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Improving the Quantification of Secondary Organic Aerosol Using a Microflow Reactor Coupled to HPLC-MS and NMR to Manufacture Ad Hoc Calibration Standards

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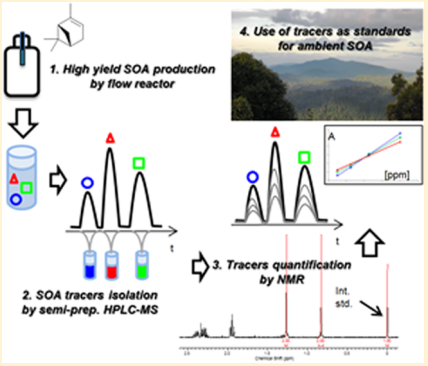
Supporting Information

ABSTRACT: Secondary organic aerosol (SOA) is a key uncertainty in quantifying the impact of humans on Earth's climate. SOA is a complex mixture of oxidized organic species, and a fundamental hurdle in determining its composition is the lack of authentic standards for comparison and quantification. Organic synthesis can be used to produce pure standards, but is limited to compounds for which there is a degree of confidence in the proposed structure and can be expensive and time-consuming. In this study, a flow reactor was developed to form SOA in sufficient quantities to be collected and pure compounds subsequently isolated from the mixture using semipreparative high performance liquid chromatography. The purity and yield of each isolated compound were obtained using proton nuclear magnetic resonance (^{1}H NMR), whereas molecular formulas were confirmed by high resolution Fourier transform ion cyclotron mass spectrometry (FTICR-MS). The effectiveness of the methodology has been evaluated here by using α-pinene as the precursor because it is the monoterpene with the most well characterized SOA chemistry. Eleven individual α-pinene SOA compounds were produced from α-pinene oxidation experiments and used for quantitative analysis of SOA formed during chamber experiments carried out close to ambient conditions. These compounds represented 25% of the total SOA mass, a significant improvement in mass balance compared to previous studies. This relatively simple approach may be extended to produce other SOA components not available commercially to improve quantification of aerosol sources.



Atmospheric aerosols impact Earth's climate by scattering and absorption of solar radiation and by acting as cloud condensation nuclei.[1] Secondary organic aerosols (SOA), formed in the atmosphere from the oxidation of gaseous organic compounds, represent a key uncertainty in determining the impact of aerosols on climate, in part due to their complexity and their continually changing composition. Two main approaches are used to investigate SOA composition. Online techniques, such as aerosol mass spectrometry (AMS), can provide quantitative and time-resolved information about the mass and degree of oxidation of organic material, but provides little information on the chemical speciation of organic aerosol.[2−4] Offline techniques, where particles are collected, usually onto filters, and analyzed back in the laboratory, allow improved molecular speciation but at the expense of time resolution. Commonly used offline techniques include gas and liquid chromatography coupled to mass spectrometry (GC−MS and LC-MS).[5] To obtain quantitative information, calibration using standards is required. This is particularly true when using electrospray ionization, where it is not possible to estimate ionization efficiencies of compounds based on structural characteristics. The use of surrogates for quantification of compounds with similar functionality, instead of specific compounds has the potential to lead to significant errors. The development of organic synthesis strategies is often required, as only a limited number of SOA tracer compounds unique to a specific source/precursor are commercially available, the majority of which are oxidation products of anthropogenic precursors. In the case of α-pinene, one of the most abundant biogenic SOA precursors emitted to the atmosphere, only limited compound specific tracers are currently available commercially (i.e., *cis*-pinonic acid, pinic

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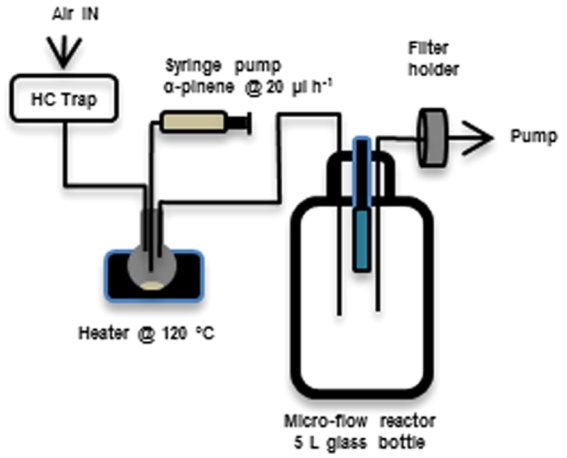
acid and terebic acid). The composition of α-pinene SOA has been explored by several modeling and laboratory studies over the past decade, and many of its potential tracers have been identified.[6−11] However, only a few studies have attempted both the identification and the quantification of α-pinene oxidation products by comparison with the corresponding authentic standards. Claeys and co-workers identified 3-methylbutane-1,2,3-tricarboxylic acid (3-MBTCA) and other terpenoic acids (terpenylic, 2-hydroxyterpenylic acid and diaterpenylic acid acetate) as tracers for α-pinene oxidation and based their characterization on the synthesis of reference compounds.[12−14] However, organic synthesis can be time-consuming, expensive and there is the risk that an incorrect structure is targeted. Preparative LC is a well-known alternative approach to organic synthesis for the production of reference compounds in many research areas and it has been recently applied in the atmospheric sciences. Recently, a number of groups have used bulk phase laboratory solutions to simulate atmospheric chemistry. Nguyen et al.,[15] used low temperature ozonolysis of monoterpenes to form keto-aldehydes (pinonaldehyde and ketolimonaldehyde), followed by semipreparative high performance liquid chromatography (HPLC) to obtain around 35 mg of material. Semipreparative liquid chromatography uses a wider bore HPLC column, capable of higher mass loadings compared to analytical columns, to isolate individual compounds. Subsequent ^{1}H NMR indicated the purity of the synthesized compounds was greater than 95%. The isolated compounds were then used to investigate aqueous reactions of ketoaldehydes with ammonium sulfate and glycine. Kitanovski et al.,[16] formed 3-methylnitrocatechol by reaction of 3-methylcatechol and $NaNO_2$ in an aqueous solution and used off-line ^{1}H NMR, ^{13}C NMR and 2D correlation spectroscopy to determine its structure and purity.

In this study, a microflow reactor was constructed to create milligram quantities of SOA and tested using the oxidation of α-pinene with ozone and OH radicals. The SOA was collected onto a filter and the water-soluble components were extracted. Semipreparative liquid chromatography was used to fractionate the mixture and obtain single compounds, ensuring the correct structure was isolated. ^{1}H NMR confirmed the purity and structure of the fractions and an internal standard was added to allow the mass of the compound to be calculated. Nine compounds were successfully isolated and used to create calibration curves for use in quantification of atmospheric simulation chamber generated aerosol.

EXPERIMENTAL SECTION

Aerosol Flow Reactor. The in-house built flow reactor consisted of a 5 L glass bottle (Duran GL 45, Fischer Scientific, UK) that was used as the mixing volume for the reactor (Scheme 1). Two PTFE 1/4 in. fittings on the bottle were used to connect the air inlet and outlet. Air was supplied to the bottle via a compressor that was scrubbed using a hydrocarbon trap (BHT-4, Agilent, UK) and passed through a 250 mL Pyrex 3-neck round-bottom flask maintained at 120 °C. The outlet of the flow reactor passed through a clean quartz microfiber filter (47 mm, QMA, Whatman, Little Chalfont, UK). Ozone was generated inside the reactor using an Hg Pen-Ray lamp (Ultra-Violet Products Ltd., Cambridge, UK) that emits a strong line at 185 nm. The lamp was suspended inside the glass reactor and the electrical wire exited through a septum connector. The Pen-Ray lamp was switched on and left for 10 min to allow ozone levels inside the bottle to increase. α-Pinene (98%, Aldrich, Gillingham, UK) was then injected into the gas stream using a syringe driver fitted with a 1 mL gastight syringe (Hamilton Bonaduz AG, Bonaduz, Switzerland) at a constant rate of 20 μL h^{-1} into the round-bottom flask. The reactor was run continuously for 3 days, consuming a total of 1.5 mL of α-pinene. After this time period, the filter paper was removed and the α-pinene SOA extracted into 4 mL of water (Fischer Optima LC-MS grade) and concentrated to 1 mL using a vacuum solvent evaporator.

Scheme 1. Schematic of the Apparatus Used to Produce α-Pinene SOA in the Microflow Reactor by Introducing α-Pinene through a Heated Inlet and Using a Hg Pen-Ray Lamp to Generate Ozone from Air



Semipreparative HPLC. The HPLC system incorporated an Agilent 1100 series HPLC (Berkshire, UK) with a diode array detector coupled to a HTC Plus ion trap mass spectrometer (IT-MS, Bruker Daltonics, Bremen, Germany). The mobile phase consisted of (A) water (Fisher Optima LC-MS grade) with 0.1% formic acid (Sigma-Aldrich, UK) and (B) methanol (Fisher Optima LC-MS grade). The analytical HPLC column was a 15 cm × 4.6 mm Pinnacle DB C18, 5 μm particle size (Thames Restek, UK) with a flow rate of 0.6 mL min^{-1}. The HPLC was run using a gradient of 10% B at t = 0 to 100% B at t = 60 min. The semipreparative HPLC column was a 15 cm × 10 mm Acentis C18, 5 μm particle size (Supelco, Australia) with a flow rate of 2.8 mL min^{-1}. The semi-preparative HPLC was run using a gradient of 10% B at t = 0 to 50% B at t = 35 min and then to 100% B at t = 45 min. The mass spectrometer was operated in alternating polarity mode, scanning from m/z 50 to 600. Tandem MS was achieved through the automated MS^2 function within the Esquire software (Bruker Daltonics, software version 5.2).

To prevent the MS being overloaded with solvent while ensuring the correct fractions were collected, the flow from the end of the column was split approximately 5:1 using a t-piece (VICI) and different lengths of PEEK capillary tubing. Individual tracer fractions were collected during multiple 100 μL injections of the SOA extract, and the aliquots resulting from each injection were then combined and reduced to dryness using vacuum solvent extraction. Each isolated compound was subsequently recovered in 500 μL of D_2O and one-fifth of this solution analyzed by ^{1}H NMR spectroscopy, while the rest was used as stock solution for both identification and quantification.

NMR Spectroscopy. ^{1}H NMR spectroscopy was used for both identification and quantification of the isolated com-

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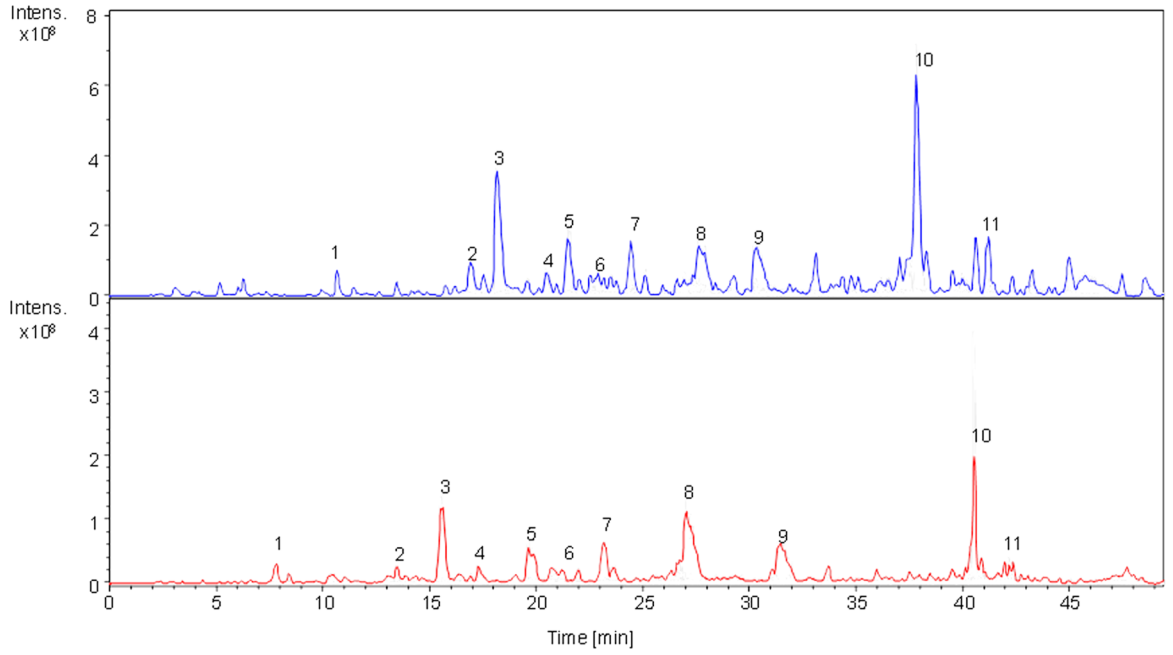

Figure 1. LC-MS base peak chromatograms (BPCs) of α-pinene SOA material formed in the microreactor and separated by analytical HPLC column (blue) or semipreparative HPLC column (red). Numbers on the top of the peaks correspond to the isolated compounds listed in Table 1.

Table 1. Overview of the Individual Species Isolated by LC-MS, Their Collection Time, MS Fragmentation Patterns and Accurate Mass Measurements

#	collection time (min)	$[M-H]^-$	MS^2 product ions	proposed compound	molecular formula	FTICR-MS error (ppm)
1	7.7–7.9	187	111, 125, 143, 169	2-hydroxyterpenylic acid[a,b]	$C_8H_{12}O_5$	0.3
2	12.9–13.4	157	113	terebic acid[c]	$C_7H_{10}O_4$	1.8
3	15.0–15.5	171	127, 153	terpenylic acid[c]	$C_8H_{12}O_4$	0.8
4	16.8–17.2	203	185	MBTCA[c,d]	$C_8H_{12}O_6$	1.1
5	19.2–19.6	199	75, 123, 137, 155, 181	10-hydroxypinonic acid[e]	$C_{10}H_{16}O_4$	0.7
6	20.2–20.7	199	73, 83, 97, 111, 137, 155, 169, 181	8-hydroxypinonic acid[e]	$C_{10}H_{16}O_4$	0.7
7	23.0–23.6	171	127, 153	norpinic acid[c]	$C_8H_{12}O_4$	0.8
8	26.4–27.5	185	123, 141, 167	pinic acid[c]	$C_9H_{14}O_4$	0.0
9	30.7–31.8	183	57, 139, 165	*cis*-pinonic acid[c]	$C_{10}H_{16}O_3$	0.0
10	40.3–40.5	357	127, 141, 171, 185	dimer pinyl-diaterpenyl ester[f]	$C_{17}H_{26}O_8$	10.2
11	41.9–42.1	367	141, 167, 185, 199, 305, 323, 349	dimer pinyl-hydroxypinonyl ester[e]	$C_{19}H_{18}O_7$	9.9

[a]Claeys et al., 2009. [b]Kahnt et al., 2014. [c]Yasmeen et al., 2011. [d]Szmigielski et al., 2007. [e]Yasmeen et al., 2012. [f]Yasmeen et al., 2010.

pounds. Sodium 3-trimethylsilyl-(2,2,3,3-d_4) propionate (TSP-d_4) was used as the internal standard for quantification by adding 50 μL of TSP-d_4 in D_2O solution (0.05%, w/w, Sigma-Aldrich) to a 5 mm probe. The ^1H NMR spectra were acquired at 400 MHz with a Jeol ECS 400. Cis-Pinonic acid (99%, Sigma-Aldrich) was used to optimize the pulse sequence and number of transients for quantitative analysis. 2D heteronuclear single quantum correlation experiments (^1H-^{13}C HSQC) were also performed for additional structural confirmation. Full NMR experiments details can be found in the Supporting Information.

Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS). Samples were also analyzed at high mass resolution using a Bruker APEX 9.4 T FTICR-MS. Extracts were sprayed at a flow rate of 2 μL min^{-1} into an Apollo II electrospray interface with ion funnelling technology. Spectra were acquired in both positive and negative ion modes over the scan range m/z 100–3000 using the following MS parameters: nebulizing gas flow, 0.9 L min^{-1}; drying gas flow, 5 L min^{-1}; drying temperature, 190 °C; collision cell accumulation, 0.05–0.5 s; data acquisition size, 2 Mb (yielding a target resolution of 130 000 at m/z 400). Data were analyzed using DataAnalysis 4.0 software (Bruker Daltonics, Bremen, Germany). The instrument was calibrated using protonated (positive ion mode) or deprotonated (negative ion mode) arginine clusters. The mass spectra were internally recalibrated with a series of prominent peaks. Background contaminants also seen in pure water and blank extracted filters were identified.

Manchester Aerosol Chamber. The Manchester aerosol chamber used for these experiments is an 18 m^3 (3 m (H) × 3 m (L) × 2 m (W)) FEP Teflon bag mounted on three rectangular aluminum frames. The air charge in the bag was dried and filtered for gaseous impurities and particles using a combination of Purafil, charcoal and HEPA filters, prior to humidification with ultrapure deionized water. α-Pinene (50 ppb) was introduced into the chamber through injection into a heated glass bulb fed with a flow of high purity nitrogen. A series of halogen lamps and a 6 kW xenon arc lamp were used to irradiate the chamber and initiate OH chemistry. Full details

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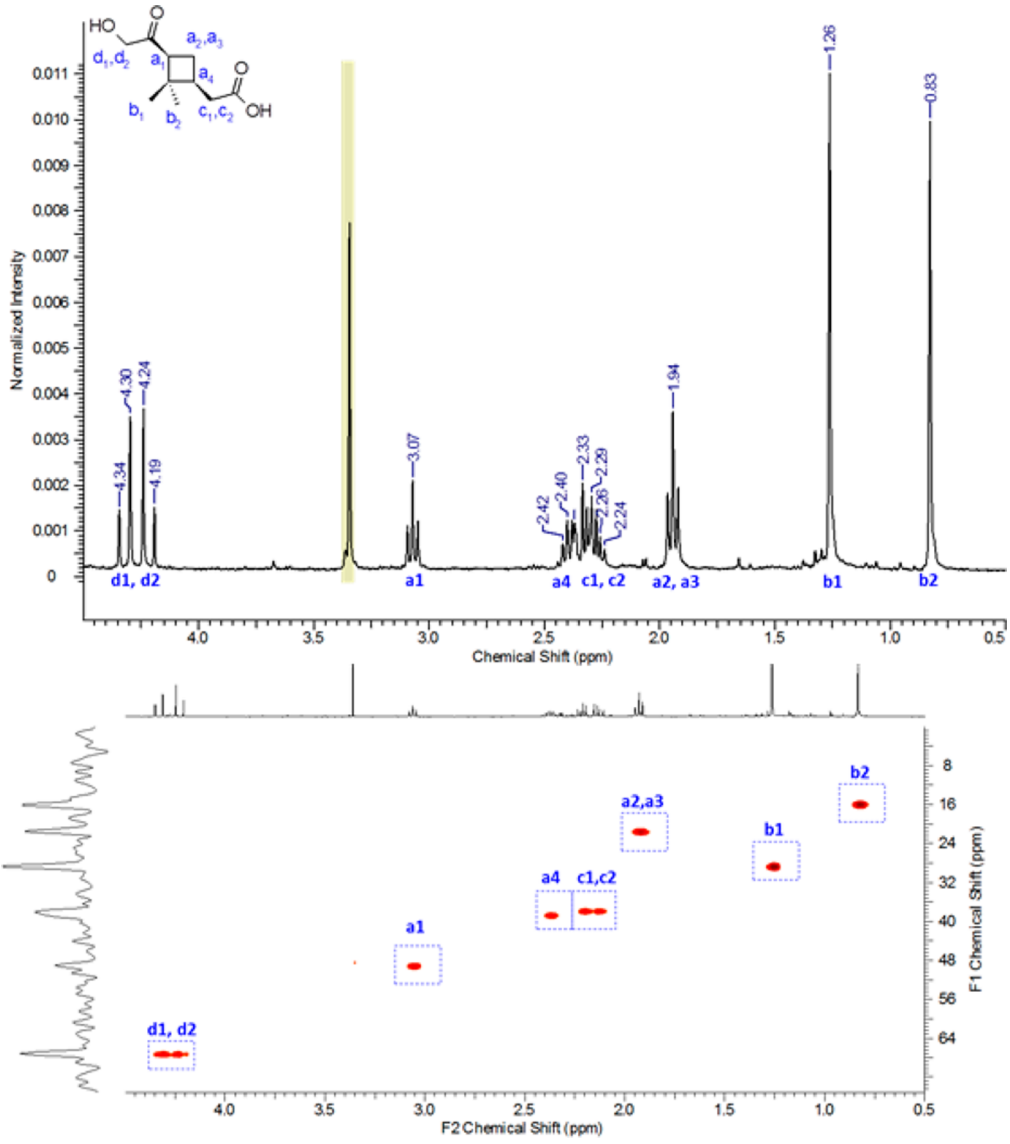

Figure 2. NMR spectra of fraction 5 identified as 10-hydroxypinonic acid. Upper: 1H-NMR between 0.5 and 4.5 ppm acquired at 400 MHz. Proton chemical shifts are shown on the top of the peaks whereas correspondences with the H atoms in the structure are indicated by the letters. The peak in the shaded region at 3.35 ppm corresponds to residual methanol. Lower: 2D ^{1}H-^{13}C NMR spectra acquired at 500 MHz. Peak labels refer to C atoms in the structure.

can be found in ref 17. The experiment was carried out during the ACID-PRUF campaign (Aerosol-Cloud Interactions, A Directed Programme to Reduce Uncertainty in Forcing) at 25 °C, with initial [α-pinene] = 50 ppb, [O_3] = 41 ppb and [NO_x] = 20 ppb, and relative humidity of around 70%.

RESULTS AND DISCUSSION

Aerosol Flow Reactor SOA Components Isolation. The aqueous extract of α-pinene SOA generated by the flow reactor was first analyzed using LC-UV/vis-MS using the analytical column. To ensure the concentrated SOA extract did not overload the HPLC column and mass spectrometer, only a 1 μL was injected, a factor of 60 lower than previously used for chamber generated SOA using α-pinene mixing ratios of 50–250 ppb. The chromatogram obtained is shown in Figure 1 (upper trace) and shows the expected α-pinene oxidation products based on previous studies, see Table 1 for assignments. The analytical column used in conventional HPLC has a fairly low loading capacity (1–5 mg) and larger injection volumes result in a loss of peak shape and ultimately separation. In order to allow larger injection volumes, the internal diameter of the column was increased to 10 mm. This larger column allows more mass to be loaded onto the column and therefore the possibility of semipreparative chromatography, where fractions of the eluent are collected. A linear scale up (factor of 4.7) was used to adjust the flow rate for the semipreparative LC method (2.8 mL min^{-1}). The chromatogram obtained with the semipreparative column, for an injection volume of 100 μL (5:1 split), is shown in Figure 1 (lower trace). Resolution factors between adjacent peaks were also slightly improved with the semipreparative method (R values much larger than 1.5) ensuring that separation between all compounds could be achieved.

Because at the high mass loading used all the compounds showed sufficiently strong UV absorptions to be observed, the UV/vis detector was used to determine elution retention time windows, shown in Table 1, to collect fractions containing

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individual α-pinene SOA compounds. Fractions were collected simply by manually diverting the flow into a clean glass vial during the retention windows. The LC method had good retention time stability, ensuring the same peak was collected from each replicate injection. A total of 11 fractions were isolated corresponding to structurally different types of SOA products with respect to the original α-pinene backbone: (a) products with the pinene cyclobutane ring still intact (norpinic acid, pinic acid, pinonic acid, two dimer esters[18]); (b) lactone-containing products (terebic, terpenylic and 2-hydroxyterpenylic acid); (c) ring-open products (MBTCA). Each fraction was subsequently analyzed using both NMR and FTICR-MS to investigate the purity and structure of the isolated material. One fifth of the mass collected from 5 × 100 μL injections was sufficient for the NMR analysis for most of the isolated compounds, whereas up to eight repeated injections were used for lower yield compounds.

Identification and Quantification of the Isolated SOA Tracers. The structural identification of the isolated fractions was carried out by NMR spectroscopy. The structures elucidated by NMR were found in good agreement with those proposed previously by tandem MS and high resolution MS data for α-pinene SOA components (previous MS spectra references are given in Table 1). High resolution FTICR-MS measurements of each fraction confirmed the molecular formulas of the isolated species, with most errors below 2 ppm. Some proton chemical shifts were available from previous literature data, i.e., for pinonic, pinic and norpinic acids.[19,20] Simulated ^{1}H NMR spectra calculated with ACD/HNMR predictor software (Advanced Chemistry Development, Inc., Toronto, ON) were also used as a supporting diagnostic tool. 2D ^{1}H-^{13}C correlation experiments were carried out for selected fractions for further confirmation of the assignments (terpenylic, 10-hydroxypinonic and pinonic acid). The 1D- and 2D-NMR spectra corresponding to fraction 5 identified as 10-hydroxypinonic acid are shown in Figure 2 for discussion, whereas the spectra obtained for the remaining fractions are reported in the Supporting Information (Figures S1−S12). The spectral region between 0.5 and 4.5 ppm corresponding to aliphatic protons is shown in all figures since no signals were observed above 4.5 ppm.

A distinctive feature characterizing the ^{1}H NMR spectrum of 10-hydroxypinonic acid (Figure 2, upper), and common to many α-pinene oxidation products, is the appearance of two intense singlets peaks between 0.5 and 1.6 ppm, corresponding to the two geminal methyls attached to the cyclobutane ring of the original α-pinene skeleton. The position of these two singlets is highly compound-specific depending largely on the shielding effect experienced by the two methyls respectively, i.e., on the different functional groups attached to the ring, allowing direct identification of each compound from their chemical shifts. Although for methyls attached to the ring, the difference between the two singlets in a spectrum can be rather large, it becomes smaller in the case of ring-opening products such as MBTCA (see Figure S4, Supporting Information). In the case of the dimers, a pair of singlets from each monomeric unit is expected in this region, i.e., four peaks in total. The ^{1}H NMR spectrum of the dimer at m/z 357, collected in fraction 10, clearly shows four singlet peaks, a pair at 0.95 and 1.20 ppm and a pair of peaks very close to each other at 1.48 and 1.51 ppm (see Figure S10, Supporting Information), confirming it contains a pinic and a diaterpenylic acid residue, respectively (see Figure S10, Supporting Information). The higher chemical shifts of the second pair, explained by the proximity of the methyls to an electron withdrawing group, are similar to the extent of the shift downfield occurring for the methyls of the lactone-containing products (see Figures S1−S3, Supporting Information). These highly compound-specific singlets peaks were integrated and used in this study for the quantification of the isolated products, as described later in the text.

The majority of the additional signals in the H NMR spectrum appears between 1.6 and 3.3 ppm, and arises from either alkylic protons attached to the ring or attached to carbonyls. The assignment of these signals is more complex due to the extent of the coupling occurring between both geminal and vicinal protons. Finally, signals of alkylic protons attached to hydroxylic groups appear at higher magnetic field, between 3.3 and 4.5 ppm, such as in the case of 10-hydroxypinonic acid (d protons in Figure 2). The 2D spectrum of fraction 5 (Figure 2, lower), where the crosspeaks indicate one-bond correlations between protons and carbon atoms (x- and y-axes, respectively), shows the presence of seven carbons at different chemical shifts as expected for the structure of 10-hydroxypinonic acid, further confirming the identification and purity of this compound.

A complete list of the protons chemical shifts and their assignments is given in the Supporting Information (Table S-1). Chemical shifts in Table S-1 are referred to the signal of the internal standard, TSP-d_4 (δ_{IS} = 0 ppm).

The internal standard (TSP-d_4) was added to the H NMR probe in a known amount to allow quantitation of the mass of each isolated compound to be obtained. The characteristic NMR singlets corresponding to geminal methyls were chosen here for quantification since they are highly compound-specific, account for three protons and appear in a relatively empty region, thus maximizing the signal-to-noise ratio and, in turn, the accuracy of the quantification. The accuracy of the quantification was tested using a commercially available cis-pinonic acid standard at a range of masses (0.01, 0.05, 0.5, 1.25 and 5.0 mg) and adding different volumes of TSP-d_4 solution 0.05% w/w (50 and 600 μL) and at the same settings used for the LC-fractions. Results of the comparison between the expected and the NMR-derived masses are summarized in Figure 3 where the ratio between the peak area used for quantification of the analyte (A_a) and that of the internal

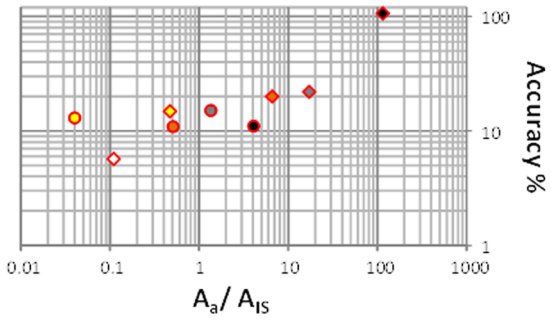

Figure 3. Accuracy (%) of *cis*-pinonic acid mass quantified with the NMR internal standard (TSP-d_4) plotted against the ratio between the peak area of the analyte (A_a) and that of the internal standard (A_{is}). Marker fill colors correspond to different amounts of *cis*-pinonic acid dissolved in the probe: 0.01 mg (white), 0.05 mg (yellow), 0.5 mg (orange), 1.25 mg (gray) and 5 mg (black) whereas diamonds and circles indicate 50 or 600 μL internal standard additions, respectively. Note that both axes are in logarithmic scale.

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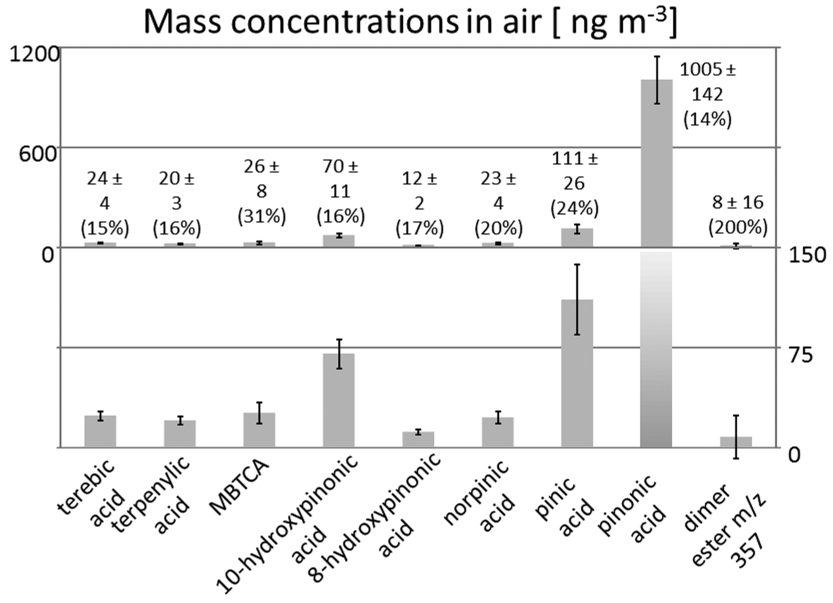

Figure 4. Absolute mass concentrations (ng m^{-3}) of the α-pinene SOA tracers in the chamber photo-oxidation experiment. Upper panel: full scale. Lower panel has been scaled for a better view of the compounds present in lower amounts. Errors bars correspond to the percentages uncertainties (given in brackets).

standard (A_{is}) is plotted against the percentage mass accuracy. The method was found to be robust and accurate within the investigated mass range (0.01−5.0 mg), with errors of around 10−20%, except for cases when the analyte peak was considerably bigger than the peak of the internal standard (i.e., $A_a/A_{is} \gg 10$). Thus, care has been taken not to exceed this value when quantifying the mass of the fractions, resulting in mass estimates within 20% of the compound mass. Good accuracy and repeatability values (≤5%) were found with a fairly short NMR acquisition time of 7 min. Moreover, the quantification of the isolated standards by using compound-specific NMR peaks offers an overall greater accuracy compared to other methods such as measuring the total carbon in the LC fractions or weighting the dried fractions, as it is not affected by the possible presence of impurities. Nevertheless, purity levels were high in most of the compounds isolated in this study. Specifically, the purity of the compounds has been estimated by dividing the peak areas of the geminal methyls used for quantification by the total area of peaks between 0.5 and 1.6 ppm. This ratio was found above 85% for terpenylic, pinic, pinonic, 10-hydroxypinonic acid and for the dimer m/z 357, with value as high as 98% for pinonic acid. Others compounds showing fairly high ratios (85−60%) included terebic, 8-hydroxypinonic and norpinic acid. Nevertheless, the compound-specific peaks in these spectra were still intense and sufficiently resolved not to affect the quantification of the corresponding standards.

The calculated masses obtained for the isolated compounds ranged from 0.04−3.0 mg of material. The majority of the isolated compounds produced well-resolved NMR spectra enabling the quantification, with the exception of 2-hydroxyterpenylic acid, MBTCA and the pinyl-hydroxypinonyl ester dimer (m/z 367) as a result of insufficient mass in the case of the former two compounds and because of the presence of larger levels impurities in the case of the dimer. Changes to the oxidant levels in the flow-reactor would likely increase the yields of MBTCA and 2-hydroxyterpenylic acid; however, no further efforts were undertaken within this study to obtain these compounds in larger amounts. Besides a low yield, in the case of the pinyl-hydroxypinonyl ester dimer (m/z 367), both the NMR and the FTICR-MS spectra indicated that this fraction contained other coeluting species (see Figure S11, Supporting Information) affecting its quantification and preventing its use as calibration standard. In summary, isolated compounds for which there was a lack of confidence in the integration due to poor NMR spectral resolution were not used as calibration standards, i.e., 2-hydroxyterpenylic acid, MBTCA and the pinyl-hydroxypinonyl ester dimer (m/z 367). However, synthetic MBTCA was available and this was used instead as a standard for quantification purposes.

Use of the Isolated Compounds as Reference Standards for Quantification. Calibration standards were made by diluting the fractions to eight different concentration levels in the range 0.01−10 ppm, in H_2O:MeOH (50:50). For all compounds, linear calibration curves ($R^2 \geq 0.99$) were obtained both in the low concentrations range (0.01−2.5 ppm) and in the high concentrations range (2.5−10 ppm) with a precision for repeated injections better than 10% for three intraday and 15% interday replicates. Calibration curves obtained using commercial cis-pinonic acid and synthetic MBTCA were tested against those obtained by the corresponding fractions. A good agreement was found for *cis*-pinonic acid, showing an error in the retrieved concentrations within the instrumental error, i.e., below 15%. On the other hand, a much higher deviation between the calibration curves was found in the case of MBTCA, as expected from the very low yields and consequently poor NMR quantification for this fraction. Thus, the synthetic compound was used as a calibration standard instead of the HPLC-isolated compound for the MBTCA quantification in the chamber samples.

The calibration data were used to quantify the abundances of these oxidation products in SOA formed in conditions close to the real atmosphere, i.e, during a photo-oxidation experiment using 50 ppb of α-pinene performed at the Manchester Aerosol Chamber as part of the NERC-funded project ACID-PRUF (Aerosol Cloud Interactions, A Directed program to Reduce

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Uncertainty in Forcing). Aerosol samples were collected onto filter papers at the end of the experiment and extracted into water using the protocols in Hamilton et al.[21] The absolute concentrations of the α-pinene oxidation products in the chamber air, obtained by dividing the filter mass loadings by the sampling volume (7 m^3), are shown in Figure 4 along with their associated errors. The associated errors were calculated by combining the standard errors in the predictions from calibration data and an additional 10% error to take into account the variability in NMR quantification of the compounds used as calibration standards. Details on the linear regressions used for their calibration are reported in the Supporting Information (Table S1). During this chamber experiment, *cis*-pinonic acid was the major identified product, with a mass loading of approximately 1 $\mu g\ m^{-3}$, followed by pinic acid and 10-hydroxypinonic acid at about 10× lower mass concentrations. The rest of the compounds were found in considerably lower amounts in the range of 10−30 $ng\ m^{-3}$. Although an extensive body of literature exists on the identification of α-pinene oxidation products, only a few publications have attempted quantification for a small subset of compounds.[6,7,22] In general, the dominant compounds quantified in this study, cis-pinonic, pinic and 10-hydroxypinonic acids, are consistent with the most abundant α-pinene SOA products observed in previous analysis.

The total SOA mass collected during this experiment was estimated to be around 37 μg using the average mass concentration measured by a differential mobility particle sizer (DMPS) within the collection period. The abundances of the quantified compounds relative to the total SOA mass are shown in Figure 5. The nine quantified products account for

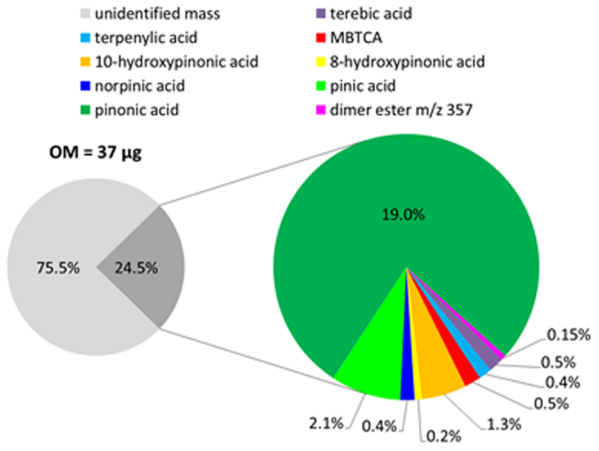

Figure 5. Percentage concentrations of the quantified α-pinene SOA tracers relative to the total OM mass collected during the chamber photo-oxidation experiment (OM = 37 μg).

25% of the chamber SOA mass. Although this result is comparable to previous attempts to determine anthropogenic SOA composition at the molecular level with GC−MS analysis (Forstner et al.[23]), it represents a large improvement compared to studies dealing with biogenic SOA where the lack of authentic standards hinders quantification efforts. Moreover, because losses due to sampling (semivolatiles) and extraction (water-insolubles) were not taken into account, the fraction of identified mass reported here represents only a lower limit. Nevertheless, an important fraction of α-pinene-SOA still eludes identification and more work is needed to understand the composition of this unresolved mass.

CONCLUSIONS

A cheap, simple and easy to implement method was developed to manufacture and isolate α-pinene oxidation products for calibration purposes. A small scale aerosol flow-reactor was used to produce a material similar to SOA, which although not likely to be identical to ambient SOA due to the high concentrations used to form it and the lack of extensive oxidation, still contains molecular markers in large quantities. This SOA-like material was extracted and 11 individual markers could be isolated by semipreparative HPLC-MS in sufficient amount for NMR analysis. NMR was used here with multiple purposes: (1) to confirm the identification of the products carried out by MS, (2) to assess the purity of the products and (3) their quantification by the use of an internal standard. The use of NMR for quantification presents some advantages with respect to the method of physical weighing, as it allows submilligram levels of collected mass to be more accurately determined with simultaneous identification and purity assessment in the same step. The distinctive NMR spectral features of α-pinene oxidation products, i.e., the clear singlets from the geminal CH_3 groups, were exploited in this case for both identification and quantification. However, this method has clear potential to isolate oxidation products from other SOA precursors. Using authentic markers as calibration standards, a much better quantification of the composition of ambient biogenic SOA becomes achievable. In this study, the quantification of SOA generated in a chamber simulating ambient conditions by using the isolated markers resulted in the identification of 25% of the total SOA mass from α-pinene oxidation, a significant improvement in obtaining a mass closure than in previous studies. However, this study highlights the fundamental gaps that still exist in our knowledge of SOA composition.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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