SUPPLEMENT for

Chemical characterization of the main secondary organic aerosol (SOA) products formed through aqueous-phase photonitration of guaiacol

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EXPERIMENTAL

S1. Chemicals

Acetonitrile (ACN) and methanol (Chromasolv gradient grade, for HPLC, ≥ 99.9%), tetrahydrofuran (THF, Chromasolv plus, for HPLC, ≥ 99.9%, inhibitor-free), diethyl ether (Puriss *p.a.*) and dichloromethane (DCM, Puriss *p.a.*) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2-propanol (LiChrosolv gradient grade, for HPLC, ≥ 99.9%), *n*-hexane (LiChrosolv gradient grade, for HPLC), nitric acid 65% (Emsure, for analysis) and sulfuric acid 98% (analysis grade) were purchased from Merck KGaA (Darmstadt, Germany). ACN and methanol for LC-MS use (Chromasolv LC-MS grade, ≥ 99.9%) were obtained from Fluka (Buchs (SG), Switzerland). High-purity water (18 MΩ cm) was supplied by a Milli-Q water purification system from Millipore (Bedford, MA, USA). Ammonia solution (25%, Suprapur) (Merck), ammonium formate and formic acid (Puriss *p.a.*, eluent additive for LC/MS) (both from Fluka), as well as ethylenediaminetetraacetic acid (EDTA, 99.995%) (Sigma-Aldrich), were used for HPLC mobile phase buffer and sample preparation. 4-nitroguaiacol (4NG) and 2-methoxy-5-nitrophenol (5-nitroguaiacol, 5NG) were purchased from Sigma-Aldrich. The standards had a purity higher than 95% and were used without further purification.

The following reagents were used for studying the aqueous phase reactivity of guaiacol: guaiacol (purity ≥98.0%), sodium nitrite (ACS reagent, ≥97.0%), catalase (from bovine liver, activity: 2000-5000 units/mg protein), sodium chloride (reagent grade, ≥98.0% + 80 mesh particle size) and magnesium sulfate (≥98.0%), and were all from Sigma-Aldrich. Hydrogen peroxide 30% (Perhydrol, *for analysis*) and sodium sulfate (anhydrous *for synthesis*) were obtained from Merck, while vitamin C (ascorbic acid, *puriss p.a.*, ≥ 99.0%) was purchased from Fluka.

Deuterated chloroform ($CDCl_3$, 99.8 atom% D stabilized with Ag, contains 0.03% *v/v* TMS) from Armar AG (Switzerland) was used as solvent for liquid-state NMR experiments.

S2. Extraction and concentration of products from guaiacol photonitration

The extraction and concentration of the products formed by photonitration of guaiacol were done only from the reaction mixtures with higer initial concentrations of reagents (see Section 2.1.2). The extraction of the products from the aqueous final reaction mixture was done using solid-phase extraction (SPE) on Oasis HLB (Hydrophilic Lipophilic Balanced) SPE cartridges (12 cm^3 barrel size, 500 mg polymeric RP-sorbent with 60 μm particles and pH range 0 - 14; Waters). The washing/conditioning solvents and solutions as well as the final reaction mixture were loaded on the cartridge using a positive pressure, at an approximate flow rate of 5 mL min^{-1}. The complete procedure of product extraction using HLB cartridge is as follows:

1. Washing of the HLB sorbent with 15 mL of ACN/methanol mixture (8/2, *v/v*);
2. Short drying of the sorbent by passing air through it;
3. Conditioning of the sorbent with 15 mL of Milli-Q water;
4. Loading of the final reaction mixture. The unretained material exiting the cartridge was directly loaded on another conditioned HLB cartridge, in order to test sample breakthrough. Significant sample breakthrough was detected after loading of cca. 100 mL of the final reaction mixture on the first HLB cartridge;
5. Washing of the sorbent with 15 mL 1% aqueous formic acid. If Milli-Q water is used instead, elution of the retained (colored) reaction products was observed;
6. Drying of the sorbent by sucking air through it for 15 min (employing negative pressure on the cartridge by using a water vacuum pump); and
7. Elution of the retained material on the sorbent with 15 mL of ACN/methanol mixture (8/2, *v/v*). The composition of the elution solvent was optimized for the highest product recoveries. After product elution using the ACN/methanol mixture, several other solvents were individually passed through the cartridge to test for highly retained compounds, such

as: 1% ammonia solution in methanol, 2-propanol, *n*-hexane and DCM. No highly retained material was observed after elution with the ACN/methanol mixture.

After SPE of the entire reaction mixture (250 mL), around 50 mL of reaction product extract in ACN/methanol (8/2, *v/v*) was obtained. The extract had a dark red to black color and was further concentrated to volume of cca. 5 mL with a rotary evaporator (bath temperature: 35 - 40 °C, vacuum pressure: 100 mbar). As the concentrated extract was opalescent, small aliquots of ACN were further added to it until a clear extract was obtained. The final extract (cca. 10 mL) was filtered through a PTFE membrane filter (Iso-Disc, 25 mm, pore size: 0.2 µm; Supelco), and thus ready for semi-preparative HPLC purification.

S3. Product isolation after semi-preparative HPLC purification

The pooled peak fractions (cca. 100 mL) were subjected to SPE to replace the organic solvent depleted aqueous buffer matrix with pure organic solvents. For that purpose, Oasis HLB cartridges (12 cm^3 barrel size, 500 mg polymeric RP-sorbent) and an extraction procedure described above (Section S2) were used. The final product isolates were dissolved in a ACN/methanol mixture (8/2, *v/v*) and were subjected to rotary vacuum evaporation until complete removal of the organic solvents. After this step, a small amount of residual water in the isolates was observed in several cases, which could result in difficulties with the preparation of the compound for NMR analysis (drying, crystallization). An additional purification procedure with the following steps was employed in such cases:

1. The wet isolate (compound) was first dissolved in cca. 10 mL of saturated aqueous NaCl solution and then 5 mL of 92 mM sulfuric acid was added;
2. It was transferred into a 50 mL separatory funnel and extracted three times with 10 mL of dichloromethane (DCM);
3. The DCM extracts were combined and dried with 1 g anhydrous sodium sulfate and 1 g of magnesium sulfate, for 10 min;
4. The dried DCM total extract was filtered through a sintered glass Büchner funnel, for salt removal;
5. The dried extract was transferred into a 50 mL conical-bottom flask and subjected to rotary vacuum evaporation (bath temperature: 40 °C and vacuum pressure: 500 mbar). After evaporation of DCM the solid residue (compound) was further dried by gradually increasing the vacuum from 500 down to cca. 15 mbar, in a period of 30 min;
6. The isolated compound was dissolved in 2 mL of DCM and quantitatively transferred into a clean, dry and weighted round-bottom flask (10 or 25 mL); and

7. The flask content was evaporated under the same conditions as described in *step 5* and the crystalized compound was further dried under high vacuum. Its mass was then determined by weighting.

S4. Preparation of the standard solutions

Standard stock solutions of 6NG and 4.6DNG with concentrations of 150 and 180 mg L^{-1}, respectively, were prepared in methanol. In addition, 100 mg L^{-1} standards of 4NG and 5NG were also prepared in methanol. Individual methanolic standard solutions were prepared by dilution of the stock standards (7.5 mg L^{-1} for 4NG and 5NG; 11.3 mg L^{-1} for 6NG and 13.5 mg L^{-1} for 4.6DNG). These individual standards were finally diluted in methanol/water mixture 3/7 (v/v) containing 5 mM ammonium formate buffer pH 3 and 200 μM EDTA, to give injection standards (150 μg L^{-1} for 4NG and 5NG; 1130 μg L^{-1} for 6NG, and 270 μg L^{-1} for 4.6DNG). The injection standards were used for the HPLC-ESI-MS/MS (SRM) identification of nitroguaiacols in ambient aerosols.

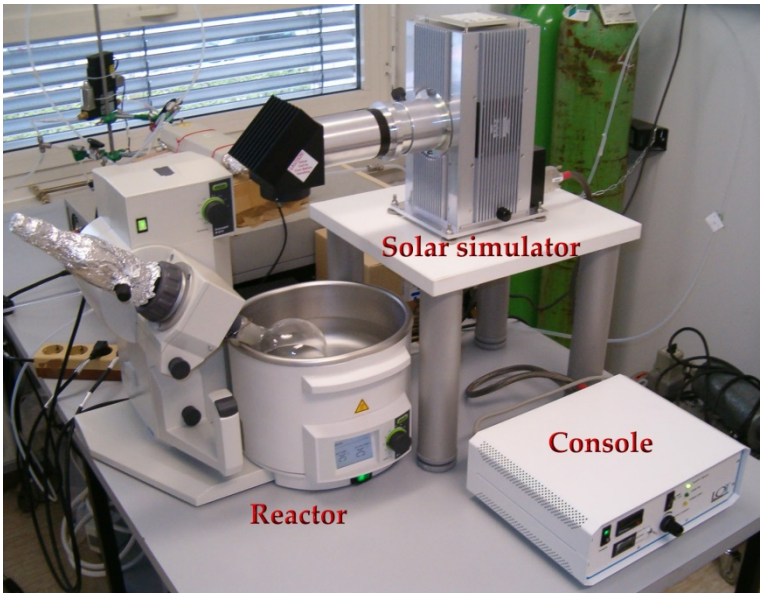
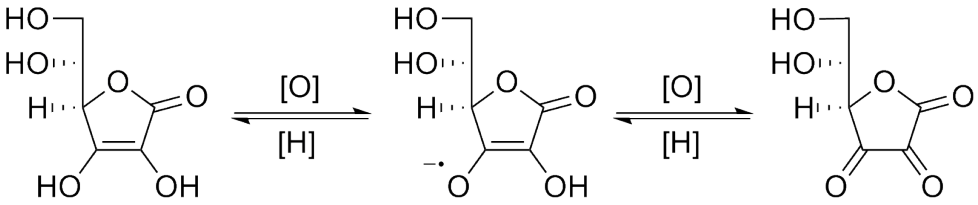


Figure S1. Experimental setup for studying aqueous phase reactions of guaiacol.



L-ascorbic acid semidehydro-L-ascorbic acid dehydro-L-ascorbic acid

Figure S2. The reversible redox system of ascorbic acid.

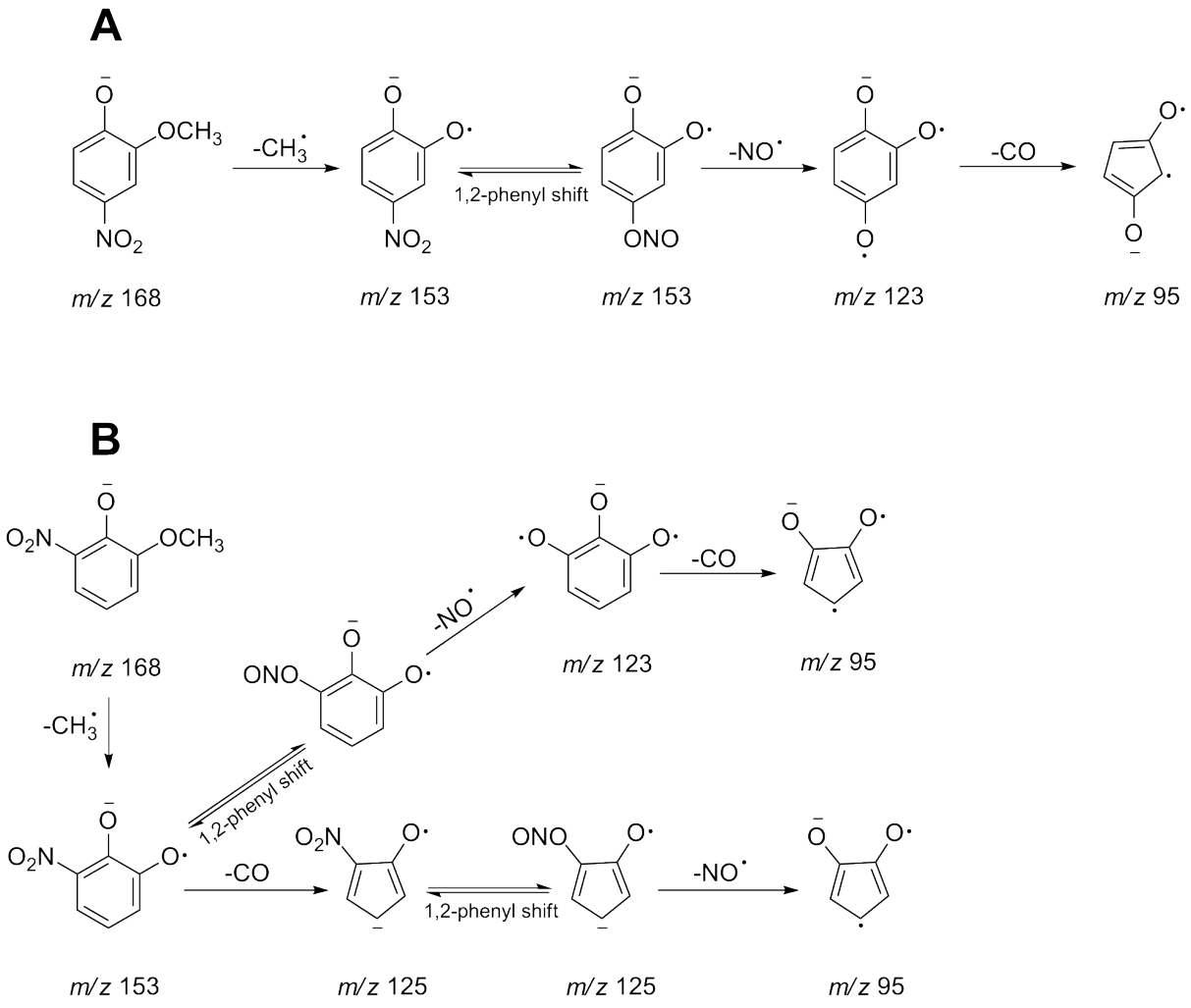


Figure S3. Proposed fragmentation pathways for 4NG (A) and 6NG (B).

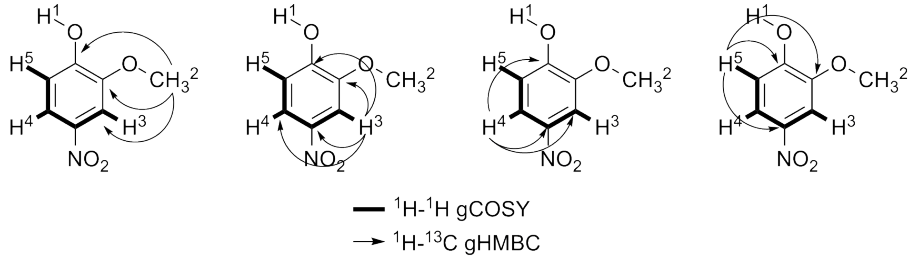


Figure S4. Important ^{1}H-^{1}H gCOSY and ^{1}H-^{13}C gHMBC correlations for 4NG.

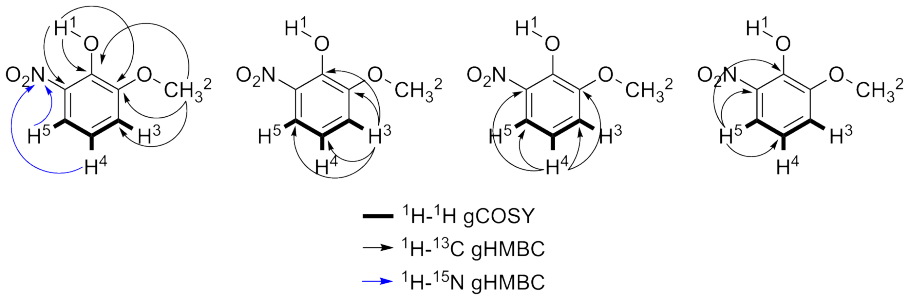


Figure S5. Important ^{1}H-^{1}H gCOSY, ^{1}H-^{13}C gHMBC and ^{1}H-^{15}N gHMBC correlations for 6NG.

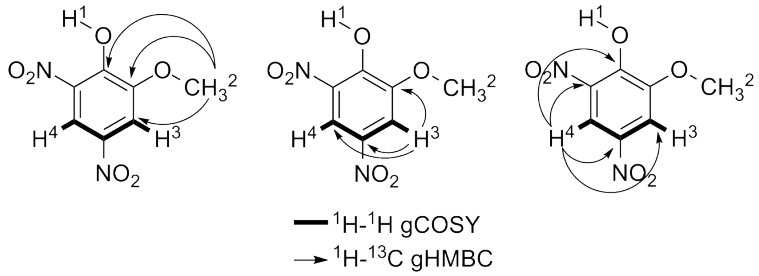


Figure S6. Important ^{1}H-^{1}H gCOSY and ^{1}H-^{13}C gHMBC correlations for 4,6DNG.

1 Table S1. Gradient elution program used for the semi-preparative method.

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Time (min)	Solvent A (%)	Solvent B (%)	Solvent C (%)
0	30	4	66
12	30	4	66
13	70	4	26
14	80	4	16
21	80	4	16
22	30	4	66

Solvent A: Acetonitrile (ACN)

Solvent B: Tetrahydrofuran (THF)

Solvent C: aqueous ammonium formate buffer, pH = 3 (final mobile phase buffer concentration: 5 mM)

* additional column re-equilibration time: 14 min

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