohexene	67	17	6	2	23	32	3	38	100
4-Isopropyl-1-methylcyclohexene	32	11	7	10	50	12		8	100
D-Limonene	4	7	18	33	20	7	49	27	100

Peak abundances less than 2% are not reported.

[a] See text.

3. Results and discussion

An α-pinene SOZ could not be synthesized, in agreement with Griesbaum et al. [8]. ASTDI and ASTDI(Me) spectra of the other SOZ are shown in Figs. 1 and 2.

3.1. ASTDI

The ASTDI ionization matrix is made up of molecular oxygen ions, $O_2^{\bullet+}$ (100% relative abundance (r.a.)) and NO^+ (30% r.a.), which produces a “soft” ionization that leads to molecular and quasi-molecular ions in the ASTDI spectra [17].

ASTDI mass spectral data are presented in Fig. 1 and Table 1. Cyclohexene was chosen as a simple model compound of a terpene with an endo-cyclic double bond. Ozonolysis hereof produced a simple symmetrical SOZ with base-peak M^+, which eliminates O, O_2 and a fragment ion 60 Da less than M^+. These losses are characteristic of all examined SOZ, see Table 1. Daughter-father scans of the latter, revealed that successive loss of $(O + CO_2)$ or (HCHO + HCHO) were the typical mechanisms leading to this fragment ion, which constituted the base-peak, except for the cyclohexene SOZ. Substitution of an H atom with a methyl group resulted in additional fragment ions for the 1-methylcyclohexene ozonide. Loss of 14 Da (Table 1) is unlikely to be formed in one step, which would involve CH_2 elimination from a highly oxidized species. However, adduct formation with NO^+ following elimination of, e.g., CO_2 or acetaldehyde is plausible. For example, $[M + NO–H_2O]^+$ fragments have been reported for acids and esters using NO chemical ionization [20]. Other major fragment ions corresponded to loss of HCHO and CO_2. Substitution of yet another H with an isopropyl group produced the same fragment ions for 4-isopropyl-1-methylcyclohexene ozonide, albeit in different abundances. For example, the neutral losses of OH and HCHO became more important than those of O_2 and CO_2. The mass spectrum of the D-limonene SOZ was expected to resemble that of 4-isopropyl-1-methylcyclohexene, since the exo double bond (located on the isopropenyl group) was the only structural difference. Characteristic losses of O and O_2 are observed as for the other ozonides. M^+ is less abundant, and a $[M + 2]^+$ quasimolecular ion appears in the spectrum, unlike in the other ASTDI SOZ spectra. Other adduct ions encountered in ASTDI (e.g., $[M + NO]^+$) were not observed for the ozonides. A significant loss of H_2O_2 was unique for the SOZ of D-limonene, as similarly reported for its electron impact spectrum [2]. It is noteworthy, that neutral loss of H_2O was not encountered for these highly oxidized species.

The D-limonene SOZ partly degraded on the capillary and transfer line, especially at high temperatures, which was evident from peak-tailing in the chromatogram. Thermal degradation and volatility introduce a special problem in the analysis. However, in this study the same analytical conditions were applied to all SOZ, rather than optimizing the parameters to obtain the highest yield of the D-limonene SOZ.

3.2. Collision activated dissociation of $[M + H]^+$ obtained by ASTDI(Me)

The ionization matrix in ASTDI(Me) is more complex than in ASTDI, with $C_2H_5^+$ present in 100% r.a. Abundant quasimolecular ions are $[M - H]^+$, $[M + H]^+$ and $[M + H_2O]^+$ [17]. However, the protonized quasimolecular ion is more abundant than $[M - H]^+$ for oxidized species other than alcohols, and because the ion current is about an order of magnitude greater than in ASTDI, ASTDI(Me) is used for CAD spectra of $[M + H]^+$. Mass spectral data are presented in Fig. 2 and Table 2.

The CAD mass spectrum of the cyclohexene ozonide $[M + H]^+$ shows losses of H_2O, $2H_2O$, $H_2O + CO$ and $2H_2O + CO$, which implies a molecular composition $C_6H_{10}O_3$. The exact same fragment ions, albeit in different abundances, have been reported in a APCI-CAD spectrum of $[M + H]^+$ of a proposed cyclohexene SOZ [10]. Daughter-father scan of the base-peak at m/z 67, revealed that direct loss of 64 Da (tentatively assigned to hydroxy-methyl-hydroperoxide, HMHP), was

Table 2
Comparison of collision activated dissociation mass spectra of secondary ozonides

Ozonide of	$[M+H]^+$	$[M-OH]^+$	$[M-H_2O]^+$	$[M-2H_2O]^+$	$[M-H_2O_2]^+$	$[M-CO_2]^+$	$[M-HMHP]^+$
Cyclohexene	28	2	2	2		4	100
1-Methylcyclohexene	47	4		4			26
4-Isopropyl-1-methylcyclohexene	95	13		3			50
D-Limonene	100	2	14	2	71		9

Peak abundances less than 2% are not reported.

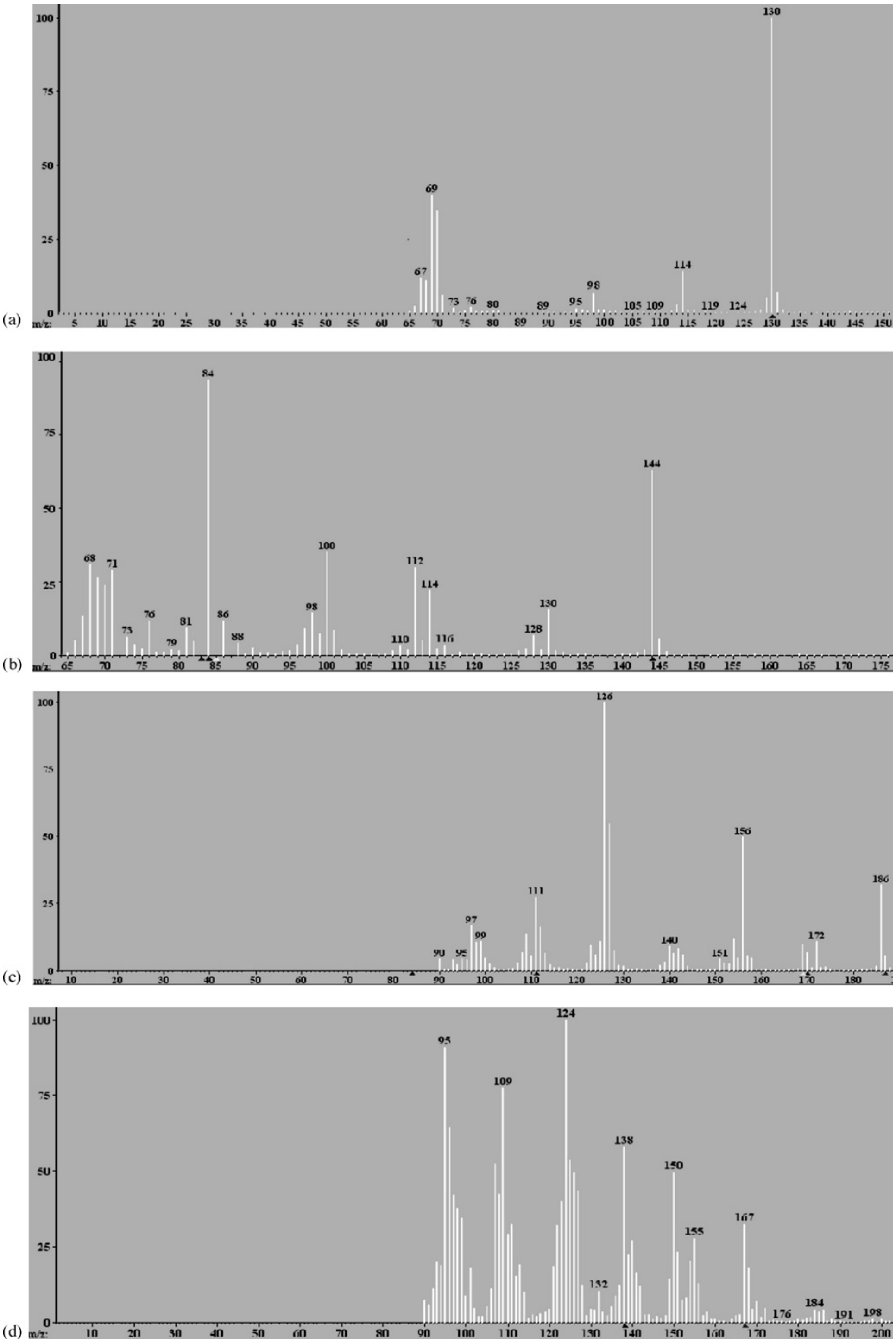

Fig. 1. ASTDI mass spectra of the secondary ozonides of (a) cyclohexene, (b) 1-methyl-cyclohexene, (c) 4-isopropyl-1-methyl-cyclohexene and (d) endo-D-limonene.

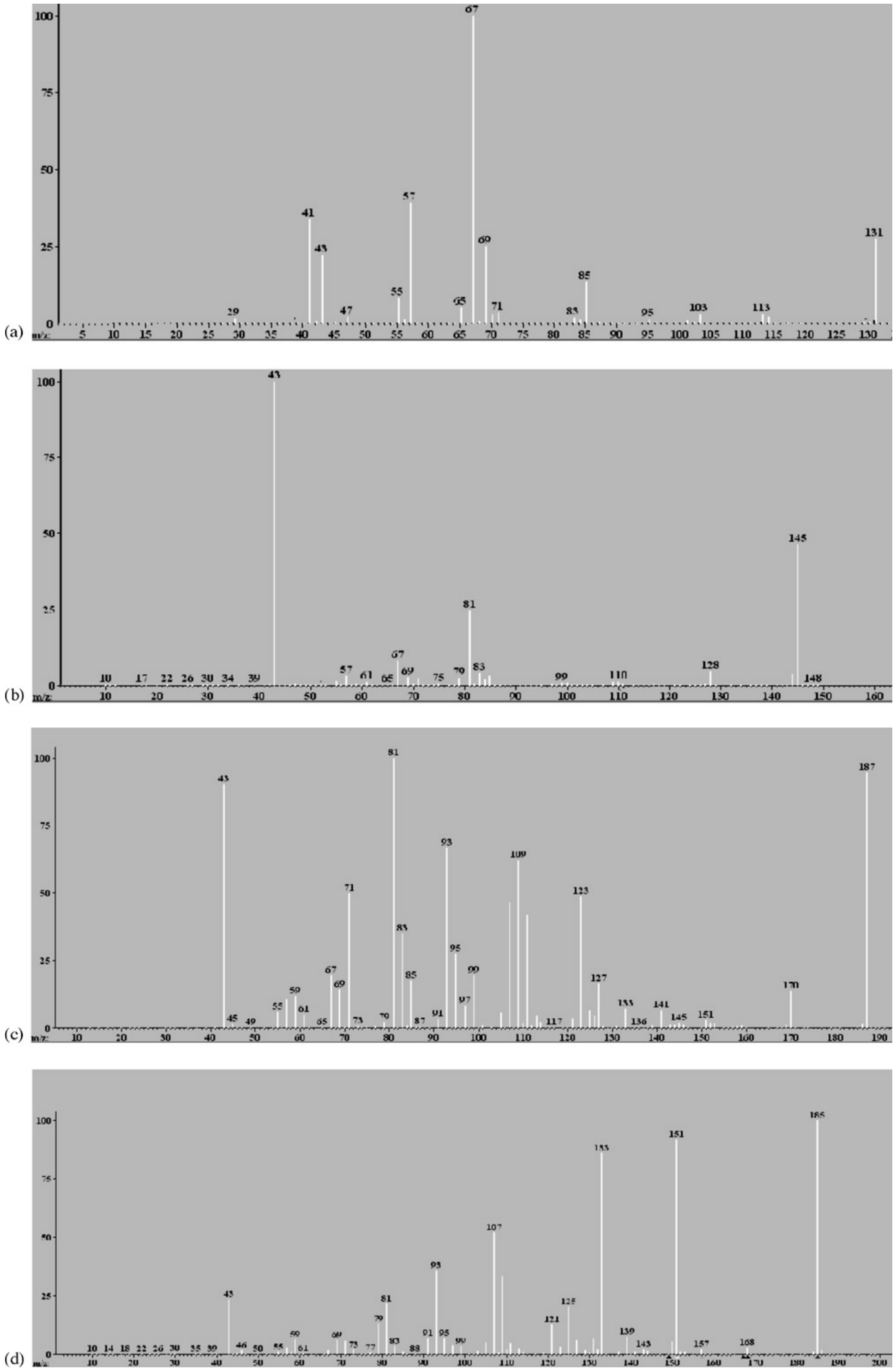

Fig. 2. ASTDI(Me) mass spectra of the secondary ozonides of (a) cyclohexene, (b) 1-methyl-cyclohexene, (c) 4-isopropyl-1-methyl-cyclohexene and (d) endo-D-limonene.

the major contributor to this base-peak, which is evident of the SOZ structure. Indeed, neutral loss of HMHP from $[M+\mathrm{H}]^+$ was observed in all SOZ spectra. In addition, consecutive losses of three O atoms was observed, although loss of the second O was minor in the spectrum of 1-methyl-cyclohexene ozonide. Unlike the other SOZ, that of D-limonene also eliminated H_2O_2.

4. Conclusion

The ASTDI and CAD mass spectra of SOZ of cyclohexene, 1-methylcyclohexene, 4-isopropyl-1-methylcyclohexene, and D-limonene showed characteristic fragment ions, evident of the ozonide structure. In ASTDI, oxygen was eliminated as, e.g., O and O_2, and consecutive losses of two formaldehyde units or $(\mathrm{O}+\mathrm{CO_2})$ corresponded to the base-peak for all SOZ, except for that of cyclohexene. The CAD spectra of $[M+\mathrm{H}]^+$ obtained in ASTDI(Me), showed consecutive losses of three oxygen. Elimination of HMHP was evident of the ozonide structure and particular important for the protonized SOZ, unlike consecutive loss of (HCHO + HCHO) or $(\mathrm{O}+\mathrm{CO_2})$. D-Limonene differed from the other SOZ by unique loss of H_2O_2. These characteristic losses appeared to be useful for identification of SOZ in gas-phase oxidation mixtures of endo-cyclic alkenes, which makes ASTDI an alternative to other on-line techniques for analysis of SOZ in alkene oxidation mixtures.

Studies of terpene oxidation mixtures aiming at the formation of SOZ are currently undertaken.

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