Carbon Suboxide, a Highly Reactive Intermediate from the Abiotic Degradation of Aromatic Compounds in Soil

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The formation of volatile compounds during abiotic degradation processes of aromatic compounds in soil has been the subject of many experimental studies but should be examined further. In this context, the present work investigates the natural formation of carbon suboxide using the model compounds catechol and 3,5-dichlorocatechol and also a soil sample from a peat bog. The measurements were performed with a purge and trap GC/MS system following various optimization steps. Under certain conditions, we obtained 16.7 ng of carbon suboxide from a 250 mg soil sample. We also found that the formation of carbon suboxide requires a definite activation energy and that it is rather short-lived in the natural environment. A subsequent reaction to malonic acid is expected in the presence of water. It is shown that iron(III), hydrogen peroxide, and chloride are prerequisites for its formation. Experimental parameters for the highest yield of carbon suboxide depend on the precise molecular structure of the model compound or on the individual soil sample, respectively. The presented results point to a new degradation process for aromatic compounds in soil.

Introduction

With four successive double bonds, carbon suboxide (O=C−C=O−C=O) is a very unstable compound. This linear and symmetric molecule, known as doubly dehydrated malonic acid, is a toxic gas with an unpleasant odor. Its escharotic property is principally due to a remarkable charge distribution. The central carbon atom is the most nucleophilic center of the molecule, and it is an important step that is expected in the presence of water. It is shown that iron(III), hydrogen peroxide, and chloride are prerequisites for its formation. Experimental parameters for the highest yield of carbon suboxide depend on the precise molecular structure of the model compound or on the individual soil sample, respectively. The presented results point to a new degradation process for aromatic compounds in soil.

Materials and Methods

Chemicals. The following chemicals were used as received: catechol (99%; Lancaster Synth), 4-chlorocatechol (97%; Aldrich), 3,5-dichlorocatechol (97%; Aldrich), 4,5-dichlorocatechol (97%; Aldrich), isopropyl (99%; Aldrich), 4-chlorocatechol (97%; Aldrich), 3,5-dichlorocatechol (97%; Aldrich), potassium chloride (99%; Merck), iron(III) sulfate (Fe 21−23%; Riedel-de-Haën), potassium chloride (99%; Merck), and sodium hydroxide (99%, Aldrich). Doubly distilled deionized water (18 MΩ cm) from an ELGASTAT UHQ PS water treatment system, was employed in all experiments. All pH measurements were made using a Mettler Toledo 320 pH meter, calibrated on the free hydrogen scale with 4.0 and 7.0 buffers.

Because of their possible emission into the atmosphere, the natural formation of volatile compounds has been the subject of numerous publications over the past years (6−9). Biotic processes through mammals, insects, fungi, plants, and bacteria leading to methane, chloromethane, and chloroform are well-known. However, abiotic processes have been attracting more and more attention. Many investigations have been performed discussing the degradation of soil organic matter (SOM) through different redox processes. As the structure of SOM is very complex, it is common to use model compounds, such as substituted phenols or catechols, which represent fragments of the humic substances in soil (7).

Hydroxyl radicals play an important part in these redox processes because they readily react with organic molecules initializing subsequent oxidation steps. Their release from the reaction of redox-active metals such as iron or manganese with members of the oxygen family (hydrogen peroxide, superoxide, molecular oxygen, etc.) is explained by the Fenton (10) and Fenton-like or Haber−Weiss (11) reactions. Small molecules such as chloroethene, chloroethene, and carbon dioxide are formed during the degradation of catechol in the presence of Fe(III) and chloride (12−15). For their formation, cleavage of the aromatic ring is an important step that is followed by further redox reactions.

Chloroethene and carbon dioxide merely comprise small fragments of the aromatic ring; thus, we expected further volatile compounds that are generated in the course of SOM decomposition. Carbon monoxide and ethene are some plausible candidates. On the basis of mechanistic models, we postulated that carbon suboxide is a conceivable intermediate among the C3-fragment molecules during the cleavage of the aromatic ring. This finally led to the assumption that the natural formation of C3O2 in soil should be possible.

To verify this hypothesis, we selected catechol as the starting model compound because it is a constituent of natural SOM. Iron as a redox-sensitive element, promoting the necessary electron transfer for the degradation reaction, and hydrogen peroxide as a strong oxidant were chosen. Additionally, chloride was used to enable halogenation processes during the degradation pathway, which may affect the formation of carbon suboxide. Preliminary results from the catechol reaction showed an elevated yield, if either chloride or chlorine covalently bound to the aromatic structure are present. A chlorinated catechol was therefore applied in a series of optimization steps, wherein the influence of different parameters on the production of C3O2 was tested: the time dependence, the concentration of hydrogen peroxide, the concentration of chlorinated catechol, the pH value, and the temperature. To confirm a possible natural formation of carbon suboxide, studies with soil samples were carried out, which are discussed in the last section of this paper.

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Experimental Procedure. The experiments were carried out in airtight closed 20 mL headspace glass vials, filled with 10 mL of aqueous solution. They were shaken for a determined time on a rotary board at 500 rpm at a precise temperature. After introduction of an inlet and an outlet stainless steel needle through the septum, the volatile compounds were purged for 4 min with a stream of helium at 40 mL/min and concentrated on a cooled trap (below 0 °C), filled with Tenax. Moisture was removed on a magnesium perchlorate water trap from the sample stream. The loaded preconcentration trap was switched into the GC carrier gas line (1 mL/min) and heated to 180 °C. The desorbed compounds were refocused at the beginning of the capillary column, which was cooled with liquid nitrogen to −90 °C. After 4 min, the GC oven temperature program started automatically: −90 to 0 °C at 40 °C/min, hold 1 min, 0 to 150 °C at 5 °C/min, hold 5 min, 150 to 210 °C at 30 °C/min, hold 15 min.

Preliminary Tests. The formation of carbon suboxide was examined using catechol as well as chlorinated catechols. In these assays, solutions of 10 mL of water with initial concentrations of 0.50 mM organic compound, 2.50 mM Fe2(SO4)3, 5.00 mM H2O2, and 5.00 mM KCl were prepared and shaken for 20 s at 40 °C.

Process Optimization. On the basis of the preliminary results, 3,5-dichlorocatechol was chosen for the next series of experiments. The yield of carbon suboxide was to be maximized by systematic sequential variation of the following reaction parameters.

Time Dependence. The first optimization step was to elaborate the time dependence of C2O3 formation and degradation. Solutions of 10 mL of water with initial concentrations of 0.50 mM 3,5-dichlorocatechol, 2.50 mM Fe2(SO4)3, and 5.00 mM H2O2 were prepared and shaken at 40 °C for different durations.

Concentration of Hydrogen Peroxide. The second series of measurements concerned the influence of hydrogen peroxide on the yield of C2O3. Therefore, solutions of 10 mL of water with initial concentrations of 0.50 mM 3,5-dichlorocatechol and 2.50 mM Fe2(SO4)3, and 5.00 mM H2O2 were prepared and shaken at 40 °C for different durations.

Concentration of 3,5-Dichlorocatechol. The third parameter to be checked was the amount of 3,5-dichlorocatechol in relation to the oxidants. For this purpose, solutions of 10 mL of water with initial concentrations of 2.50 mM Fe2(SO4)3 and 0.71 mM H2O2 were prepared together with different concentrations of 3,5-dichlorocatechol and shaken for 1 min at 40 °C.

pH Value. To test for a possible pH dependence of the carbon suboxide production, solutions of 10 mL of water with initial concentrations of 0.50 mM 3,5-dichlorocatechol, 2.50 mM Fe2(SO4)3, and 0.71 mM H2O2 were prepared. The pH was adjusted by adding different quantities of NaOH. The solutions were shaken for 1 min at 40 °C.

Temperature. Finally, the applied reaction temperature was varied. Solutions of 10 mL of water with initial concentrations of 0.50 mM 3,5-dichlorocatechol, 2.50 mM Fe2(SO4)3, 0.71 mM H2O2, and 12.5 mM NaOH were prepared and were shaken for 1 min at different temperatures.

Catechol. Using the results of the optimization procedure on 3,5-dichlorocatechol, a further enhancement of the observed C2O3 production from catechol was assayed. For this, solutions of 10 mL of water with initial concentrations of 0.50 mM catechol, 2.50 mM Fe2(SO4)3, 5.00 mM H2O2, and 5.00 mM KCl were prepared and shaken for 1 min at 40 °C.

Soil Sampling. The next logical step was to apply the knowledge gained with the model compounds to natural samples. The employed peatland soil was sampled in March 1996 from the natural reserve Rotwasser, Odenwald/Germany (49°36′39″ N, 8°53′11″ E). After the collection, it was freeze-dried and milled to a fine powder. A total of 250 mg of this powdered soil was used for each experiment. The characterization of the soil is shown in Table 1.

Identification and Quantification of Carbon Suboxide. For a qualitative identification, C2O3 was produced by the thermolysis of malonic acid bis(trimethylsilyl) ester (17) and introduced into the purge and trap GC/MS system. The C2O3 peak appeared after a retention time of 13.40 min showing m/z 68 (M+) and 53 (M+ − O + H) as the most prominent fragments of its mass spectrum. Nevertheless, quantification of this compound seems to be simple, due to its escharotic behavior and to the lack of standard reference material. Therefore, to estimate the response factor of carbon suboxide, according to previous arguments in a related case (14), we used a roughly similar molecule: isoprene. Both compounds were assumed to have an identical recovery during the different steps of the purge and trap GC/MS method. Furthermore, both analytes have comparable ionization energies: 10.6 eV for carbon suboxide and 8.86 eV for isoprene (18). The mass spectrometric response of a compound is determined by two factors: the ionization cross-section for 70 eV electrons, which is largely dependent on the ionization energy of the molecule, and the relative abundance of the ions in the spectra selected for quantification. The difference in the ionization potentials of both compounds should be of minor importance, as electrons with an energy of 70 eV are used in the MS. The selected masses for chromatographic quantification were m/z 68 in the case of carbon suboxide and m/z 53 for isoprene.
Therefore, the response factor of C₃O₂ can be estimated according to eq 1

\[ \text{rf (C}_3\text{O}_2) = \frac{\text{abundance (m/z 68)}}{\text{abundance (m/z 53 + 67)}} \text{rf (isoprene)} \quad (1) \]

The relative abundances were calculated from reference spectra with a mass scan range from m/z 10 to 249, recorded at different compound concentrations. For isoprene, the relative weight of the quantitation ions m/z 53 + 67 remained approximately constant at 33.7%, whereas in the case of carbon suboxide, the computation turned out to be more complicated. At higher concentrations, we observed the decrease of m/z 68 and the formation of an ion at m/z 80 that was generated by collision processes in the ion trap and that has already been observed by Bortolini et al. (1).

Therefore, the abundance of m/z 68 was calculated for each measurement individually, assuming that the error becomes more important at higher concentrations.

**Results and Discussion**

**Preliminary Tests.** The results of the preliminary assays on carbon suboxide formation from catechol and its chlorinated analogues are shown in Figure 1. Because 3,5-dichlorocatechol as well as 4,5-dichlorocatechol were the most effective starting compounds for C₃O₂ production, the former was selected for the optimization test series.

**Optimization.**

**Time Dependence.** The measurements show that the optimum time for highest carbon suboxide yield is very short, approximately 30 s to 1 min. An exponential decrease is observed, and C₃O₂ is no longer detectable after 30 min (Figure 2A). This agrees with its known property of...
being rather unstable and readily reacting with nucleophilic compounds such as H2O to form malonic acid. To reduce these losses, yet affording a practicable experimental protocol, an agitating time of 1 min was chosen for the next optimization steps.

Concentration of Hydrogen Peroxide. When the concentration of hydrogen peroxide is varied, an optimal production of carbon suboxide is achieved at a molar ratio of 1:7 as compared to the concentration of iron(III) (Figure 2B). No C3O2 is detected in the absence of H2O2, which indicates that, for the production of carbon suboxide, the presence of OH radicals is necessary. They are generated via Fenton reaction from H2O2 and Fe(II), the latter through the preceding reduction of Fe(III) by the organic substrate. On the other hand, H2O2 concentrations exceeding a 1:7 molar ratio to iron are not favorable for an optimal yield. Presumably, side reactions, leading to higher oxidized product compounds (e.g., CO and CO2 (15)) become more efficient under these conditions. Therefore, a ratio of 1:7 (H2O2/Fe) was used to evaluate the other parameters.

Concentration of 3,5-Dichlorocatechol. In the third optimization step, the yield of C3O2 has to be set in relation to the concentration of the organic matter employed because higher concentrations deliver more material for the degradation. This ratio (amount of carbon suboxide / employed molar quantity of 3,5-dichlorocatechol) shows a distinct maximum at a molar concentration of 3,5-dichlorocatechol, which is very close to 1/10 of the Fe(III) molarity (Figure 2C). Small deviations of the 3,5-dichlorocatechol concentration from this value result in lower amounts of carbon suboxide. Hence, a definite stoichiometric ratio between iron and 3,5-dichlorocatechol seems to be as important as an appropriate hydrogen peroxide concentration. Consequently, an initial concentration of 0.25 mM 3,5-dichlorocatechol was used for the next optimization steps.

pH Value. Thus far, the pH value of the solution was mainly determined by the concentration of iron(III) sulfate. The addition of sodium hydroxide shows that the optimized C3O2 yield from 3,5-dichlorocatechol is more favorable at pH values ranging from 2.7 to 3.5 (Figure 2D). The production of carbon suboxide increases by a factor of 10 with respect to the case where no sodium hydroxide is used (pH 2.2). Moreover, the measurements reveal a very sensitive system under these conditions, especially regarding iron speciation. By adding sodium hydroxide to the solution, a part of the Fe(III) precipitates as hydroxide, and the amount of Fe(III) available for the reaction is reduced. Particularly small differences in the preparation, the handling, the shaking, or the transferring of the samples lead to fluctuating yields of C3O2. Therefore, reproducible results are significantly more difficult to achieve than at lower pH values, explaining the larger error bars in the figures. Hence, the employed NaOH concentration for the last optimization step was chosen to be 12.5 mM.

Temperature. The experiments performed at room temperature up to 70 °C show approximately constant amounts of carbon suboxide above 30 °C, with a distinct decrease to lower temperatures (Figure 2E). This finding could be explained by an appreciable activation energy necessary for the production of carbon suboxide, which competes with C3O2-destroying processes at higher temperatures.

Finally, starting from 5 µmol of 3,5-dichlorocatechol in the preliminary tests, the different optimization steps of the carbon suboxide production permitted us to accomplish a yield of 7.9 µg of C3O2, which is 1000 times higher than before the optimization. Next to the time dependence of the reaction, the influence of the pH value proves to be the most important. Nevertheless, it is also obvious that in this sensitive system, further maxima of C3O2 can be expected from small simultaneous parameter changes.

Catechol. The experiments with catechol demonstrate that a production of carbon suboxide is possible. However, the conditions are somewhat different as compared to those employed with 3,5-dichlorocatechol. An important factor is potassium chloride. In the absence of KCl, no carbon suboxide is detected. However, when larger amounts of KCl are added, the C3O2 yield increases significantly. In comparison, the experiments with 3,5-dichlorocatechol do not show any effect of potassium chloride addition. This indicates that the presence of chlorine in the system, either as substituent or anion, is necessary for the production of C3O2. However, it remains difficult to decide whether covalently bound Cl atoms or anionic Cl− are more efficient for the formation of carbon suboxide. Indeed, the higher yield obtained from 3,5-dichlorocatechol (approximately 109 ng) as compared to that obtained with catechol (approximately 8.8 ng) under similar conditions might favor the covalently bound chlorine.

The necessity of chloride for the carbon suboxide formation from catechol points to a chlorinated intermediate (e.g., chlorocatechol) that is subsequently hydroxylated to form highly unstable tetrahydroxybenzene leading to two C3O2.
molecules after ring fission (Scheme 1). But so far, experimental data do not convincingly imply that chlorinated catechols or quinones are always required as intermediates.

**Soil Samples.** In contrast to 3,5-dichlorocatechol, catechol can be found as a natural constituent of SOM. Therefore, this compound is a more adequate representative for soil, and it is plausible to apply reaction conditions that have been optimized for a maximum C$_{3}$O$_{2}$ yield from catechol rather than from 3,5-dichlorocatechol. The corresponding GC chromatogram and mass spectrum are shown in Figure 3 A,B.

As with catechol, the addition of potassium chloride is crucial. The small amounts of carbon suboxide obtained without KCl could arise either from chloride (4.5 ppm) or from covalently bound chloride already present in the soil samples.

In summary, in the catechol experiments, we obtained 8.8 ng of C$_{3}$O$_{2}$ from 0.36 mg of carbon, whereas 16.7 ng of C$_{3}$O$_{2}$ resulted from 4.1 mg of carbon in the soil sample (Table 1). In addition, the concentrations of Fe(III), H$_{2}$O$_{2}$ and Cl$^{-}$ required to obtain the elevated carbon suboxide yields from the natural soil samples, were slightly higher than those from the model compound catechol. The following hypotheses are conclusive, due to the complex structure of SOM: the organic carbon of the soil sample basically consists of other non-degradable organic compounds, or the formed carbon suboxide is intercepted before it is liberated into the gas phase, or the oxidants are consumed by other constituents in the soil. Therefore, the study of these processes has to be individually optimized for different soils.

This work suggests that the formation of C$_{3}$O$_{2}$ points to a new degradation pathway for aromatic structures that also takes place in soil. Up to now, cleavage of the C–C bond between the hydroxyl groups of catechols, leading to dicar-bonic acids such as muconic acid (7, 15, 19–21), or the complete destruction of the aromatic ring into small compounds such as CO$_{2}$, vinyl chloride, and chloroethyne (7, 12–15) has been known. From the present study, it seems conceivable that the electron-rich phenolic C$_{6}$ ring could also be split symmetrically, resulting in two C$_{3}$O$_{2}$ molecules without a loss of carbon atoms. Further investigations should focus on polar intermediates to elucidate the precise degradation pathway.

**Acknowledgments**

We thank B. Breuninger and D. Pieroth for execution and assistance in a portion of the experimental research during this work as well as A. Cheburkin and S. Rheinberger for their technical support. We also thank J. Pracht and F. Keppler for collecting the Rotwasser soil sample. This work was supported by the German Research Foundation (DFG).

**Literature Cited**


Received for review June 22, 2007. Revised manuscript received August 30, 2007. Accepted September 7, 2007.

ES071530Z