ELSEVIER

Journal of Chromatography A, 833 (1999) 121–135

JOURNAL OF CHROMATOGRAPHY A

Determination of polar terpene oxidation products in aerosols by liquid chromatography–ion trap mass spectrometry

Marianne Glasius, Matthew Duane, Bo R. Larsen*

Environment Institute, EC Joint Research Centre, I-21020, Ispra (VA), Italy

Received 1 October 1998; received in revised form 30 November 1998; accepted 2 December 1998

Abstract

A new method for the determination of polar terpene oxidation products in secondary aerosols is described. It is based on collection of particles on PTFE filters and extraction with dichloromethane followed by analysis with liquid chromatography–ion-trap mass spectrometry (MS^n) using pneumatically assisted electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) with the ion-trap operated in the product scan mode. Separations were achieved on a C_{18} reversed-phase column with methanol–water (0.1% acetic acid) as eluent. The method has a high sensitivity (instrument detection limit 0.7–7 pg/μl at $S/N=3$) and precision (5–10%) and a good linearity. Acidic oxidation products produce strong signals with ESI. They appear as negative quasimolecular ions $[M-H]^-$, acetate adducts $[M+CH_3COO]^-$ and molecular clusters $[2M-H]^-$. In the MS^2 mode these acids show strong signals from neutral loss of CO_2: $[M-H-CO_2]^-$, and/or weaker signals from loss of H_2O: $[M-H-H_2O]^-$, $[M-H-H_2O-CO_2]^-$. Mass spectra were recorded by APCI for a number of oxygenated terpenoid standards containing keto groups, hydroxy groups, aldehyde groups, or epoxy groups. These compounds give intense signals as their positive quasimolecular ions $[M+H]^+$, methanol adducts $[M+H+CH_3OH]^+$ and fragments from loss of water, such as $[M+H-H_2O]^+$, $[M+H+CH_3OH-H_2O]^+$ and $[M+H-2H_2O]^+$. By MS^2 and MS^3 neutral loss of H_2O and $2H_2O$ is observed. The method has been tested in analysis of aerosol from O_3–α-pinene and O_3–myrtenol and has been proved to compare well with classical methods based on derivatisation and gas chromatography mass spectrometry. Three new compounds, tentatively identified in aerosol are reported here for the first time: 10-hydroxypinonic acid, 9-hydroxynorpinonic acid and pinalic 4-acid.

Keywords: Aerosols; Mass spectrometry; Terpenes; α-Pinene; Carboxylic acids

1. Introduction

Smog chamber experiments carried out over the past two decades have identified secondary organic aerosol (SOA) from gas-phase oxidation of volatile organic compounds (VOC) as an important contributor to the atmospheric burden of particulate matter [1] (review) [2–17]. Recent reviews on global VOC emissions estimate that the magnitude of VOCs of biogenic origin exceeds that of anthropogenic origin with an order of magnitude [18,19]. Although observations of blue haze in the atmosphere derived from compounds emitted by trees date back to 1960 [20], organic aerosol research has mainly been focused on anthropogenic VOCs and their SOA forming potential with relevance to urban air quality. The growing concern about biogenically emitted compounds such as terpenes, recently estimated to amount to 120–480 Tg/year on a global scale

*Corresponding author. Tel.: +39 332 785453; fax: +39 332 785704; e-mail: bo.larsen@jrc.it

0021-9673/99/$ – see front matter
PII: S0021-9673(98)01042-5

[18,19], has triggered investigations of the SOA forming potential of these compounds. The first results indicate that biogeochemical sources of SOAs may play an important role in atmospheric chemistry [21,22].

Very little is known about the mechanism for the photochemical gas-to-particle conversion of terpenes. It is conceivable that the formation of oxidation products with considerably lower vapour pressures than the mother compounds plays an important role. Until recently, mainly carbonyl compounds have been identified in secondary organic aerosol from gas phase reactions of terpenes with O_3, OH or NO_3 [23] (review). Carbonyls generally possess relatively high vapour pressures and thus cannot reach super-saturation to nucleate homogeneously or condense directly under the experimental conditions used. Therefore, in order to explain the particle burst in most SOA experiments, attention must be turned to secondary products such as carboxylic acids, ketocarboxylic acids, hydroxyketones, diols, and dicarboxylic acids with stronger inter-atomic forces and much lower vapour pressures. Analysis of such polar compounds by gas chromatography–mass spectrometry (GC–MS) necessitates the inclusion of one ore more chemical derivatisation steps, which are time consuming and may imply intrinsic technical problems – not only with yields, reproducibility, and chemical interference but also during GC injection due to presence of residual reactants in the final extracts. Furthermore, derivatisation is directed towards single classes of compounds (e.g., carbonyls, acids or alcohols) and no information is obtained for other classes of compounds. An ideal method for screening of polar compounds is liquid chromatography coupled with mass spectrometry (LC–MS). Until the recent development of atmospheric pressure ionisation sources such as pneumatically assisted electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI), LC–MS had not offered sufficient sensitivity and robustness to allow for routine analysis. In the present paper the use of LC–ESI-MS and LC–APCI-MS with an ion trap mass spectrometer (MS^n) is investigated for the analysis of polar terpene oxidation products from smog chamber experiments with α-pinene and myrtenol. The latter compound is not a significant compound in biogenic emissions [18,19] but was investigated because of its structural similarity to α-pinene with the aim of (tentatively) identifying polar oxidation products by analogy to the known products [15–17] from the α-pinene ozonolysis.

2. Experimental

2.1. Secondary organic aerosol samples

Particles of secondary aerosols formed by ozonolysis of terpenes were collected from 40 to 500 l air on PTFE fibre filters (2.5 cm in diameter with a 0.5 μm pore size) at a flow-rate of 1–2.5 $l\,min^{-1}$. The air with its content of particles was drawn from smog chamber reactors in which monoterpenes had been oxidised by ozone. To avoid sampling artefacts [24,25] ozone was used in a slight deficit. The compounds used in this investigation were the cyclic monoterpenes α-pinene and its hydroxy derivative myrtenol used in initial mixing ratios around 2 ppm (11–13 $\mu g\,l^{-1}$) The terpenes were obtained from Fluka (Milan, Italy) in a purity greater than 99%. After sampling, the filters were stored at 5°C in sealed glass vials. A detailed description of the reaction chambers [26] and the experimental conditions [27] can be found elsewhere.

Sampling of aerosol is connected with a number of inherent errors. These aspects have not been quantified in the present study. First of all, the moment for sampling (3–6 h) combined with a relatively high surface-to-volume ratio of the PTFE bags used as reaction chambers may have led to loss of particles by coagulation and subsequent settling on the reactor walls. Secondly, some organic vapours may adsorb on the reactor walls and onto particles already collected on the filter surface and other organic compounds may evaporate or desorb from these particles [27]. The reported reaction yields in this investigation should thus be taken as first indications of the approximate levels of polar terpene oxidation products in aerosols. A validation of the complete analytical procedure can only be carried out when all measured products have been synthesised as authentic standards. This is out of the scope of the present investigation.

2.2. Extraction

The PTFE fibre filters were sonicated for 30 min

with 7 ml dichloromethane at room temperature, the filters were removed, the solvent was evaporated under a gentle stream of nitrogen, and the residues were redissolved in 1 ml 17.5 m*M* acetic acid containing 25% methanol for direct LC–MS analysis. A second extraction step with methanol–water (1:1, v/v) was applied to search for highly polar compounds such as tricarboxylic acids. No such compounds were found. For comparison selected samples were derivatised and analysed by GC–MS. The derivatisation was done with 300 μl 10% BF_3–methanol at 50°C for 30 min in order to esterify organic acids [16,28]. The solution was cooled, 300 μl distilled water was added and the esters were extracted with 2 ml pentane containing 2 ng μl^{-1} bornyl acetate as internal standard.

The analytical procedure was evaluated in preliminary experiments for pinonaldehyde, pinonic acid, pinic acid and 1,2,4-butanetricarboxylic acid spiked onto the used PTFE filters and proved to produce quantitative results.

2.3. Analysis

Non-derivatised extracts of the filters were analysed by LC–MS. Aliquots (50 μl) of this solution were loop injected into a ThermoSeparation HPLC coupled to a Finnigan Mat LCQ ion–trap mass spectrometer. The HPLC was equipped with a thermostated (20°C) 25 cm×4.6 mm C_{18}-coated silicagel (4 μm) column (Waters, Milan, Italy) run in the gradient mode (0.8 ml min^{-1}). The eluents were 17.5 m*M* acetic acid in H_2O (A eluent) and methanol (B eluent). The gradient was programmed from 25% B to 90% B in 30 min.

For analysis of acidic products the outlet of the HPLC was split (3:1) to the ESI interface. The sheath and auxiliary gases were N_2 (81 ml min^{-1}) and He (9 ml min^{-1}), respectively. The capillary temperature was 265°C, and the spray voltage −4.1 kV. In a first run the ion-trap was scanned from 70 to 400 *m/z* with 3 scans s^{-1}. In a second run the ion-trap was operated in the MS^2 daughter ion scan mode (15% relative energy) with the first cycle locked on the *m/z* value corresponding to the quasimolecular ion $(M-H)^-$ of the suspected target compounds and the daughter ions scanned from *m/z* 70 to ~30 above the molecular mass of the target compound. Total data acquisition time was 20 min.

For analysis of neutral products the HPLC was interfaced to the ion-trap through an APCI source run at 450°C at a corona discharge voltage of +5 kV. The sheath and auxiliary gases were nitrogen (81 ml min^{-1}) and helium (9 ml min^{-1}), respectively. Data were acquired from the ion-trap in two separate runs for each sample (MS and MS^2) as described above. Total data acquisition time was 30 min.

The comparative analyses with a traditional method was carried out by GC–MS. Methyl ester derivatives of organic acids in the extracts were analysed in the chemical ionisation (isobutane) mode and the electron impact (70 eV) mode. Sample introduction was achieved either by thermal desorption of 50 μl aliquots of the extract spiked onto Tenax traps using a Perkin Elmer ATD400 thermal desorber coupled to an HP 5890/5970 GC–MSD system or by large volume injection of 50-μl aliquots on a MEGA 2 GC coupled to a TRIO 1000 MSD [16,26].

To facilitate the interpretation of ESI(−) and APCI(+) data individual mass spectra (MS, MS^2 and MS^3) were recorded for a number of oxygenated terpenoids representing different classes of functional groups. Carboxylic acids: *cis*-pinic acid, *trans*-pinic acid, 1,2,4-butanetricarboxylic acid; ketocarboxylic acids: *cis*-pinonic acid; aldehydes: *trans, trans*-hepta-2,4-dienal, myrtenal, neral, citronellal; ketoaldehydes: pinonaldehyde; epoxides: α-pinene oxide, β-pinene oxide, limonene-2-oxide; hydroxyepoxides: linalool oxide; alcohols: verbenol, myrtenol, linalool; hydroxyketones: hydroxycarvone, 2-hydroxy-3-pinanone; diols: menth-6-ene-2,8-diol, pinanediol; ketones: nopinone, menthone, camphor, limona ketone; ethers: 1,9-cineol; esters: terpinyl acetate, geranyl acetate, linalyl acetate, bornyl acetate. All compounds were obtained from Roth (Milan, Italy), Aldrich (Milan, Italy) and Fluka as high purity compounds (>95%) except pinonaldehyde, which was synthesised [29] in the laboratory at a 95% purity. The structure of the used compounds are depicted in Tables 2 and 3. The nomenclature for oxidation products has been adopted from Larsen et al. [30]. New compounds are mentioned for the first time in the text with their names according to this nomenclature together with their IUPAC names. Stock solutions of each individual compound were made in methanol at the 1–10 μg μl^{-1} level and diluted into 50% methanol in 17.5 m*M* acetic acid at the 100 ng μl^{-1} level immediately before use. The

mass spectra were recorded using the fusion injection technique at 3 and 50 μl min^{-1} for ESI and APCI, respectively. Each compound was injected individually. Its full scan MS spectrum (50–400 *m*/*z*) was first recorded followed by its daughter scan MS^2 spectrum of the quasimolecular ion (or in absence of this, the major fragment ion) and its product scan MS^3 spectrum of important daughter fragments. The collision energy for the MS^2 and MS^3 was varied in order to obtain maximum fragmentation while preserving the parent ion at around 10%.

3. Results and discussion

3.1. HPLC–MS analysis of carboxylic acids

3.1.1. Development of the method

In a series of preliminary experiments with pure standards of acidic oxidation products of α-pinene [15–17] the LC–MS was run under different conditions with respect to eluent gradient, eluent pH, and ionisation techniques. The best results were obtained with a combination of methanol–water–acetic acid using electrospray in the negative ion mode. In Fig. 1 is shown the LC–MS total ion chromatograms (TIC) of *cis*- and *trans*-pinic acid at basic (17.5 m*M* ammonium acetate), neutral, and acidic (17.5 m*M* acetic acid) conditions (pH~8, pH~6 and pH~3, respectively). It is evident from the figure that the best separation is obtained under acidic conditions. It is interesting, however, to observe the behaviour of the *cis*-pinic acid at neutral conditions at which the compound elutes in the chromatogram as a split peak. An explanation for this can be found in the intra-molecular forces, which prevail in this molecule (Fig. 2). Such forces are sterically favoured in the *cis*-isomer as opposed to the *trans*-isomer, and have a notable effect of the pK_a value(s) of the dicarboxylic acid. The peak splitting of the *cis*-isomer may be explained by a partial dissociation of the acidic group(s).

A distinct difference between the *cis*- and the *trans*-isomers is also verified in the ion chemistry of

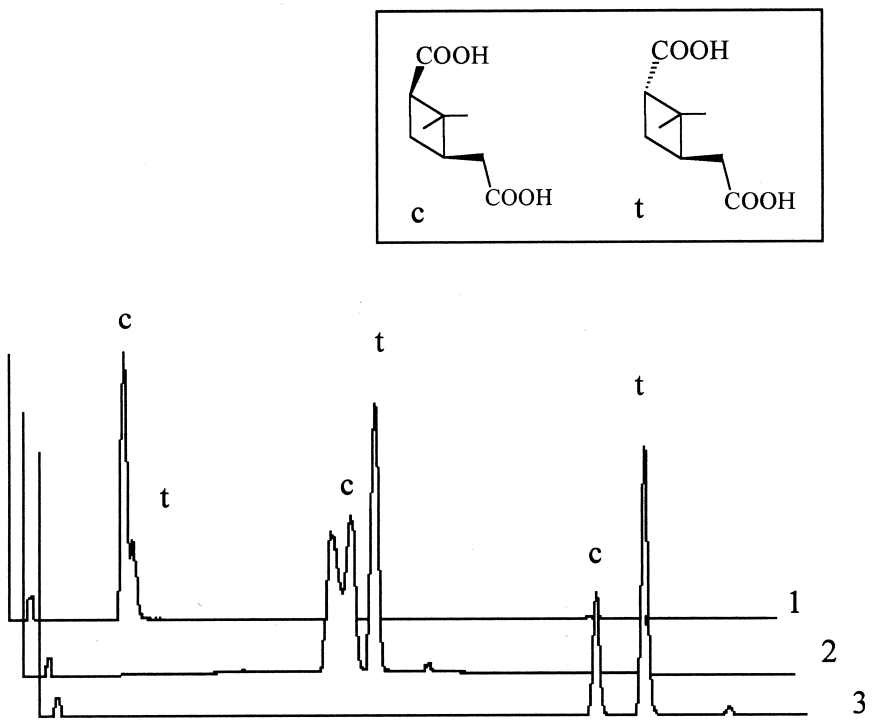


Fig. 1. LC–MS chromatograms (0–15 min) of *cis*- and *trans*-pinic acid at basic (1), neutral (2) and acidic (3) conditions. For details see text.

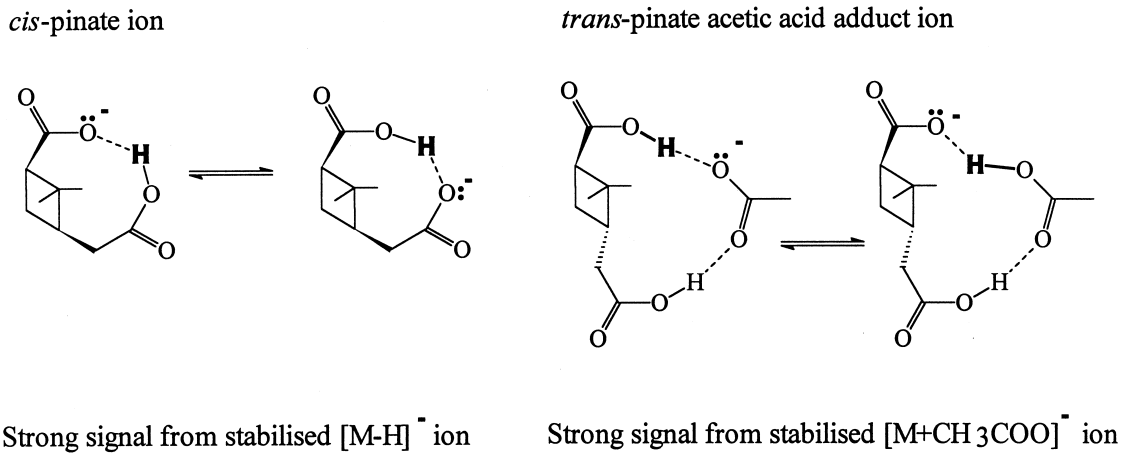


Fig. 2. Intra- and inter-molecular forces stabilise the quasimolecular ion $[M-H]^-$ for *cis*-pinic acid and the acetate adduct ion $[M+CH_3COO]^-$ for *trans*-pinic acid.

the compounds inside the ion-trap mass spectrometer. An LC–MS chromatogram (acidic conditions) of mono- di- and tricarboxylic acids is depicted in Fig. 3. The mass spectrum for the *cis*-isomer of pinic acid (b) shows an intense quasimolecular ion $[M-H]^-$ of $m/z=185$ and a solvent adduct ion of the possible

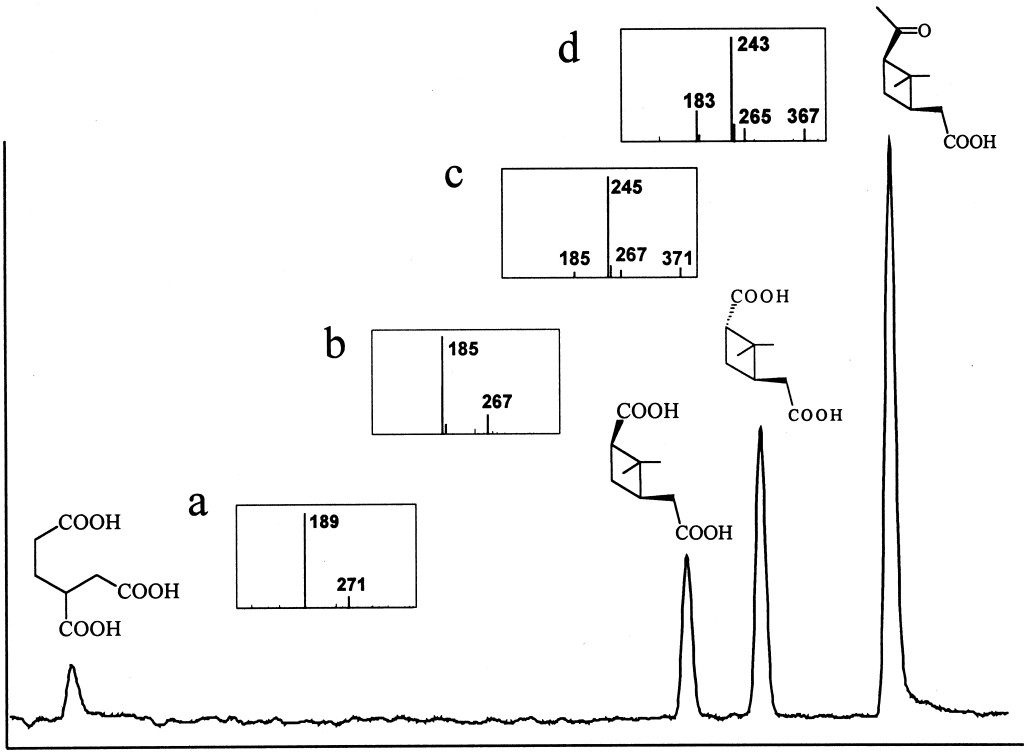


Fig. 3. LC–MS chromatograms (0–18 min) and mass spectra (ESI−) of (a) 1,2,4-butanetricarboxylic acid (5 ng μl^{-1}), (b) *cis*-pinic acid (2.4 ng μl^{-1}), (c) *trans*-pinic acid (2.4 ng μl^{-1}), and (d) *cis*-pinonic acid (4.5 ng μl^{-1}). For details see text.

composition $[M-H+2CH_3OH+H_2O]^-$ with an intensity of 25% compared to the base peak. The *trans*-isomer (c) with its free acidic groups not involved in intra-molecular coupling shows an intensive acetate adduct ion $[M+CH_3COO]^-$ but a very weak quasimolecular ion $[M-H]^-$ and solvent adduct ion $[M-H+2CH_3OH+H_2O]^-$. Furthermore, a molecular cluster ion $[2M-H]^-$ is present for the *trans*-isomer.

Also included in the mixture of Fig. 3 are a tricarboxylic acid and a keto-carboxylic acid. The 1,2,4-butane-tricarboxylic acid elutes very early in the chromatogram, due to dissociation of two of the carboxylic acids ($pK_{a1}\approx 6$, $pK_{a2}\approx 4.5$, $pK_{a3}\approx 3$). Its mass spectrum contains an intense quasimolecular ion $[M-H]^-$ of $m/z=189$ and a weak solvent adduct ion $[M-H+2CH_3OH+H_2O]^-$. The keto-carboxylic acid, *cis*-pinonic acid, elutes late in the chromatogram, probably as the non-dissociated species. Its mass spectrum contains the quasimolecular ion $[M-H]^-$ of $m/z=183$, the acetate adduct ion $[M+CH_3COO]^-$ as base peak, plus weak peaks from the solvent adduct ion $[M-H+2CH_3OH+H_2O]^-$ and the molecular cluster ion $[2M-H]^-$. The difference in ability of the four compounds to produce secondary ions is reflected in the intensity of the signal in the TIC trace. Thus, *cis*-pinonic acid shows the highest relative molar response (100%) followed by *trans*-pinic acid (93%), *cis*-pinic acid (27%) and 1,2,4-butane-tricarboxylic acid (10%).

For quantitative determinations with MS the best sensitivity is generally obtained with single ion monitoring (SIM). For ion-trap mass spectrometers the gain in sensitivity of SIM as compared to full scan is not as drastic as for sector instruments or quadrupoles. However, the signal-to-noise ratio is considerably lower in SIM with ion-traps and a gain in detection limits of approximately a factor 10 is not unusual [31]. In the case of screening for unknown compounds it is necessary to operate the mass spectrometer in the full scan mode. In this case, the signal-to-noise ratio can be strongly improved by reconstruction of extracted ion traces from the full scan data. In the absence of chemical background noise, the best sensitivity is obtained with the most intensive ion in the mass spectrum for each analyte. This may be the quasimolecular ion for some compounds and an adduct ion for other compounds.

Table 1
Instrument detection limits (pg μl^{-1} at $S/N=3$) for polar terpene oxidation products with HPLC–MS run in full scan mode using extracted ion data

Compound	ESI−	APCI+
1,2,4-Butanetricaboxylic acid	5 (m/z 189)	−
cis-Pinonic acid	1.3 (m/z 243)	4 (m/z 185)
cis-Pinic acid	4 (m/z 185)	−
trans-Pinic acid	0.7 (m/z 245)	−
cis-Pinonaldehyde	−	7 (m/z 169)

In Table 1 instrument detection limits are shown for selected compounds previously reported in secondary aerosols from the α-pinene O_3/OH reaction [15,16,25] (review). The detection limits were determined by sequential dilution of a standard until a signal with the intensity three times that of the background noise had been reached ($S/N=3$). The best sensitivity was obtained for the two compounds which form strong acetate adducts, *trans*-pinic acid and *cis*-pinonic acid. Injections of the compounds over a broad concentration range produced identical mass spectra which shows that adduct formation is independent of the analyte concentration. Thus, the response curve of the adduct ion is expected to be linear as shown in Fig. 4. For the molecular cluster ion $[2M-H]^-$ such linearity was not observed. In fact, the relative intensity of this ion increased with the square of the concentration of the analyte. Secondary ion formation is a well-known phenomenon in LC–MS using APCI and ESI coupled to ion-traps, and may occur in the interface as well as the ion-trap. The intensity of secondary ions depends on parameters such as interface temperature, and flow, ion storage period, ion ejection period, and concentration (and chemical properties) of precursors (ions and neutral molecules). This complexity makes molecular cluster ions, $[2M-H]^-$, unsuitable for quantitation, and may also complicate the structure elucidation of unknown compounds when based on relative intensities of ions in the mass spectrum. Indeed great caution should be taken when interpreting mass spectra obtained with API sources. Hoffmann et al. have recently developed an elegant method for the online analysis of SOA based on direct inlet APCI–MS^n. In SOA from α-pinene/O_3 three compounds were detected [15]. Based on

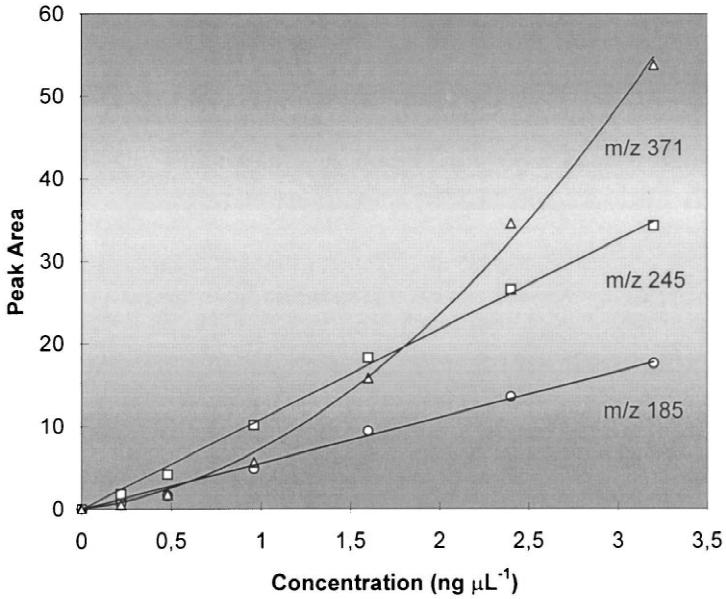


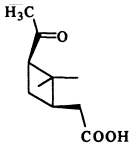
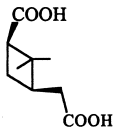
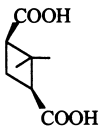
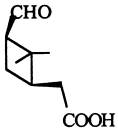
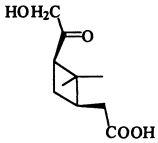
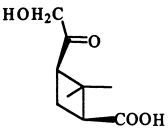
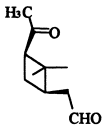
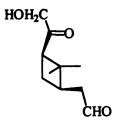
Fig. 4. Calibration curves for *trans*-pinic acid using extracted ion data for the quasimolecular ion $[M-H]^-$ (m/z 185), the acetate adduct ion $10\times$ $[M+CH_3COO]^-$ (m/z 245), and the molecular cluster ion $[2M-H]^-$ (m/z 371).

reaction mechanistic considerations, the molar masses, and the neutral loss of CO_2 and H_2O in the MS^2 analysis these compounds were assigned the structure of pinonic acid, pinic acid, and norpinic acid (3-carboxy-2,2-dimethyl-cyclobutylmethanoic acid). With this technique peaks are also observed in the mass spectra at higher m/z values, corresponding to molecular clusters $[2M-H]^-$ and adducts $[M_1+M_2-H]^-$. Without further experimental evidence such peaks cannot be interpreted as original aerosol components [27]. In the present investigation, where aerosol components are efficiently separated by HPLC before detection by MS, we have not found any evidence for terpene reaction products in SOA at higher molecular masses.

Quantitative determination of polar reaction products from smog chamber experiments is possible for compounds that have standards available such as pinonic and pinic acid. A comparison of the quantitative results by the LC–MS method and the classical GC–MS method was satisfactory. Molar reaction yields of the acidic products from the smog chamber experiments agreed within 10–25% for pinic acid and within 20–35% for pinonic acid (only the *cis*-isomers were formed). Also regarding the precision did the LC–MS method perform well. Repeated injections of carboxylic acid standards at the concentration level 10 times the detection limit and sample extracts produced standard deviations in the order of 5–10%.

The qualitative analyses by MS^n in the daughter ion scan mode provided excellent information on functional groups of the compounds (Table 2). Using 15% energy (~5 eV) with the first cycle of the ion trap locked on the m/z value corresponding to the quasimolecular ion $(M-H)^-$ and the daughter ions scanned from m/z 70 to ~30 above the molecular mass of the target compound it was easy to recognise carboxylic acids by their strong signal for the neutral loss of CO_2 (m/z 44). The dicarboxylic acids, *cis*- and *trans*-pinic acid, were characterised by a strong signal for the neutral loss of CO_2 (m/z 44) plus a weak signal for the neutral loss of a H_2O (m/z 18). In the MS^3 mode the keto-carboxylic acid, pinonic acid, was characterised by a further neutral loss (weak signal) of H_2O (m/z 18) from the $[M-H-CO_2]^-$ ion and the dicarboxylic acids were characterised by their further neutral loss of H_2O from the $[M-H-CO_2]^-$ ion and CO_2 from the $[M-H-H_2O]^-$ ion.

Table 2
Chromatographic and mass spectrometric data for compounds found in aerosol from ozonolysis of α-pinene and myrtenol

Identified product	Structure	Precursor	ESI(−)MS	ESI(−)MS^n	APCI(+) MS	APCI(+) MS^n	GC–CI(+)MS
Pinonic acid[c]		α-Pinene	367 (15)	$-CO_2$	217 (7)	$-H_2O$	199 (100)
t_R (LC)=14.9 min, M_r=184			265 (14)	$-H_2O$ (trace)	185 (100)→	$-H_2O_8-H_2O$	181 (20)
t_R (GC)=24.8 min, M_r=198[a]			243 (100)		167 (24)		167 (15)
			183 (29)→				129 (81)
Pinic acid[c]		α-Pinene	267 (26)	$-CO_2$ (strong)	No signal	No signal	215 (100)
t_R (LC)=11.5 min, M_r=186		Myrtenol	185 (100)→	$-H_2O$ (weak)			183 (91)
t_R (GC)=25.5 min, M_r=214[b]				$-CO_2\rightarrow-H_2O$			
				$-H_2O\rightarrow-CO_2$			
Norpinic acid[d]		α-Pinene	343 (62)	$-CO_2$ (strong)	No signal	No signal	201 (100)
t_R (LC)=5.7 min, M_r=172		Myrtenol	231 (100)	$-H_2O$ (weak)			169 (33)
t_R (GC)=22.7, M_r=200[b]			171 (88)→	$-CO_2\rightarrow-H_2O$			
				$-H_2O\rightarrow-CO2$			
Pinalic 4-acid[d]		α-Pinene	251 (32)	$-CO_2$ (strong)	185 (20)	$-H_2O$	185 (100)
t_R (LC)=11.1 min, M_r=170		Myrtenol	229 (100)	$-H_2O$ (strong)	171 (100)→	$-H_2O\rightarrow-H_2O$	167 (32)
t_R (GC)=22.6 min, M_r=184[a]			169 (41)→		153 (21)		153 (18)
							107 (29)
10-Hydroxypinonic acid[d]		Myrtenol	399 (11)	H_2O (strong)	233 (20)	$-H_2O$	215 (100)
t_R (LC)=7.5 min, M_r=200		α-Pinene	259 (100)	$-CO_2$ (strong)	201 (100)→	$-H_2O-H_2O$	197 (51)
t_R (GC)=25.5 min, M_r=214[a]			199 (32)→	$-H_2O$ and CO_2 (weak)	183 (37)		183 (27)
					165 (18)		129 (46)
9-Hydroxynorpinonic acid[d]		Myrtenol	371 (10)	H_2O (strong)	187 (100)→	$-H_2O$	201 (100)
t_R (LC)=4.5 min, M_r=186			267 (18)	$-CO_2$ (strong)	169 (55)		183 (51)
t_R (GC)=22.7 min, M_r=200[a]			245 (41)	$-H_2O$ and CO_2 (weak)	151 (20)		169 (27)
			185 (100)→				115 (46)
Pinonaldehyde[c]		α-Pinene	No signal	No signal	183 (12)	$-H_2O$	169 (100)
t_R (LC)=15.3 min, M_r=168					169 (100)→	$-H_2O\rightarrow-H_2O$	151 (40)
t_R (GC)=21.6 min, M_r=168					151 (28)		123 (20)
					107 (12)		107 (8)
10-Hydroxypinonaldehyde[d]		Myrtenol	No signal	No signal	199 (46)	$-H_2O$	185 (100)
t_R (LC)=7.1 min, M_r=184					185 (100)→	$-2H_2O$	167 (16)
t_R (GC)=22.3 min, M_r=184					167 (24)		153 (26)
					149 (10)		135 (11)

[a] Methyl ester.
[b] bis-Methyl ester.
[c] Confirmed by pure standard.
[d] Tentatively identified.
$-A_8\rightarrow-B^{\#}$ implies MS^3 of the daughter ion $[M\pm H-A]^{\pm}$.

3.1.2. Application of the method for acidic compounds in aerosol

In the LC–ESI(−)MS analysis of aerosol from the O_3–α-pinene reaction five carboxylic acids were detected, two of which could be positively identified as pinonic acid and *cis*-pinic acid. Their concentrations in the aerosol corresponded to relative molar yields around 0.2% and 1%, respectively, in good accordance with previously published data [16]. The three other compounds are tentatively identified as norpinic acid (*cis*-3-carboxy-2,2-dimethylcyclobutylmethanoic acid), 10-hydroxypinonic acid (*cis*-2,2-dimethyl-3-hydroxyacetylcyclobutylethanoic acid), and pinalic 4-acid (*cis*-3-formyl-2,2-dimethylcyclobutylethanoic acid):

The major compound formed in aerosol from myrtenol was expected to be *cis*-10-hydroxypinonic acid by the analogy to *cis*-pinonic acid in aerosol from O_3/α-pinene. The LC–ESI(−)MS^n analysis (Fig. 5, top) showed one major peak eluting 7 min before pinonic acid with a molecular mass, M_r=200, and which forms intensive acetate adducts and molecular clusters, and produces strong signals from neutral loss of H_2O and CO_2 and a weaker signal from the neutral loss of H_2O and/or CO_2. All these data point to 10-hydroxypinonic acid.

The GC–MS analyses showed two major compounds (eluting at 22.3 and 25.5 min) and a number of minor peaks including pinic acid bis-methyl ester and the tentatively identified norpinic acid bis-methyl ester. The former of the major peaks is tentatively identified as *cis*-10-hydroxypinonaldehyde and will be discussed in the chapter on APCI(+). The latter of the major peaks indicates a M_r of 214, the presence of one carboxylic acid methyl ester (fragment ion $[M+H-CH_3OH]^+$) and at least one functional group able to produce neutral loss of H_2O. The GC–EI spectrum of this compound has the fragment ion $[M-CH_2C(O)OCH_3]^-$, which indicates that the carboxylic group is placed on a methyl group. This information strongly points to (the methyl ester of) 10-hydroxypinonic acid.

This hydroxyketo acid was also detected in aerosol from α-pinene, albeit at a much lower level. Using the acetate adduct ion for quantitation in the LC–ESI(−)MS analysis and the total ion current (TIC) for quantitation in the full scan GC–EI-MS analysis and by assuming equal response factors for pinonic acid and 10-hydroxypinonic acid the estimated concentration of the latter in aerosol corresponds to a relative molar yield around 3% for myrtenol and 0.004% for α-pinene. In the myrtenol experiment the decarbonylised form of *cis*-10-hydroxypinonic acid was also tentatively identified (see Table 1) at a ten times lower concentration level, namely *cis*-9-hydroxynorpinonic acid (*cis*-2,2-dimethyl-3-hydroxyacetyl-cyclobutylmethanoic acid). This compound could not be detected in aerosol from α-pinene. As for norpinic acid, a low yield of this compound is explainable by a mechanistic pathway following ozonolysis and subsequent reaction with secondary OH radicals 10-hydroxypinonic acid and 9-hydroxynorpinonic acid are reported here for the first time. Norpinic acid has recently been tentatively identified in other investigations as a minor component of aerosol from α-pinene oxidation [15,17,27]. The identification of norpinic acid in the present study is based upon its LC–MS and GC–MS retention times and mass spectral data compared with pinic acid. Assuming equal response factors for the quasimolecular ion of pinic and norpinic acid in the LC–MS analysis and equal response factors for these compounds in the full scan GC–EI-MS analysis the estimated concentration of norpinic acid in the aerosol from α-pinene corresponds to a relative molar yield of ≈0.008%. Such a low yield is explainable by a mechanistic pathway following ozonolysis and subsequent reaction with secondary OH radicals. Norpinic acid was also observed at a very low yield in aerosol from myrtenol, which is to be expected if the mechanistic pathway is similar for α-pinene and myrtenol.

The fifth acidic compound in the O_3–α-pinene aerosol was the least intensive carboxylic acid peak in the LC-ESI(−)MS chromatogram. Based on the molecular mass, the retention time and the MS, and MS^2 mass spectrum three likely structures can be assigned to this compound: norpinonic acid (*cis*-3-acetyl-2,2-dimethyl-cyclobutylmethanoic acid), pinalic 3-acid (*cis*-2,2-dimethyl-3-formylmethylcyclobutylmethanoic acid), and pinalic 4-acid (*cis*-2,2-dimethyl-3-formylcyclobutylethanoic acid). The ESI(-)MS^2 and GC–CI data showing a strong signal after neutral loss of H_2O points to an aldo-acid rather than a keto-acid. A fragment ion at m/z=111 in the GC–EI analysis explained by the

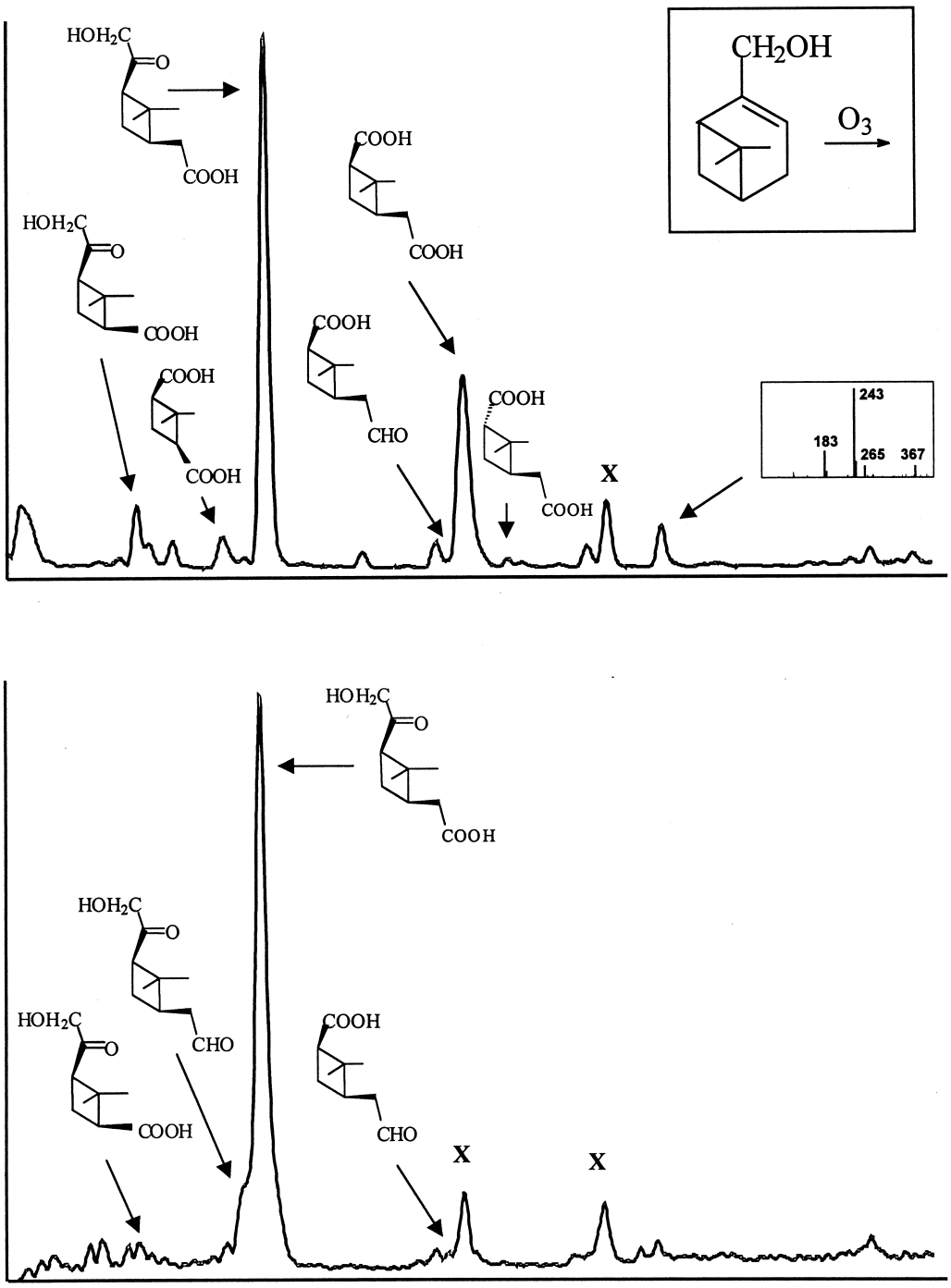


Fig. 5. HPLC–MS analysis of secondary aerosol from the O_3–myrtenol reaction. Top: ESI(−). Bottom APCI(+).

neutral loss of $-CH_2C(O)OCH_3$ points to a cyclobutylethanoic acid methyl ester rather than a cyclobutylmethanoic acid methyl ester. On this basis the suggested structure for this carboxylic acid is pinalic 4-acid. Assuming equal response factors for the quasimolecular ion of pinonic and pinalic 4-acid in the LC–MS analysis and equal response factors for these compounds in the full scan GC–EI-MS analysis the estimated concentration of pinalic 4-acid in the aerosol from α-pinene corresponds to a relative molar yield of ≈0.003%. Pinalic 4-acid is tentatively identified here for the first time. Yu et al. [17] have recently found a compound with the same molecular mass in aerosol from α-pinene–O_3 and proposed the structure of pinalic 3-acid. This was done on a purely mechanistic basis. The data presented by Yu et al. and our data in the present paper does not give a certain basis for distinguishing between the two isomers of this aldo-acid.

In the LC–ESI(−)MS chromatogram of the O_3–myrtenol aerosol (Fig. 5top) a peak appears with a similar mass spectrum to *cis*-pinonic acid but with a 2-min longer retention time. It has not been possible to assign a structure to compound. A possible

Table 3
Mass spectral data (APCI+) for oxygenated terpenoids

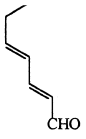
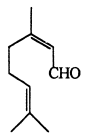
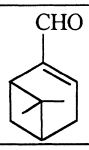
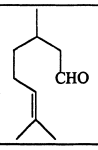
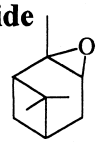
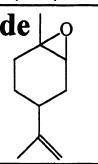
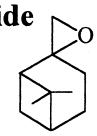
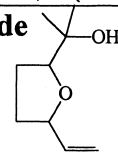
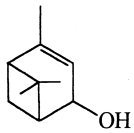
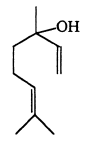
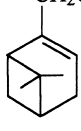
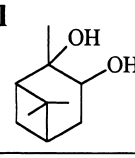
Compound	APCI(+)MS	MS^n	Compound	APCI(+)MS	MS^n
Response factor Molecular mass					
heptadienal  F_R =0.39 MW 152	111 (100) ▸ 93 (16) 67 (4)	93 (100) 83 (7) 67 (11)	**neral**  F_R =0.13 MW 152	153 (100) ▸ 135 (34) 95 (51)	135 (100) 109 (18) 95 (21) 81 (29)
myrtenal  F_R =0.63 MW 150	151 (100) ▸ 133 (7) 123 (6) 107 (8)	133 (100) 123 (45) 109 (51) 107 (45) 93 (23)	**citronellal**  F_R =0.26 MW 154	155 (100) ▸ 137 (42) 95 (9) 81 (23)	137 (100) 113 (26) 95 (4) 81 (18)
α-pinene oxide  F_R =0.14 MW 152	185 (24) 167 (48) 153 (100) ▸ 135 (71) 109 (33)	135 (100) 109 (45) 95 (8) 81 (7)	**limonene oxide**  F_R =0.31 MW 152	187 (13) 167 (6) 153 (100) ▸ 135 (26) 107 (20)	135 (100) 109 (34) 107 (25) 95 (8) 93 (15)
β-pinene oxide  F_R =0.14 MW 152	185 (18) 167 (11) 153 (70) ▸ 135 (100 107 (37)	135 (100) 107 (19) 93 (6) 81 (5)	**linalool oxide**  F_R =0.16 MW 154	153 (50) ▸ 135 (19) 107 (5) 93 (5)	135 (100) 107 (9) 93 (15) 71 (6)
verbenol  F_R =0.013 MW 152	153 (50) ▸ 152 (20) 151 (100) 135 (92) 109 (48)	135 (100) 109 (10) 107 (12) 93 (20)	**linalool**  F_R =0.07 MW 154	152 (6) 151 (5) 137 (100) ▸ 95 (36) 81 (72)	95 (100) 81 (71)
myrtenol  F_R =0.06 MW 152	153 (16) ▸ 152 (21) 151 (7) 135 (100) 108 (34)	135 (100) 109 (40) 83 (12)	**pinanediol**  F_R =0.26 MW 170	170 (8) 169 (42) 153 (100) ▸ 151 (30) 109 (100)	135 (100) 109 (47) 95 (21) 81 (11)

Table 3. Continued

Compound	APCI(+)MS	MS^n	Compound	APCI(+)MS	MS^n
Response factor Molecular mass					
menth-6-ene-2,8-diol F_R =0.16 MW 170	170 (43) 169 (11) 153 (41) ▸ 135 (100) 107 (49)	135 (100) 109 (35) 95 (41) 81 (10)	**2-hydroxy-3-pinanone** F_R =0.20 MW 168	183 (68) 169 (37) ▸ 151 (73) 123 (21) 109 (100)	154 (84) 151 (100) 141 (33) 123 (37) 109 (8)
hydroxy-carvone F_R =0.57 MW 168	183 (9) 169 (60) ▸ 151 (100) 123 (6) 109 (40)	154 (100) 153 (6) 152 (10) 151 (11) 141 (38)	**menthone** F_R =0.69 MW 154	155 (100) ▸ 137 (15) 95 (5) 81 (12)	137 (100) 95 (4) 81 (13)
nopinone F_R =0.47 MW 138	139 (100) ▸ 121 (18) 93 (4) 83 (7)	121 (100) 95 (12) 93 (13) 83 (32)	**limona ketone** F_R =0.14 MW 138	139 (100) ▸ 121 (6) 95 (17)	121 (100) 95 (90) 81 (19)
camphor F_R =0.67 MW 152	153 (100) ▸ 135 (8) 109 (3) 95 (9)	135 (100) 109 (52) 95 (32)	**1,8-cineol** F_R =0.11 MW 154	155 (14) ▸ 137 (100) 95 (20) 81 (62)	137 (100) 127 (20) 111 (39) 93 (15)
terpinyl-acetate F_R =0.006 MW 196	273 (7) 166 (21) 137 (100) ▸ 95 (25) 81 (68)	119 (5) 109 (16) 95 (100) 81 (55) 57 (5)	**geranyl-acetate** F_R =0.022 MW 196	273 (81) 166 (12) 137 (100) ▸ 95 (32) 81 (46)	109 (13) 95 (100) 81 (64) 57 (11)
linalyl-acetate F_R =0.03 MW 196	273 (100) 166 (18) 137 (100) ▸ 95 (61) 81 (42)	109 (17) 95 (100) 81 (66) 57 (12)	**bornyl-acetate** F_R =0.027 MW 196	166 (4) 137 (100) ▸ 109 (4) 95 (22) 81 (53)	109 (9) 95 (100) 81 (56) 57 (8)

candidate is *trans*-pinonic acid. However, it is not easy to envisage a mechanistic pathway for the formation of the *trans*-isomer from myrtenol.

3.2. HPLC–MS analysis of polar oxidation products other than carboxylic acids

3.2.1. Development of the method

Atmospheric oxidation of terpenes by OH, NO_3 and O_3 mainly leads to non-acidic products containing carbonyl groups and to some extend hydroxy groups. A typical example is pinonaldehyde from oxidation of α-pinene. Despite their higher vapour pressure such compounds are also found in secondary aerosol due to gas–solid partitioning processes. The lack of acidity renders these compounds insensitive in the electrospray interface. However, their functional groups may induce a signal in the APCI(+) mode depending on the ability of the compound to accept a proton. Good candidates for APCI(+) analysis are ketones, aldehydes, and alcohols which have electric dipole moments around 3 D, 2.5 D and 2 D, respectively and may form the ions $R'R''–C=OH^+$, $R–HC=OH^+$ and ROH_2^+. The

APCI(+) spectra were recorded for a number of compounds representing various classes of functional groups (see Table 3). All compounds were similar in their in fragmentation patterns. Intense signals from the positive quasimolecular ions $[M+H]^+$ were present together with strong signals of methanol adducts $[M+H+CH_3OH]^+$ and weaker signals from fragments produced by neutral loss of water, such as $[M+H-H_2O]^+$, $[M+H+CH_3OH-H_2O]^+$, and $[M+H-2H_2O]^+$. Also the molecular cluster ion $[2M+H]^+$ was frequently seen. The sensitivity varied over almost two orders of magnitude for the investigated compounds and followed by large the number and polarity of functional groups in the analyte (Fig. 6). A few useful rules can be derived for the fragmentation pattern of each class of compounds:

Ketones: strong signals from the $R'R''–C=OH^+$ ions and weak signals (2–20%) from the $[M+H-H_2O]^+$ ions. MS^2 produces strong signals from neutral loss of H_2O and some fragmentation of R′ and R″.

Aldehydes: strong signals from the $R–HC=OH^+$ ions and signals (10–40%) from the $[M+H-H_2O]^+$ ions and weak signals from the $[M+H+CH_3OH-H_2O]^+$ ions (5–10%) and the $[M+H+CH_3OH]^+$ adduct ions (10–15%). MS^2 produces strong signals from neutral loss of H_2O and some fragmentation of R.

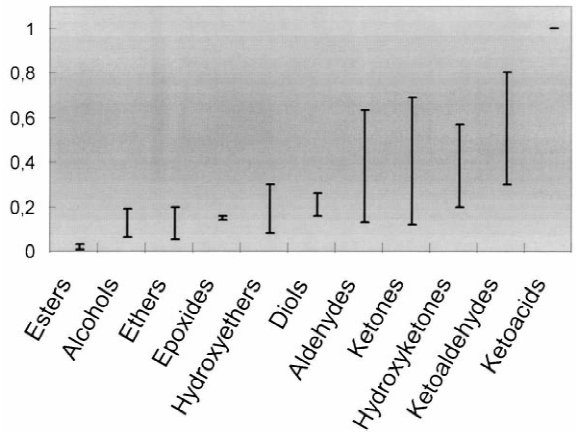


Fig. 6. Relative response factor of oxygenated terpenoids by APCI(+). Pinonic acid=1.

Alcohols: typical clusters of ROH_2^+, ROH^+, and RO^+ of lower intensity (10–90%) than the strong signals from $[M+H-H_2O]^+$ ions (90–100%). MS^2 produces strong signals from neutral loss of H_2O and distinct fragmentation of R.

Diols: typical clusters of $HOR'R''OH^+$/$HOR''R'OH^+$, and $HOR'R''O^+$/$HOR''R'O^+$ of lower intensity (30–40%) than the strong signals from the $[M+H-H_2O]^+$ and the $[M+H-2H_2O]^+$ions (50–100%). MS^2 from M^+ and $[M+H-H_2]$ produce ambiguous ions. MS^2 from $[M+H-H_2O]^+$ produce strong $[M+H-2H_2O]^+$ ions and distinct fragmentation of R′ and R″.

Hydroxy-ketones: good signals from the $HOR'R''–C=OH^+$/$R'C(O)R''=OH^+$ ions (40–60%), the $[M+H-H_2O]^+$ ions (75–100%), and the $[M+H+CH_3OH]^+$ adduct ions (10–70%). A strong signal is also seen for the fragment $[M+H-C_2H_4O_2]^+$ (40–100%). MS^2 produces strong signals from $[M+H-H_2O]^+$ and $[M+H-2H_2O]^+$.

Epoxides: good signals from the $R'CH[OH^+]CHR''$ ions plus strong signals from the $[M+H-H_2O]^+$ ions (30–100%) and clear signals from the $[M+H+CH_3OH-H_2O]^+$ ions (10–50%) and the $[M+H+CH_3OH]^+$ adduct ions (10–30%). MS^2 produces strong signals from neutral loss of H_2O and some fragmentation of R′ and R″.

Ethers: weak quasimolecular ion (15%), strong neutral loss of H_2O (100%) and some fragmentation of R.

Esters (acetates): very low response, quasimolecular ion absent, and a base peak from the loss of the acetate group $[M+H-O(CO)CH_3]^+$. MS^2 of this peak produces fragmentation of R.

Carboxylic acids: mono-carboxylic, dicarboxylic and tricarboxylic acids give no response in APCI(+) if no other functional group is present.

The keto-aldehyde, pinonaldehyde and the keto-acid, pinonic acid, proved to be the most sensitive compounds analysed by APCI(+). They give strong quasimolecular ions $[M+H]^+$ (100%) and a weaker signal (25–30%) from the $[M+H-H_2O]^+$ ions (see Table 2). Pinonaldehyde also gives a signal for the $[M+H+CH_3OH-H_2O]^+$ adduct ion (10%). MS^2 of the quasimolecular ions of these compounds produces strong signals from neutral loss of H_2O and some fragmentation of R. MS^3 of the $[M+H-H_2O]^+$ daughter ions produces a further loss of H_2O.

3.2.2. Application of the method for polar compounds other than acids in aerosol

To avoid complications identical chromatographic conditions were used for LC–APCI(+)MS analysis as for LC–ESI(−)MS analysis of the organic aerosols. These conditions are optimised for carboxylic acids. Thus, to minimise the risk of losing later eluting compounds the data acquisition was allowed to continue for 10 min longer when APCI(+) was used. Pinonaldehyde elutes as a broad peak partly overlapping with pinonic acid. Based on this observation it was expected that the analogue compound in the myrtenol experiment, *cis*-10-hydroxypinonaldehyde (*cis*-2,2-dimethyl-3-hydroxyacetyl-cyclobutylethanal) would co-elute with *cis*-10-hydroxypinonic acid. In fact a compound is seen in Fig. 5 bottom as a shoulder to the peak tentatively identified as 10-hydroxypinonic acid. The APCI(+) mass spectral data for this compound is in accordance with the proposed structure. (100% $[M+H]^+$, 46% $[M+H+CH_3OH-H_2O]^+$ adduct, 24% $[M+H-H_2O]^+$ and 10% $[M+H-2H_2O]^+$ neutral loss fragments by MS, and the strong signals from $[M+H-H_2O]^+$ and $[M+H-2H_2O]^+$ by MS^2). Also the GC–MS data points to this compound. *cis*-10-Hydroxypinonaldehyde has been reported previously as a gas-phase reaction product [32] and a trace compound in aerosol from α-pinene oxidation [17]. In the present experiments this product was not detected in aerosol from α-pinene. However, in a recent study with OH radical oxidation of α-pinene we have detected small amounts of 10-hydroxypinonaldehyde in the evolved aerosol [27].

The mass spectral data for peaks eluting in the LC–APCI(+)MS chromatograms at the same retention time as in the LC–ESI(−)MS chromatograms supported the tentative identifications (Fig. 5). The major peak proposed as 10-hydroxypinonic acid shows 100% $[M+H]^+$, 20% $[M+H+CH_3OH]^+$, 37% $[M+H-H_2O]^+$ and 18% $[M+H-2H_2O]^+$ MS, and strong signals from $[M+H-H_2O]^+$ and $[M+H-2H_2O]^+$ by MS^2. The concentration of the decarbonylised form of this acid, 9-hydroxy-norpinonic acid, in the myrtenol experiment was high enough to give a clear mass spectrum: 100% $[M+H]^+$, 55% $[M+H-H_2O]^+$ and 20% $[M+H-2H_2O]^+$ by MS, and strong signals from $[M+H-H_2O]^+$ and $[M+H-2H_2O]^+$ by MS^2. The minor peak at 11.1 min, proposed as pinalic 4-acid shows 100% $[M+H]^+$, 5% $[M+H+CH_3OH-H_2O]^+$, and 7% $[M+H-H_2O]^+$ pointing to an aldo-acid rather than a keto-acid, thereby giving support to the tentative identification rather than norpinonic acid.

4. Conclusion

A new method for analysis of polar terpene oxidation products in aerosol has been developed based on LC coupled with ion-trap MS (MS^n). The use of both pneumatically assisted electrospray ionisation and APCI facilitates the analysis of carboxylic acid as well as neutral compounds such as ketones, aldehydes, alcohols, epoxides, and combinations of these. For compounds with pure standards available this method can be used as a sensitive, precise and accurate tool for quantitations; it compares favourably with classical methods based on derivatisation and successive analysis by GC–MS. For structure elucidation of new compounds LC–MS with APCI and ESI is a useful complementary technique to GC–MS with electron impact ionisation and chemical ionisation.

The method has been used to analyse polar oxidation products in aerosols deriving from ozonolysis of α-pinene and its hydroxy-derivative, myrtenol. Five carboxylic acids were found in aerosol in concentrations corresponding to relative molar yields ranging from 0.003% to 3%. Evidence for high-molecular-mass oxidation products was not found. The low yields are in agreement with expectations from known reaction mechanisms. Although these levels do not seem very high, it is possible that carboxylic acids owing to their extremely low vapour pressures may play a significant role in nucleation processes for secondary aerosol. The present method will be used throughout the NUCVOC project to address the importance of carboxylic acids in secondary aerosol formation from a branch of terpenes emitted in high quantities from natural vegetation. The first results of this investigation are already available [27,33] and point to carboxylic acids as ubiquitous components of secondary organic aerosol.

Acknowledgements

The present work has been carried out as a part of

the European Commission funded NUCVOC project (Nucleation Processes from Oxidation of Biogenic Volatile Organic Compounds, ENV4-CT97-0391). The collaboration of A. Calogirou in the myrtenol experiment is gratefully acknowledged. The authors thank D. Kotzias for useful discussions.

References

[1] P. Saxena, L.M.J. Hildemann, Atm. Chem. 24 (1996) 57.
[2] J.J. Bufalini, R.R. Arnts (Eds.), Atmos. Biog. Hydrocarbons, Vol. 2, 1981, Ann Arbor Science, Ann Arbor.
[3] Y. Yokouchi, Y. Ambe, Atmos. Environ. 19 (1985) 1271.
[4] S. Hatakeyama, K. Izumi, T. Fukuyama, H.J. Akimoto, Geophys. Res. 94 (1989) 13013.
[5] S.E. Paulson, S.N. Pandis, U. Baltensperger, J.H. Seinfeld, R. Flagan, E.J. Palen, D.T. Allen, W. Giger, A.J. Portmann, Aerosol. Sci. 21 (1990) 245.
[6] S.N. Pandis, S.E. Paulson, J.H. Seinfeld, R.C. Flagan, Atmos. Environ. 25A (1991) 997.
[7] S.N. Pandis, R.A. Harley, G.R. Cass, J.H. Seinfeld, Atmos. Environ. 26A (1992) 2269.
[8] D. Grosjean, Atmos. Environ. 26A (1992) 953.
[9] E.J. Palen, D.T. Allen, S.N. Pandis, S.E. Paulson, J.H. Seinfeld, Atmos. Environ. 26A (1992) 1239.
[10] S.-H. Zhang, M. Shaw, J.H. Seinfeld, R.J. Flagan, Geophys. Res. 97 (1992) 20717.
[11] H. Hakola, J. Arey, S. Aschmann, R. Atkinson, J. Atmos. Chem. 18 (1994) 75.
[12] J.R. Odum, T. Hoffmann, F. Bowman, D. Collins, R.C. Flagan, J.H. Seinfeld, Environ. Sci. Technol. 30 (1996) 258.
[13] M. Lahaniati, Ch. Nanos, C. Konidari, B. Niccolin, D. Kotzias, in: B.R. Larsen, B. Versino, G. Angeletti (Eds.), Physicochemical Behaviour of Atmospheric Pollution. European Commission, Luxembourg, EUR 17482 EN. Proceedings 7th European Symposium, Venice, 2–4 October, 1997, pp. 542–546.
[14] T. Hoffmann, J.R. Odum, F. Bowman, D. Collins, D. Klockow, R.C. Flagan, J.H.J. Seinfeld, Atmos. Chem. 26 (1997) 189.
[15] T. Hoffmann, R. Bandur, J.H. Seinfeld, in: Workshop on Biogenic Hydrocarbons. American Meteorological Society, University of Virginia, 24–27 August, 1997, pp. 131–134.
[16] T.S. Christoffersen, J. Hjorth, O. Horie, N.R. Jensen, D. Kotzias, L. Molander, P. Neeb, L. Ruppert, R. Winterhalter, A. Virkkula, K. Wirtz, B.R. Larsen, Atmos. Environ. 32 (1998) 1657.
[17] J. Yu, R.C. Flagan, J.H. Seinfeld, Environ. Sci. Technol. 32 (1998) 2357.
[18] F. Fehsenfeld, J. Calvert, R. Fall, P. Goldan, A. Guenther, N.C. Hewitt, B. Lamb, S. Liu, M. Trainer, H. Westberg, P. Zimmerman, Global Biogeochem. Cycles 6 (1993) 389.
[19] A. Guenther, N.C. Hewitt, D. Erickson, R. Fall, C. Geron, T. Graedel, P. Harley, L. Klinger, M. Lerdau, W.A. Mckay, T. Pierce, B. Scholes, R. Steinbrecher, R. Tallamraju, J. Taylor, P.J. Zimmerman, Geophys. Res. 100(D5) (1995) 8873.
[20] F.W. Went, Nature 187 (1960) 641.
[21] T. Novakov, J.E. Penner, Nature 365 (1993) 823.
[22] M.O. Andreae, P.J. Crutzen, Science 276 (1997) 1052.
[23] A. Calogirou, B.R. Larsen, D. Kotzias, Atmos. Environ., in press.
[24] A. Calogirou, B.R. Larsen, C. Brussol, M. Duane, D. Kotzias, Anal. Chem. 68 (1996) 1499.
[25] A. Calogirou, M. Duane, D. Kotzias, M. Lahaniati, B.R. Larsen, Atmos. Environ. 31 (1997) 2741.
[26] P. Fruekilde, J. Hjorth, N.R. Jensen, D. Kotzias, B.R. Larsen, Atmos. Environ. 32 (1998) 1893.
[27] M. Glasius, M. Lahaniati, A. Calogirou, D. Di Bella, N.R. Jensen, J. Hjorth, M. Duane, D. Kotzias, B.R. Larsen, Environ. Sci. Technol. submitted for publication.
[28] E. Hargesheimer, T. Satchwill, JWSRT-Aqua 38 (1989) 345.
[29] M. Glasius, A. Calogirou, N.R. Jensen, J. Hjorth, C.J. Nielsen, Int. J. Chem. Kinet. 29 (1997) 527.
[30] B.R. Larsen, M. Lahaniati, A. Calogirou, D. Kotzias, Chemosphere 37 (1998) 1207.
[31] D. Baglio, D. Kotzias, B.R. Larsen, J. Chromatogr., submitted for publication.
[32] L.A. Hull, in: J.J. Bufalini, R.R. Arnts (Eds.). Atmos. Biog. Hydrocarbons, Ann Arbor Science, Ann Arbor, Vol. 2, 1981.
[33] M. Glasius, M. Lahaniati, A. Calogirou, D. Di Bella, N.R. Jensen, J. Hjorth, M. Duane, D. Kotzias, B.R. Larsen, in: Proceedings of the 5th EUROTRAC Symposium. Garmisch-Partenkirchen, 23–27 March 1998.