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Studies of complex reactions using modern hyphenated methods: α-Pinene ozonolysis as a model reaction

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This contribution is dedicated to Dr. E.H. Korte on the occasion of his 60th birthday.

Abstract

Modern analytical equipment, in this case the combinations of gas chromatography (GC) with mass spectrometry (MS) and infrared spectroscopy (IR) and liquid chromatography (LC) with mass spectrometry, nuclear magnetic resonance (NMR) spectroscopy and infrared spectroscopy, respectively, have been used to monitor complex reactions that do not only form one or two but a larger number of products. Additionally, side reactions of one primary product with a reactant form a second line of secondary products. To be able to propose formation pathways or even mechanistic interpretation of reactions like these, sophisticated analytical instrumentation is necessary to be able to observe all steps of such a reaction. In this case, the gas phase reaction of α-pinene with ozone has been used as a model reaction. A number of both volatile and low-volatile reaction products could be characterized and formation pathways for a reaction with ozone and OH radicals were proposed.

Keywords: Mass spectrometry; Hyphenated techniques; Infrared spectroscopy; Chromatography

1. Introduction

The tasks of analytical chemistry have become much more complex and difficult in the last decade. One example is the human genome project (HGP), which was largely completed in 2003. Considering that at the beginning a finish was not expected until 2010 [1], the early results are also indicative of the development of analytical techniques.

In comparison, the HGP is not the only problem where analytical chemistry is faced with complex problems. The investigation of proteins in all kinds of living organisms has a strong impact on future therapies of diseases [2] although the identification of proteins, their structure and the working mechanism remains a difficult task. Other complex problems in environmental or combinatorial chemistry need a powerful analytical methodology to be solved [3].

Here, we want to report the use of modern analytical methods for the investigation of complex reactions. The term "complex" is in this case not meant to include coordination compounds, but rather mixtures from reactions that consist of a large number of reaction products. If a chemical reaction of the formula

$$A + B \rightarrow C + D + X_n \quad (1)$$

does not only produce a product C or D but an additional number of products i.e. more than 20, the formation pathways and reaction mechanisms are more difficult to identify. If now, additionally, one reactant reacts with one of the first stage products to produce a second line of reaction products, like shown in Eq. (2), the complexity of the product distribution

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makes identification of mechanisms and formation pathways even more difficult.

$$A + D \rightarrow Y_n \quad (2)$$

The observation of reactions like this in regard to identification of mechanisms and formation pathways is only possible when sophisticated analytical instruments are available.

We have chosen the reaction of α-pinene with ozone as a model reaction. This reaction is of interest since the first reports about the "blue haze" phenomenon observed on sunny days above forests [4]. Monoterpenes are emitted in large quantities from plants and react with atmospheric oxidants (ozone, OH- and NO_3-radicals). The reaction products are considered to undergo a so-called gas-to-particle conversion to become a part of the secondary organic aerosol [5]. Particles in the atmosphere react different than gaseous compounds, because they can absorb or scatter solar radiation, serve as cloud condensation nuclei or be involved in multiphase atmospheric chemistry [6,7]. From these biogenic emissions α-pinene is the most abundant monoterpene.

A number of studies have been carried out recently to observe the reaction and identify reaction products [8–12]. Additionally, we recently reported the identification of different volatile and less volatile reaction products formed in the gas phase ozonolysis of α-pinene [13,14]. The initial step of this reaction is the formation of two so-called Criegee-intermediates **IIIa** and **IIIb** [15] proposed as shown in Fig. 1. For linear and exocyclic alkenes, the carbonyl and biradical functions are not connected and two separate molecules are formed, a carbonyl and a Criegee intermediate. The Criegee intermediates are formed in an excited state [16,17]. The stabilization and decomposition of these intermediates have been the subject of numerous kinetic and product studies of mainly simple alkenes [17–19]. Not many mechanistic studies have been carried out for monoterpenes. At this point, there is no detailed mechanism that is generally accepted. One problem is that a large variety of different oxygenated compounds are formed in gas phase ozonolysis [20]. In some cases, as for α-pinene, not all reaction products have even

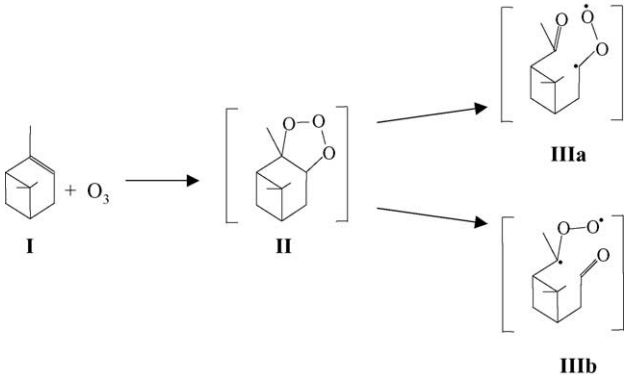


Fig. 1. Reaction pathway of α-pinene oxidation: formation of the molozonid which reacts to the reactive Criegee intermediates **IIIa** and **IIIb**.

been identified [9,11,21,22]. On the basis of a number of studies, Atkinson [17] suggested four general pathways for the reaction of the biradical:

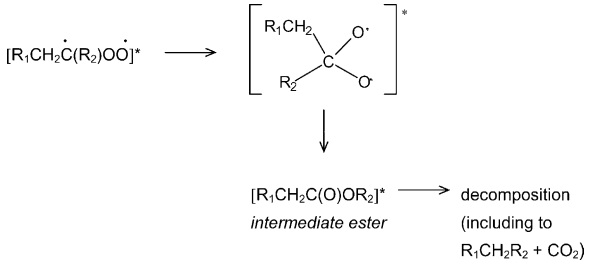
I. Stabilization by collision:

$$[R_1R_2\dot{C}O\dot{O}]^* + M \rightarrow R_1R_2\dot{C}O\dot{O} + M$$

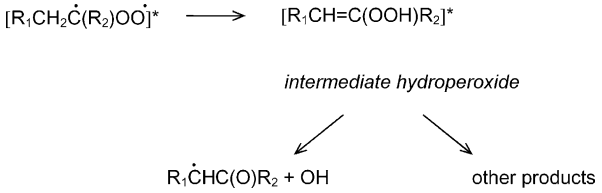
II. O-atom elimination:

$$[R_1CH_2\dot{C}(R_2)O\dot{O}]^* \rightarrow O(^3P) + R_1CH_2C(O)R_2$$

III. Ester channel:



IV. Hydroperoxide channel:



These pathways might give an idea of the complexity of the reaction mechanisms and they are only the first step from the "hot" Criegee intermediate to the product. Especially the ester and the hydroperoxide channels still offer some options for further decomposition.

In regard to the reaction products from α-pinene ozonolysis, just a few compounds have been associated with proposed mechanisms. The reaction pathway leading to the main reaction product pinonaldehyde is ambiguous [16,17,21,23,24], since it is considered to be formed through different pathways. However, stabilization and reaction of the stabilized Criegee radicals with water probably representing the major one. The formation pathway of another major product, nor-pinonaldehyde has been suggested by Jang and Kamens [21] to be formed out of Criegee intermediate **IIIa**. Two of the products that are considered to be formed through the ester channel pathway are (acetyl-2,2,3-trimethyl)cyclobutane and (2,2-dimethyl-3-acetyl-cyclobutyl)methyl-formate. For α-pinene oxide, an epoxide, there have been two different formation pathways suggested. It was reported to be formed from the OH-radical reaction [21] or by direct oxidation of the parent alkene [16,23,25].

Nevertheless, up to now there are only few products for which reaction pathways have been suggested at all.

An important aspect for the understanding of monoterpene ozonolysis is the hydroperoxide channel, which is also used to explain the formation of OH-radicals from alkene ozonolysis. The high molar OH yield in the ozonolysis of α-pinene (0.7–0.85) illustrates the significant contribution of

the hydroperoxide channel to the overall reaction [26–28]. Therefore, if not suppressed, OH-radicals can act as additional reaction partner for both primary and secondary reactions.

For molecules like α-pinene, where further rearrangements are possible [29,30], product studies are often the only way to establish a mechanism or to furnish proof, e.g. for the existence and relevance of the pathways. For this purpose, it is necessary to identify all reaction products and to determine their yields.

Here, studies on volatile and low-volatile reaction products have been carried out using modified and optimized modern analytical instrumentation to characterize products and find indications of formation pathways of this complex reaction.

We have employed the combination of gas chromatography (GC) with infrared spectroscopy (IR) and mass spectrometry (MS), respectively, for volatile products and liquid chromatography (LC) coupled with mass spectrometry, infrared spectroscopy and nuclear magnetic resonance spectroscopy (NMR) and using Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) for low volatile products to get some insights of this reaction. The use of modern analytical technology allows to characterize a large number of products and to suggest a reaction scheme that is based primarily on characterized products.

2. Experimental

The reaction of α-pinene and ozone was carried out in a 10 L glass chamber with a diameter of about 10 cm. It was wrapped in aluminum foil to prevent any influence of daylight radiation [31]. The initial α-pinene concentration was 550 ppbv throughout all the experiments, realized by a test gas generator with a constant diffusion rate of 220 μg/h. The initial ozone concentration was varied between 0 and 1000 ppbv by an ozone generator (Peripheral COM; Anseros, Tübingen, Germany) and analyzed by an ozone analyzer (Ozomat MP; Anseros, Tübingen, Germany) at the entrance to the chamber before and after each experiment. During the experiments with an OH-radical scavenger two uncovered vials filled with *n*-pentane were additionally placed in the test gas generator leading to an *n*-pentane concentration of 650 ppmv. Further details of the experimental set-up and sampling are described elsewhere [13]. For the quantitative experiments, the areas of the detector signal from the flame ionization detection (FID) system were analyzed in order to calculate the yields of the compounds according to Eq. (3):

$$y_{\text{compound}} = \frac{1}{\text{RRF}_{\text{compound}}} \times \frac{a_{\text{compound}}}{a_{\text{api0}} - a_{\text{api1}}} \quad (3)$$

where, y_{compound} = yield of the compound, $\text{RRF}_{\text{compound}}$ = relative response factor of the compound referred to α-pinene, a_{compound} = FID area of the compound, a_{api0} = FID area of α-pinene initially measured without ozone, a_{api1} = FID area of α-pinene.

The values for the RRF were experimentally determined for all compounds where standards were commercially available. For (acetyl-2,2,3-trimethyl)cyclobutane and (2,2-dimethyl-3-acetyl-cyclobutyl)methyl-formate the ECN concept proposed by Scanlon and Willis [32] was used to determine the RRF. Identification of the respective compounds has been done by using GC-FT-IR as well as GC–MS data [13].

For GC-cryocondensation-FTIR measurements a Bio Rad FTS-60-A interferometer equipped with a Tracer-Unit (Digilab Division, Krefeld, Germany) and a Fisons gas chromatograph series 8060 (Mainz, Germany) were used. Both columns are attached to a pneumatically actuated four-way-valve (Valco, GAT Analysentechnik, Berlin), located inside the GC oven, which allows to use the FID system as well as the Tracer as detection devices for both columns. GC–MS experiments were carried out with a Dani 6500 gas chromatograph coupled to a Finnigan MAT ITD 700 ion trap mass spectrometer (Finnigan MAT, Bremen, Germany).

Samples for the volatile products were taken at the end of the reaction chamber on adsorption tubes containing Tenax TA material, which has been investigated in detail elsewhere [31].

The particulate reaction products were sampled at the outlet of the chamber on boron silicate filter (GF6, diameter 55 mm, thickness 0.35 mm, pore size 0.5–1.5 μm) (Schleicher und Schüll, Dassel, Germany) using a laboratory-built holder made of Teflon. The products were sampled with a flow rate of 65 L/h for 8 h. In the experiment 6 ppm α-pinene were reacted with 5 ppm ozone. The filter was extracted after sampling with a Soxhlett extractor in 50 mL methanol for 24 h and the resulting solution was concentrated to about 0.5 mL. This solution was reduced to 0.2 mL and in the final step 0.3 mL water were added. The high concentrations were necessary to obtain enough material for NMR analysis.

2.1. LC-NMR-MS analysis

The LC system consisted of a Bruker LC22 quaternary low-pressure gradient pump and a Bruker diode array detector (Bruker Biospin, Rheinstetten, Germany). The separation was carried out using a standard C_{18} column (250 mm × 4.6 mm, 5 μm).

Eluent composition: A: 0.3 ml of 95% formic acid in 500 ml of ^2H_2O (99.8%) (Deutero GmbH, Kastellaun, Germany); B: acetonitril-d3 (99.5%). The gradient was: starting conditions: 100% A; after 5 min: 85% A 15% B; after 30 min: 40% A 60% B; after 35 min: 100% B.

The used interface was a commercially available Bruker Peak Sampling Unit (BPSU-36—Bruker Biospin, Rheinstetten, Germany). The NMR-spectra were recorded on a Bruker DRX 500-spectrometer, at 500.13 MHz, equipped with a ^1H–^{13}C inverse flow probe (o.d. = 4 mm). MS data were recorded on a Bruker Esquire-LC Ion Trap mass spec-

trometer using electrospray ionization (ESI) (Bruker Daltonics, Bremen, Germany).

LC-IR measurements were carried out using a laboratory-built interface, which has been described in detail elsewhere [33].

2.2. LC–MS

A Bruker Esquire 3000 Ion Trap MS was coupled with a WellChrom Maxi-Star K-1001 gradient system HPLC (Knauer Berlin, Germany). Separations were carried out using a 1 mm standard C_{18} column at a flow rate of 10 μl/min. Data were obtained in the negative mode using ESI–MS.

High-resolution mass spectrometry experiments were carried out using a Bruker APEX III Fourier-transform ion cyclotron resonance mass spectrometer (Bruker Daltonics, Bremen, Germany) with a 7 T actively shielded super conducting magnet. The instrument was equipped with an Agilent ESI source and measurements were performed using infusion of a liquid sample with a flow rate of 2 μl/min in methanol/water (1:1, v/v). For a better signal-to-noise ratio up to 100 scans were added using a size of 512,000 data points.

3. Results and discussion

The reaction products from the α-pinene/ozone reaction can be separated into two classes: volatiles and low-volatiles. This categorization is based on the volatility of each compound. If they are volatile enough to evaporate quantitatively in a gas chromatograph they belong to the volatile fraction. The low-volatile products were analyzed using liquid chromatography.

The results of the gas phase reaction showed a number of products that have been formed. The combination of infrared and mass spectrometric detection clearly allows the characterization of a large number of products, which has been reported elsewhere [13].

One of the main interests was to determine which of the products are primary products or if they react further to form secondary products. Mechanistic and kinetic studies for such complex reactions are challenging. However, experiments on the influence of different ozone concentrations and OH radicals on the product distribution are feasible and can provide valuable information on the formation pathways of individual products as can be seen in the diagrams of Fig. 2 and Table 1.

The evaluation of the results gave indications for the occurrence of secondary processes, i.e. the formation of

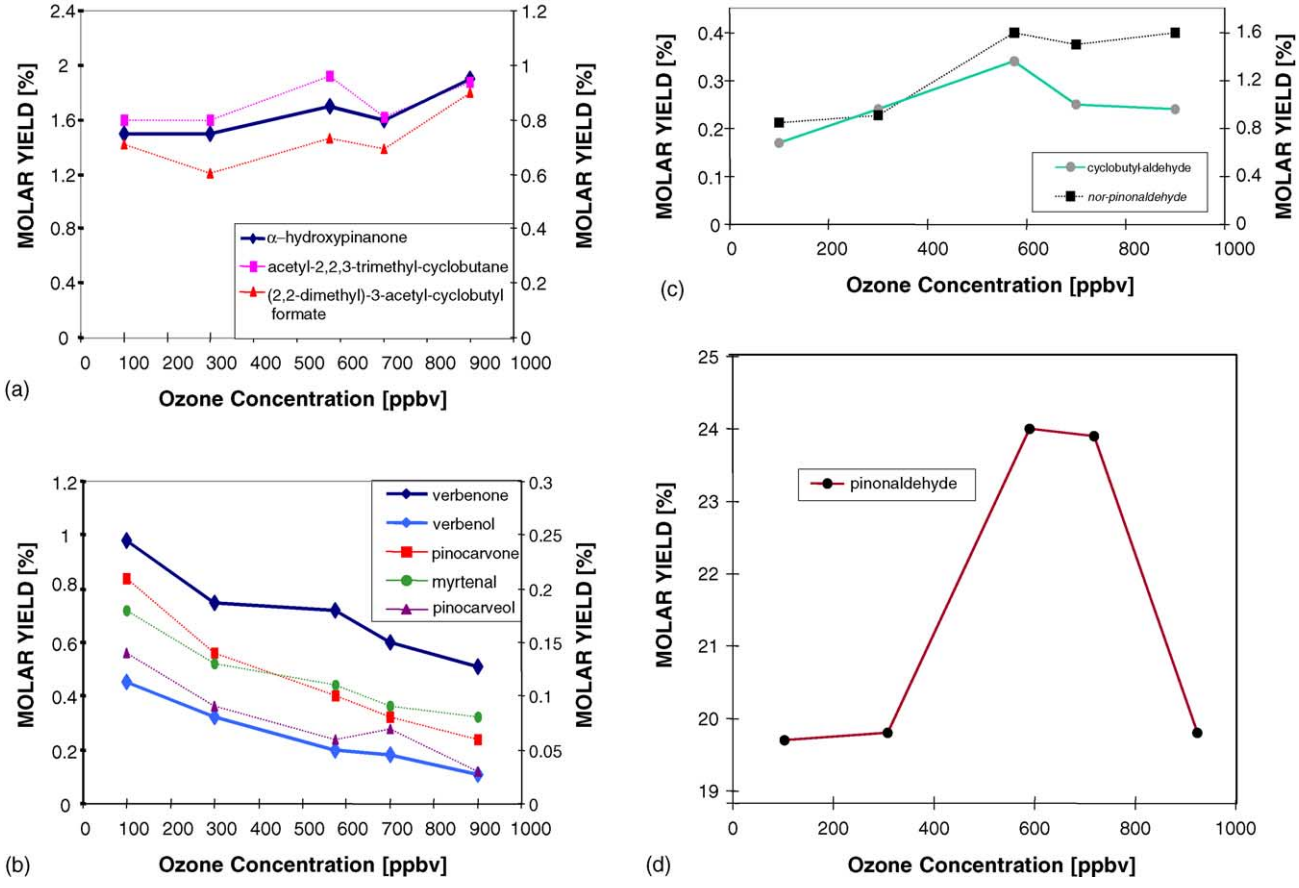
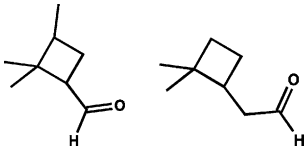
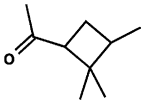
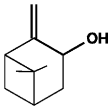
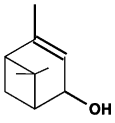
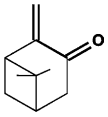
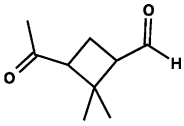
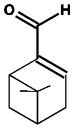
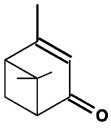
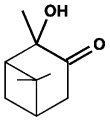
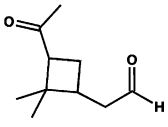
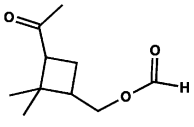


Fig. 2. Molar yields of the reaction products of the α-pinene ozonolysis as a function of the ozone concentration. (broad lines on left scale, pointed lines at right scale). (The error for measurements at one specific ozone concentration has been found to be less than 10% for all data).

second-generation products from the degradation of first generation products. A constant yield over the whole ozone range is expected for products that are formed directly in the α-pinene/ozone reaction and that are inert towards further reaction with the oxidant. This is the case for the compounds displayed in Fig. 2a. For (acetyl-2,2,3-trimethyl)cyclobutane, α-hydroxypinanone and (2,2-dimethyl-3-acetylcyclobutyl)methyl formate, the yields

Table 1
Molar yields of the reaction products obtained at different ozone concentrations

Peak no.	Compound	Structure	RRF	Yield %					Fig. 2
				100[a]	300[a]	580[a]	700[a]	900[a]	
1	a: (2,2,3-Trimethyl-cyclobutyl)-formaldehyde; b: (2,2-Cyclobutyl)-acetaldehyde		0.76	0.17	0.24	0.34	0.25	0.24	(c)
2	(Acetyl-2,2,3-trimethyl)-cyclobutane		0.78	0.80	0.80	0.96	0.81	0.94	(a)
5	*trans*-Pinocarveol		0.89	0.14	0.09	0.06	0.07	0.03	(b)
6	*trans*-Verbenol		0.89	0.45	0.32	0.20	0.18	0.11	(b)
7	Pinocarvone		0.81	0.21	0.14	0.10	0.08	0.06	(b)
8	Nor-pinonaldehyde		0.62	0.85	0.91	1.6	1.5	1.6	(c)
9	Myrtenal		0.81	0.18	0.13	0.11	0.09	0.08	(b)
10	Verbenone		0.81	0.98	0.75	0.72	0.60	0.51	(b)
11	α-Hydroxy-pinanone		0.67	1.5	1.5	1.7	1.6	1.9	(a)
12	Pinonaldehyde		0.65	19.7	19.8	24.0	23.9	19.8	(d)
13	(2,2-Dimethyl-3-acetyl-cyclobutyl)methyl formate		0.52	0.71	0.60	0.73	0.69	0.90	(a)

[a] Ozone concentration (ppbv).

show only minor fluctuations. Since the three reaction products are all saturated compounds, this kind of behavior is expected. However, for the compounds shown in diagram 2b, the individual product yields show a continuous decrease with an increasing ozone concentration. This kind of behavior is expected when secondary reactions of first generation products are occurring. Since all compounds displayed in diagram 2b contain a double bond, the experimental observation displayed in the diagram is readily explained by further oxidation reactions.

From the curves of the two compounds in diagram 2c, it can be concluded that different processes influence the yields. For both compounds in the beginning the yields increase with increasing ozone concentrations. This should be the case for compounds that are secondarily formed. It is difficult to determine from the data, whether these compounds are exclusively formed in a secondary process. Since especially for nor-pinonaldehyde the yield at 100 ppb is already rather high, it is more likely that this compound can also be formed directly, showing formation as both a primary as well as a secondary product. The fact that for cyclobutylaldehyde at high ozone concentration the yield decreases again indicates that an additional process takes place, which can be explained, as discussed above, by oxidation of the compound.

The main reaction products pinonaldehyde is a prime example to be involved in secondary reactions after formation. This curve in Fig. 2d has a tendency that shows the influence of more than one parameter. Here, not only the concentration of ozone plays a significant role in reacting further with other reactants. A reduced yield of pinonaldehyde at higher ozone concentrations can be caused by reactions of pinonaldehyde with OH radicals. Considering all data, it seems that more parameter are involved in the formation of pinonaldehyde. This has been shown before [34,35] were additional influence of water was found to play a significant role in the formation mechanism.

The fact that OH-radicals are being formed in the reaction of α-pinene with ozone is making things more complicated. The products found and described above can thus result from the α-pinene/ozone as well as from the α-pinene/OH-radical reaction. To distinguish between these two reactions an OH-radical scavenger, *n*-pentane, was added in excess. Due to the high pentane concentration most of the formed OH radicals will react with the scavenger molecules. Estimates suggest that the concentration of *n*-pentane used for this experiments should scavenge about 99% of OH radicals.

Therefore, the products found in these experiments can be considered to be almost exclusively formed in the reaction of α-pinene with ozone.

Fig. 3 shows the GC-FT-IR functional group chromatograms of samples taken during an experiment without (top trace) and with *n*-pentane (bottom trace) as scavenger. The comparison suggests that the six compounds not detected in the presence of the scavenger are formed from reaction with OH-radicals.

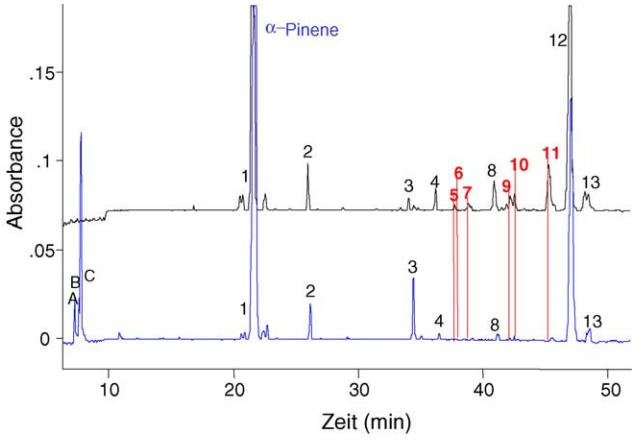


Fig. 3. GC-FT-IR functional group chromatograms of the CH-bands between 3000 and 2750 cm^{-1}; Compounds A, B and C were identified as 2-pentanone, 3-pentanone, and a signal containing both 2-pentanol and 3-pentanol, respectively; compounds 1 either a: (2,2,3-trimethylcyclobutyl) formaldehyde or b: (2,2-cyclobutyl) acetaldehyde, 2 (acetyl-2,2,3-trimethyl)cyclobutane, 3 α-pinene oxide, 4 campholene aldehyde, 8 nor-pinonaldehyde, 12 pinonaldehyde and 13 (2,2-dimethyl-3-acetyl-cyclobutyl)methyl-formate are major products formed from reaction of α-pinene with ozone, while compounds 5 *trans*-pinocarveol, 6 *trans*-verbenol, 7 pinocarvone, 9 myrtenal, 10 verbenone, 11 α-hydroxypinanone are formed by OH-radical reaction. It has to be noted that the intensities of 2 and 3 can vary, because 2 is thermally labile and decomposes to 3 in the injector of the GC depending on the injection parameters.

Additionally, four compounds were found in the reaction with *n*-pentane that were identified as 2-pentanone (A), 3-pentanone (B), and 2-pentanol and 3-pentanol (C) (not separated, occurring together in one signal) [36,37]. These are the main compounds reported in the reaction of *n*-pentane with OH radicals [38]. Their formation in the reaction chamber can therefore be taken as experimental evidence of the presence of OH radicals.

The compounds exclusively formed in the α-pinene/OH-radical reaction are *trans*-pinocarveol, *trans*-verbenol, pinocarvone, myrtenal, verbenone, α-hydroxypinanone. Among these six compounds, α-hydroxypinanone has so far not been reported to be formed in gas phase oxidation of biogenic VOCs or to be present in plant emissions. This result has an important practical aspect, since α-hydroxypinanone might be used in future field studies as a tracer compound for the occurrence of α-pinene/OH-radical reactions. To our knowledge, a distinction between α-pinene/ozone and α-pinene/OH-radical reaction from the measurement of the product distribution was not realized up to date.

Two principal mechanisms for the reaction of α-pinene with OH radicals are suggested and illustrated in Fig. 4: H-atom abstraction leading to the formation of water and an allylic radical and, secondly, addition of the OH radical to the double bond leading to the formation of β-hydroxyalkyl radicals. Addition to the double bond is considered to represent the dominant reaction pathway [17,20]. This is confirmed in this study. Nonetheless, there are five products, *trans*-pinocarveol, *trans*-verbenol, pinocarvone, myrtenal and ver-

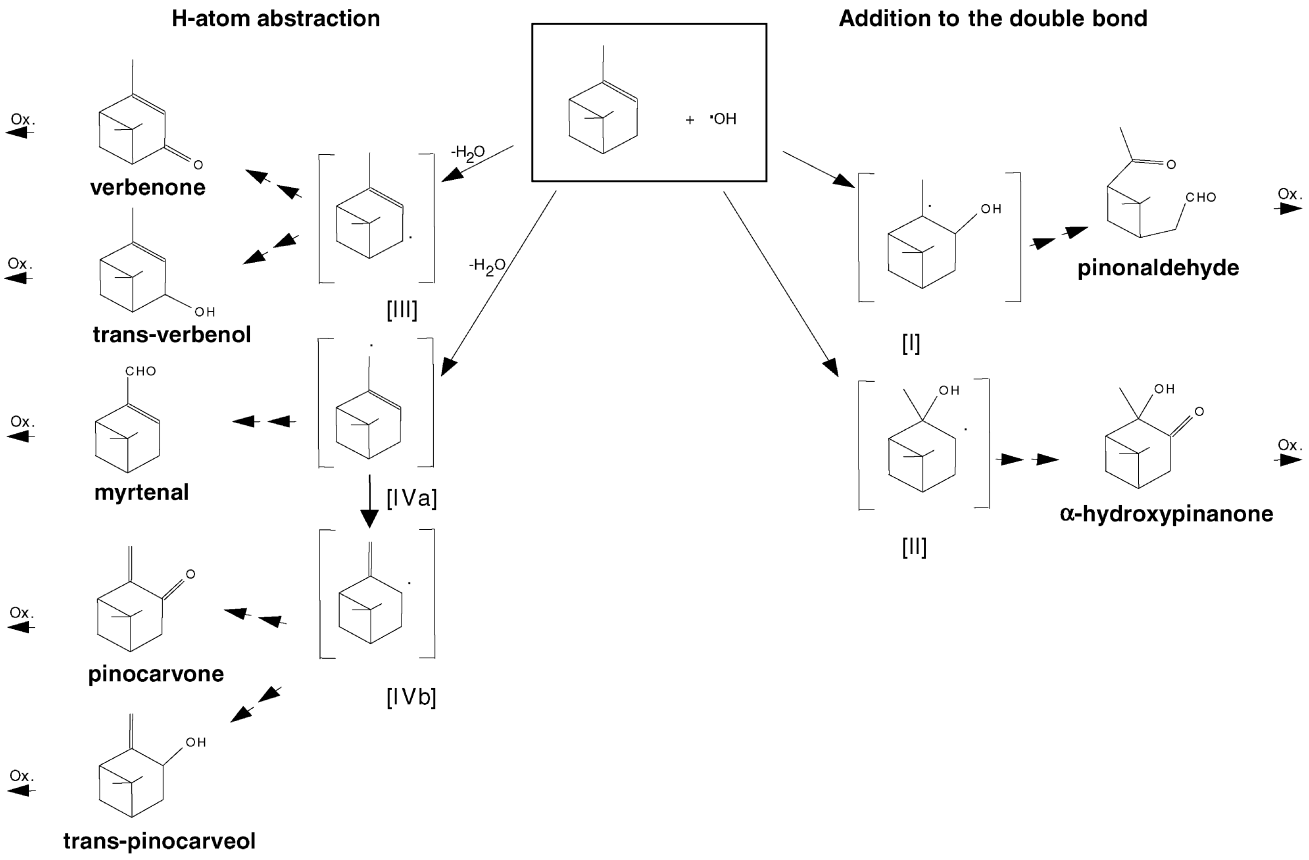


Fig. 4. Proposed reaction pathway for the reaction of α-pinene with OH-radicals. Yields were calculated by assuming an average OH-radical yield of 80% in the α-pinene/ozone reaction and that all OH radicals react with α-pinene [39].

benone, whose formation can best be explained by the H-atom abstraction mechanism. The five compounds formed via H-atom abstraction still contain a double bond and are therefore subject to further oxidation by ozone and OH radicals. Since the initial yield, especially of verbenone, is still rather high for a minor compound, it is a prime example for a compound that can undergo secondary reactions.

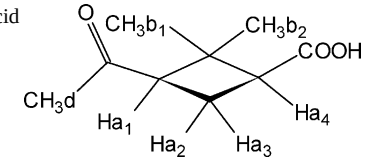
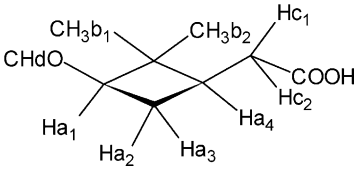
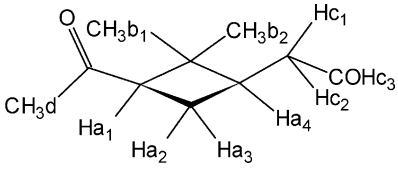
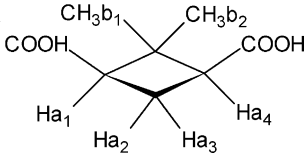
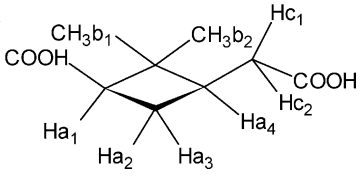
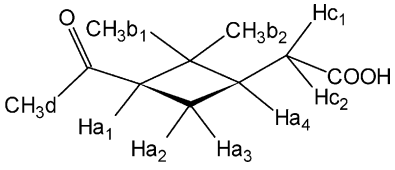
The addition of OH radicals to the double bond can lead to the formation of two different β-hydroxyalkylradicals. While α-hydroxypinanone can be formed via the intermediate radical **II** [6,15], reaction pathways via the intermediate radical **I** have been suggested for the formation of pinonaldehyde [40,41].

The data from this study indicate that the reaction of OH radicals with monoterpenes, in this case α-pinene, lead to a number of products for which reactions pathways could be suggested. They are in agreement with theoretical studies reported recently by Peters et al. [42].

The higher oxidized and therefore, less volatile reaction compounds were analyzed using methods implementing liquid chromatography as a separation technique. The good results obtained from GC-IR studies lead to the development of an interface between liquid chromatography and infrared spectroscopy [33]. The successful testing of the LC-IR combination made it possible to analyze the low-volatile fraction of the reaction products, which has not been shown before, because usually the kind of deposition interface used for LC-IR has been employed only for very low volatile compounds like polymers. Here, even though the low volatile reaction compounds from α-pinene ozonolysis are less volatile than the precursors, they are much more volatile than polymers. Unfortunately, the results from the reaction products revealed that infrared spectroscopy is more suited for the identification of the volatile fraction because there are more differences in the molecular structure to distinguish the reaction products. The higher oxidized compounds all contain a carboxylic group within the molecule, and to distinguish a C_9- from a C_{10}-molecule infrared spectroscopy is not the method of choice. Here NMR spectroscopy and mass spectrometry give more information and allow to characterize a number of products. Nonetheless, the strength from IR is to distinguish structural elements that allowed characterization of certain compounds. Molecules containing functional groups like hydroxyl, ether or dimer groups could be characterized.

The low volatile products that have been characterized during this study correspond to the same basic structure. As shown in Fig. 1, the ozonolysis is carried out by addition of ozone to the double bond of α-pinene. The first step is the formation of the molozonid **II** that forms reactive Criegee intermediates **IIIa** and **IIIb**. The oxidation of the two side chains lead to the formation of several products that have the cyclobutyl ring as a basic structural element.

Table 2
^{1}H NMR-data of compounds found in the filter extract

Molecule	Proton(s)	Chemical shift
cis-Norpinonic acid 	Hd	3.100 (dd, 1H)
	Ha_4	2.886 (dd, 1H)
	Ha_2	2.352 (q, 1H)
	Ha_1	2.090 (s, 3H)[a]
	Ha_3	1.852 (qd, 1H)
	Hb_1	1.411 (s, 3H)
	Hb_2	0.868 (s, 3H)
cis-Pinornalic acid 	Hd	9.631 (s, 1H)
	Ha_1	2.817 (t, 1H)
	Hc_1	2.575 (dd, 1H)
	Hc_2	2.476 (dd, 1H)
	Ha_4	2.408 (m, 1H)
	Ha_2	Possibly obscured
	Ha_3	1.810 (dd, 1H)
	Hb_1	1.193 (s, 3H)
	Hb_2	0.917 (s, 3H)
cis-Pinonaldehyde 	Hc_3	9.631 (s, 1H)
	Ha_1	3.004 (t, 1H)
	Hc_1	2.497 (dd, 1H)
	Hc_2, Ha_4	2.349–2.490 (m, 2H)
	Hd	2.092 (s, 3H)[a]
	Ha_2, Ha_3	1.816–1.877 (m, 2H)
	Hb_1	1.262 (s, 3H)
	Hb_2	0.774 (s, 3H)
cis-Norpinic acid 	Ha_1, Ha_4	2.863 (dd, 2H)
	Ha_2	2.304 (q, 1H)
	Ha_3	1.981 (q, 1H)[a]
	Hb_1	1.291 (s, 3H)
	Hb_2	0.964 (s, 3H)
cis-Pinic acid 	Ha_1	2.769 (dd, 1H)
	Ha_4, Hc_1, Hc_2	2.246–2.401 (m, 3H)
	Ha_3	2.018–2.069 (m, 1H)[a]
	Ha_2	1.794 (q, 1H)
	Hb_1	1.167 (s, 3H)
	Hb_2	0.913 (s, 3H)
cis-Pinonic acid 	Ha_1	2.990 (dd, 1H)
	Ha_4, Hc_1, Hc_2	2.187–2.348 (m, 3H)
	Hd	2.032 (s, 3H)[a]
	Ha_2, Ha_3	1.782–1.905 (m, 2H)
	Hb_1	1.266 (s, 3H)
	Hb_2	0.797 (s, 3H)

[a] Partly obscured by signal of acetonitrile.

NMR spectroscopy allows directly to distinguish *cis* and *trans* isomers as the Hb_1 and Hb_2 singlet signals from the protons of the methyl groups of *cis*-pinic acid standard compound show a large chemical shift difference, while the difference in the *trans*-isomer is rather small (see Table 2).

Since the products from the gas phase reaction are rather similar in structure, it reduces the chromatographic separation and did not allow to fully distinguish all compounds. Often the NMR spectra showed a mixture of two or three reaction products. Parallel to the LC-NMR results MS data were recorded that made it easier to differentiate the NMR data. One example is shown in Fig. 5a. Here, the ^{1}H and TOCSY NMR can be compared directly with the MS data from the ion trap. For this setup, the Bruker peak sampling unit allows to split the effluent flow to be diverted into two different streams. The main flow is directed to the flow cell of the NMR spectrometer, where it can be used in the on-flow mode, where the effluent is continuously flowing through the cell, or the stop-flow mode, where the chromatographic flow is stopped while the analyte is located inside of the flow cell. Additionally, the desired compounds can be sampled in a fractionation sampler for further analysis.

The second part of the chromatographic flow, about 5%, is being connected to the ESI source of an ion trap mass spectrometer. When the chromatographic signal is wide enough, it is possible to implement collision activated dissociation

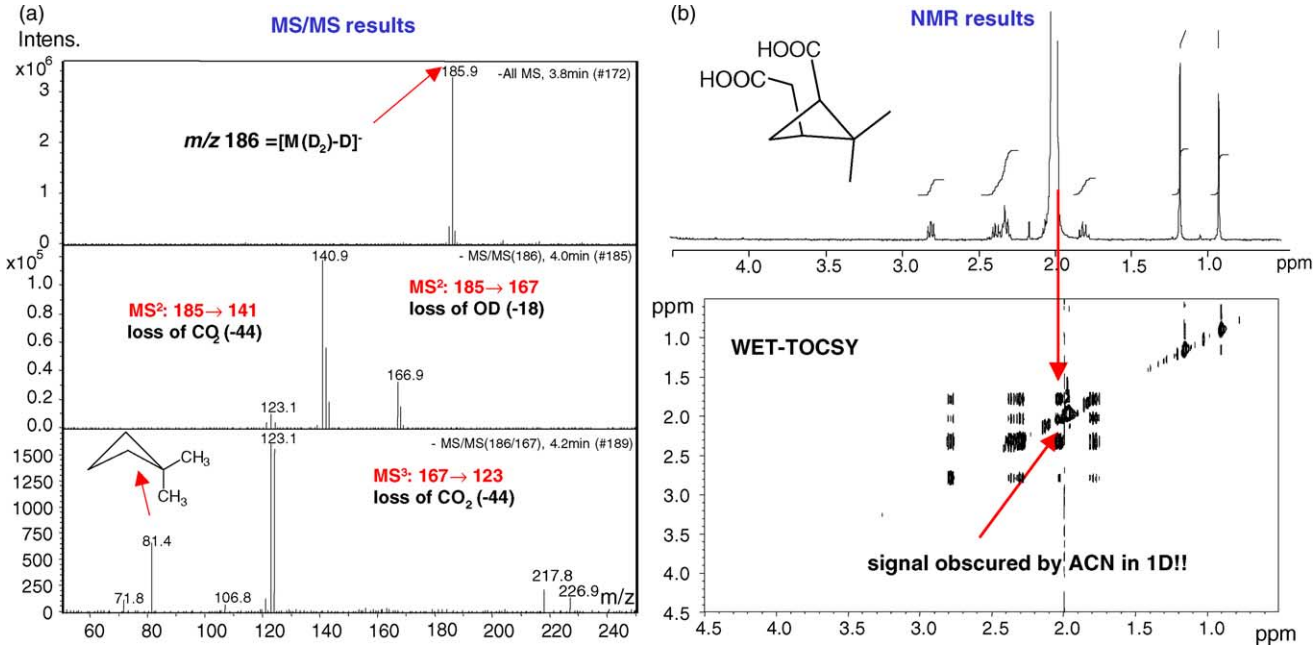


Fig. 5. LC-NMR spectrum of pinic acid, NMR results include WET TOCSY 2D spectrum; MS spectra after CAD from pinic acid; $M = 186$; $[M(D1) - D]^- = 185$; online LC-NMR-MS experiment, showing MS^3 in negative ion mode; proposed fragmentation as shown.

(CAD) studies. These experiments with up to MS5 were possible with online LC-separation. The results of the reaction product pinic acid in Fig. 5b shows the fragmentation pattern from ESI-MS while the chemical shifts from the NMR can be correlated. Not all compounds gave clear and undistinguished NMR data as pinic acid because some of the signals were obscured by the large signal from the mobile phase.

The use of FT-ICR-MS allows to characterize the compounds from a very complex reaction even without chromatographic separation. The high resolution makes it possible to distinguish the different products. In addition to the data from standard MS method, FT-ICR-MS allows to differentiate compounds that appear at one nominal mass as can be seen in Fig. 6. At some nominal masses not only one signal appears, but multiple signals differing by 0.036 Da, which is exactly the difference between CH_4 and an oxygen atom. The high accuracy in combination with the high resolution from the FT-ICR-MS makes it possible to characterize the

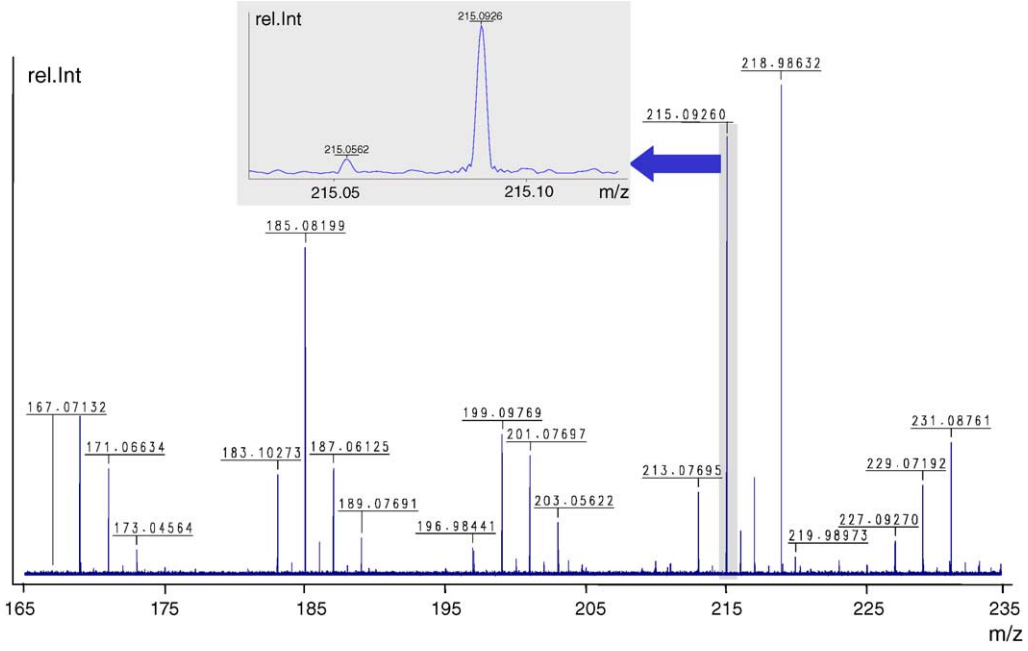


Fig. 6. FT-ICR-MS spectrum of reaction products; obtained after adding 32 scans with 512,000 data points.

Table 3
Compilation of gas-phase ozonolysis products from α-pinene and their formula composition (elemental composition was calculated from $[M-H]^-$-ion)

$[M-H]^-$	Accurate mass	Corresponding formula	Accuracy (ppm)
m/z 167	167.0714	$C_9H_{11}O_3$	3.5
m/z 169	169.08704	$C_9H_{13}O_3$	0.2
m/z 171	171.06628	$C_8H_{11}O_4$	0.04
m/z 173	173.08219	$C_8H_{13}O_4$	1.5
	173.04564	$C_7H_9O_5$	0.5
m/z 183	183.10273	$C_{10}H_{15}O_3$	0.36
	183.06626	$C_9H_{11}O_4$	0.1
m/z 185	185.08198	$C_9H_{13}O_4$	0.3
	185.04559	$C_8H_9O_5$	0.25
m/z 187	187.06125	$C_8H_{11}O_5$	0.3
	187.09760	$C_9H_{15}O_4$	0.11
m/z 189	189.0768	$C_8H_{13}O_5$	0.3
	189.04053	$C_7H_9O_6$	0.4
m/z 197	197.08204	$C_{10}H_{13}O_4$	0.5
	197.04583	$C_9H_9O_5$	1.4
m/z 199	199.06132	$C_9H_{11}O_5$	0.65
	199.09769	$C_{10}H_{15}O_4$	0.5
m/z 201	201.07697	$C_9H_{13}O_5$	0.6
	201.11330	$C_{10}H_{17}O_4$	0.3
	201.04064	$C_7H_9O_6$	0.9
m/z 203	203.05589	$C_8H_{11}O_6$	1.1
	203.09235	$C_9H_{15}O_5$	0.75
m/z 213	213.07949	$C_{10}H_{13}O_5$	0.5
	213.11332	$C_{11}H_{17}O_4$	
m/z 215	215.05623	$C_9H_{11}O_6$	0.56
	215.09260	$C_{10}H_{15}O_5$	0.47
m/z 217	217.07186	$C_9H_{13}O_6$	0.5
	217.10814	$C_{10}H_{17}O_5$	0.9
m/z 229	229.07192	$C_{10}H_{13}O_6$	0.7
m/z 231	231.08737	$C_{10}H_{15}O_6$	0.4
m/z 247	247.08267	$C_{10}H_{15}O_7$	1.4

different compounds and calculate the elemental composition. The insert in Fig. 6 shows the signals at *m/z* 215. The two signals are easily distinguished using FT-ICR-MS while with standard MS just one nominal peak appears. The list of the signals is summarized in Table 3.

One example of how the data can help to determine a formation pathway is illustrated in Fig. 7, which combines the MS/MS as well as the IR spectrum of one compound at *m/z* 215.

The main fragment ion with *m/z* = 157 shows a mass difference of 58 compared to the parent ion. A neutral compound with a molecular mass of 58 can have the empirical formula $C_2H_2O_2$. The loss of $C_2H_2O_2$ can be explained by α-cleavage at the carbonyl group, which has been reported for esters of secondary alcohols, and proton transfer from the hydroxyl group [43]. The determination of the exact mass using the FT-ICR-MS allowed to calculate the elemental composition $C_{10}H_{15}O_5$ of the ion at 215.09260 with an accuracy of 0.47 ppm.

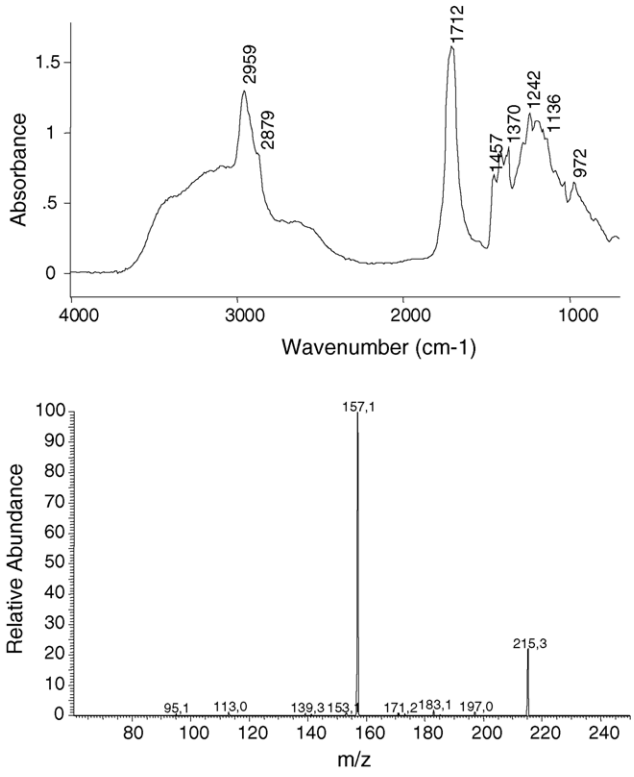


Fig. 7. IR- (above) and MS–MS (below) spectra of compound [hydroxyacetic acid-((2,2-diemethyl)cyclobutyl) ester]acetic acid; see text for details.

Additionally, IR bands at 1457, 3400 and 1712 cm^{-1} indicate a formate ester group, a hydroxy and a carboxylic group, respectively. All of this data lead to the proposal of a molecular structure of [hydroxyacetic acid-((2,2-dimethyl)-cyclobutyl)-ester]-acetic acid and allows to suggest a formation pathway. Assuming decomposition of Criegee intermediate **IIIb**, according to the ester channel, formation of a compound with the proposed structure in the α-pinene/zone reaction seems possible, as illustrated in Fig. 8.

All analytical data combined allow to propose a reaction scheme that is based on the characterization of reaction products. The determination of the products resulting from the OH-radical reaction make the scheme a little less complex,

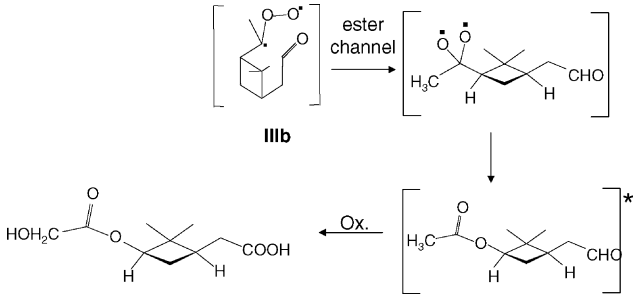


Fig. 8. Proposed reaction scheme leading to a tentatively identified ozonolysis product.

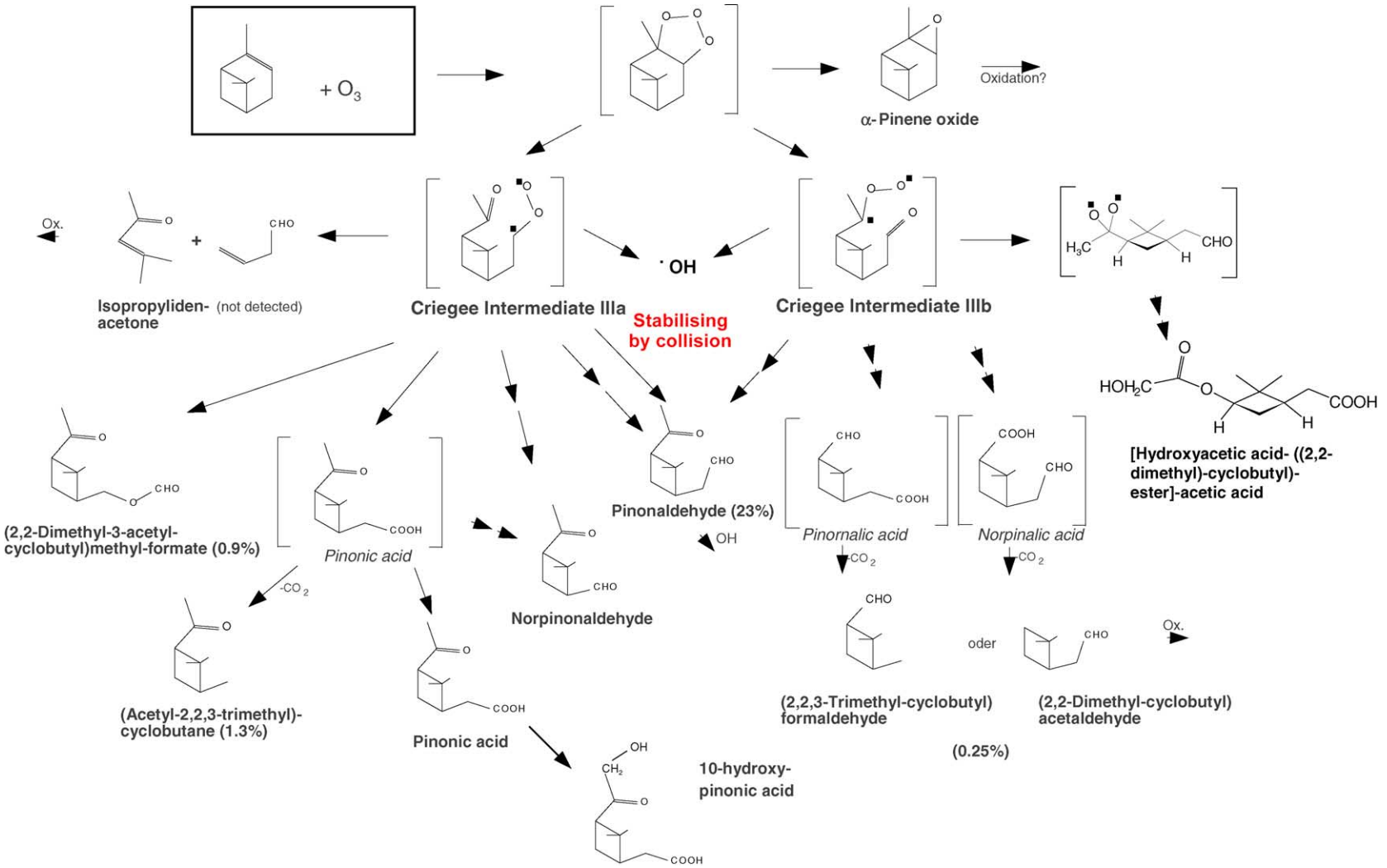


Fig. 9. Reaction scheme that combines all products studied during this investigation and that are based on analytical data.

but nonetheless, without the implementation of different analytical techniques this study would not have been possible. Here, we have one set of tools available that allows to distinguish formation pathways of very complex reactions and characterizing even minor products of the reaction. The proposed scheme is shown in Fig. 9 and displays only reaction products that were detected during this study. It has to be noted that not all compounds can be observed properly using this set of analytical equipment. One exception is α-pinene oxide, which is thermally unstable. It partially decomposes in the injector of the GC to campholene aldehyde and pinocamphone. A quantitative determination of this product is therefore not possible. On the other side it is too volatile to be analyzed by electrospray MS.

4. Conclusion

The main goal of this study was the development and application of analytical tools for product studies of very complex reactions. One focus was placed on the identification of reaction products to identify individual oxidation pathways. The concentrations were high for some experiments to directly correlate them to atmospheric conditions and therefore, the yields given above have to be confirmed by experiments in "large scale" reaction chambers. Nonetheless, it was possible to give insights of a very complex gas phase reaction. Additionally, with α-hydroxypinanone as a relatively long-living compound exclusively formed following the OH-radical addition to α-pinene, a potential marker compound for the distinction between ozone and OH-radical reaction for field studies has been identified. At the same time (acetyl-2,2,3-trimethyl)cyclobutane and (2,2-dimethyl-3-acetyl-cyclobutyl)methyl-formate could be used as marker compounds for the ozone reaction.

In general, the use of modern hyphenated techniques allows an insight even of complex reactions like monoterpene ozonolysis. The next step is to utilize these tools on the determination of mechanisms leading to the secondary organic aerosol.

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