Oxidation of Aminodinitrotoluenes with Ozone: Products and Pathways

RONALD J. SPANGGORD,* C. DAVID YAO, AND THEODORE MILL

SRI International, 333 Ravenswood Avenue, Menlo Park, California 94025

An investigation of the products from the reaction of ozone with aminodinitrotoluenes (ADNTs) provides information about the oxidation pathway. Studies conducted at low conversions of 2- and 4-ADNT show 2:1 ozone/ADNT stoichiometries, prompt formation of glyoxylic and pyruvic acids, and NO_2^- and NO_3^- (NO_x) ions. Reaction schemes to account for these results involve a 1,3-dipolar cycloaddition of ozone to selected double bonds of the aromatic ring, leading to ring cleavage. ¹⁵N-Labeling experiments indicate that the amino function is not involved in the initial ozone oxidation and eventually is incorporated into pyruvamide (2-ADNT) and oxamic acid (4-ADNT) before being oxidized to nitrate.

Introduction

Aminodinitrotoluenes [2-amino-4,6-dinitrotoluene (2-ADNT) and 4-amino-2,6-dinitrotoluene (4-ADNT)] are environmental pollutants that arise from the microbial biotransformation of 2,4,6-trinitrotoluene (TNT). These chemicals are unique to soils, lagoons, and groundwaters near TNT production and handling facilities. In the remediation of such environments, consideration must be given to the behavior of these pollutants toward the remediation technology. In the previous paper (1), we investigated the kinetics of the ozone-hydrogen peroxide (peroxone) oxidation of 2- and 4-ADNT and found that, under most peroxone usage conditions, the oxidation of ADNTs is controlled by ozone. In using an oxidation remediation process, it becomes important to know the final end products formed under the treatment conditions. In this investigation, we determined the products resulting from ozone oxidation of 2- and 4-ADNT and evaluated products from oxidation of ¹⁵N-amino-labeled ADNTs, from which multiple potential oxidation pathways can be advanced.

Many of the mechanistic approaches to the oxidation of organic compounds by ozone can be described by the Criegee mechanism (2, 3). The initial steps for ozonation of an aromatic compound appear to involve sequential formation of a π -complex (I), an addition product (II), a zwitterion (III), and an isoozonide (IV) as shown here for benzene.

In aqueous systems, water can play a major role in the decomposition of intermediates I-IV to form aldehydes or ketones and hydroxyhydroperoxides (V). If the aromatic ring has substituents, reaction with ozone will be significantly



accelerated with electron-donating groups and retarded with electron-withdrawing groups. Reaction pathways and prod-



ucts can become complex, depending on the nature and number of the substituents (*3*). Both electron-donating (amino, methyl) and electron-withdrawing (nitro) groups in ADNTs create reactive centers for ozonide decomposition in water and lead to a multiplicity of products. The ADNTs serve as interesting examples of ozone oxidations in aromatic systems with multiple reaction sites and the products provide pathway information in electron-deficient systems. With the exception of a few simple aromatics and polycyclic aromatics, detailed characterization of polysubstituted aromatic ozone reactions has not been done.

Experimental Section

Chemicals. 2- and 4-ADNT were prepared in our laboratory at 99% purity using the method of Zbarskii (4). ¹⁵N-Labeled aminodinitrotoluenes were prepared by the method of Spanggord and Clizbe (5). The remaining chemicals were obtained from Aldrich Chemical Co. (Milwaukee, WI) or synthesized where indicated. An authentic standard of oxamic acid aldehyde was prepared using the method of Tits and Bruylant (6); pyruvamide was prepared using the method of Claison and Shadwell (7). Both standards yielded chromatographic retention volumes and UV spectral data identical to those for ozonation products from ADNTs.

Ozone Oxidations. Ozone oxidations were conducted in aqueous solutions adjusted to pH 5.0 with phosphate buffer. Ozone was generated from a Welsbach ozone generator and bubbled into water. Ozone concentrations were measured using the indigo colorimetric method (ϑ).

An ozone stock solution was added in 20 μ M increments to a rapidly stirred stock solution of ADNT (150 μ M). After each addition, the ADNT was analyzed by reverse-phase high-performance liquid chromatography (HPLC), using a gradient program developed for aldehydes and ketones as their 2,4-dinitrophenylhydrazones (DNPHs, *9*) and using an Alltech Altima C18 column (5 μ m, 4.6 \times 250 mm) and UV detection at 350 nm. Under these conditions, the ADNTs eluted at 11.4 min. Carboxylic acids were analyzed by ion chromatography using a Supelcogel 610H column and UV detection at 210

VOL. 34, NO. 3, 2000 / ENVIRONMENTAL SCIENCE & TECHNOLOGY = 497

^{*} Corresponding author phone: (650)859-3822; fax: (650)859-4321; e-mail: ronald.spanggord@sri.com.



FIGURE 1. HPLC profile of carbonyl-containing products resulting from the ozonation of 2-ADNT. B, glyoxylic acid; C, 2,4-dinitrophenylhydrazine; H, pyruvamide; D, pyruvic acid; I, 2-ADNT, J, glyoxal; F, pyruvaldehyde.

nm or a Dionex PAX-500 column with conductivity detection. Structural identifications were made by mass spectrometry and/or chromatographic retention times coupled with UV spectral data obtained from a Waters photodiode array detector (PDA) as compared to authentic standards.

 NO_x *Ions.* Nitrite and nitrate were analyzed by ionexchange liquid chromatography with UV detection at 210 nm according to the method of Thayer and Huffaker (*10*). ¹⁵N-Labeled nitrite and nitrate were analyzed by mass spectrometry using the method of Glover and Hoffsommer (*11*) after conversion of each anion to nitrobenzene.

Results

Products at High (95%) ADNT Conversions. The oxidations of 2- and 4-ADNT by ozone were followed by HPLC by

monitoring the formation of carbonyl-containing compounds (as their 2,4-dinitrophenylhydrazones), carboxylic acids, and nitrite and nitrate using incremental additions of ozone to oxidize up to 95% of the ADNT. Typical HPLC profiles of carbonyl-containing compounds formed at 95% conversion of 2- and 4-ADNT are shown in Figures 1 and 2. Products identified from 2-ADNT include glyoxylic acid, pyruvic acid, pyruvamide, glyoxal, and pyruvaldehyde. From 4-ADNT, the products include pyruvic acid, glyoxylic acid, oxamic acid, oxamic acid aldehyde, pyruvaldehyde, and a compound tentatively identified as a tricarbonyl-containing amide. The formation and decline of these products as a function of ozonation time are shown in Figure 3 for 2- and 4-ADNT. It appears that, for both compounds, pyruvic acid is generated as a stable end product.



FIGURE 2. HPLC profile of carbonyl-containing products resulting from the ozonation of 4-ADNT. A, oxamic aldehyde; B, gloxylic acid; C, 2,4-dinitrophenylhydrazine; D, pyruvic acid; G, 4-ADNT; E, product 1; F, pyruvic aldehyde.

HPLC profiles, showing a similar set of short-chain carboxylic acids for each ADNT, are found in Figure 4. These acids include acetate, glycolate, formate, glyoxylate, pyruvate, malonate, and oxalate. Most of these acids, with the exception of glyoxylic acid, are terminal end products from the ozonation of ADNT intermediates.

Nitrogen Balances. Nitrogen balances in oxidized ADNTs are largely composed of NO_x ions at low (6–45%) and high (69–99%) conversions of ADNTs, with the exception of pyruvamide, which appears in significant amounts in the high conversions of 2-ADNT (Table 1). NO_x ions together

account for only 50% of the available nitro groups at low conversions of 2-ADNT and 25-35% of oxidized 4-ADNT. They account for >90% of the nitro groups at high conversions (Tables 1 and 2).

Ozonation of ¹⁵N-labeled amino-2- and 4-ADNT produced NO_x ions that were converted to nitrobenzene and analyzed for ¹⁵N content by mass spectrometry by comparing the ratio of m/z 123/124 (¹⁴N-labeled nitrobenzene/¹⁵N-labeled nitrobenzene). No ¹⁵N-labeled nitrobenzene was detected, indicating that amine nitrogen is not involved in the initial ozonation of ADNTs. ¹⁵N-Labeled oxamic acid was identified



FIGURE 3. Quantitative determination of 2,4-dinitrophenylhydrazone derivatives of products from (A) 2-ADNT and (B) 4-ADNT.

TABLE 1. Nitrogen Balance^a on Products from the Ozonation of 2-ADNT (μ mol)

ADNT	∆ADNT	nitrate	nitrite	pyruv- amide	total product N	% N balance	% N based on NO _x
160	0	0	0	0	0		
50.8	109	200	20	18.9	240	73	67
20.4	140	390	30	27.2	447	106	100
10.3	150	410	22	25.9	458	102	96
2.0	158	425	18	21.8	465	98	93

^{*a*} Sample calculation: At the first point, 109 μ mol of ADNT consumed corresponding to 327 μ mol of nitrogen produced (3 × 109). Total micromoles of nitrogen from products = 240 μ mol. Percent nitrogen balance = 240/327 × 100 = 73%. Percent N balance from NO_x = (200 + 20)/327 = 67%.

in the reaction mixture from ozonation of 4-ADNT by mass spectrometry after converting it to the methyl ester. Its mass spectrum showed a molecular ion one mass unit higher than reported for methyl oxamate by Woodburn et al. (*12*). After extended ozonation, >95% of the nitrogen in both ADNTs appear as NO_x ions, showing that pyruvamide and oxamide intermediates are eventually oxidized further.

Equations 1 and 2 illustrate the pathways for conversion of ADNT nitrogens to products at low conversions.



Carbon Balances. Carbon balances were determined by quantifying known products as a function of the amount of ozone added to the ADNT solutions. Data for 2-ADNT, in Table 3, show that recovery of carbon in products is nearly constant at about 48% over the conversion range of 67–99%. Data for 4-ADNT, in Table 4, show a lower recovery of carbon between 32 and 40%. Over the course of the oxidations, total organic carbon (TOC) was found not to change significantly, indicating that little carbon was oxidized to CO₂. We believe several four- and five-carbon carbonyl products from ring cleavage account for the missing carbon; these products appear in HPLC profiles of product dinitrophenylhydrazone (DNPH) derivatives but could not be identified or quantified due to a lack of available standards.

Products at Low ADNT Conversions. Studies were conducted with ozone as the limiting reagent to determine

ADNT	ADNT	nitrate	nitrite	oxamic acid aldehyde	oxamic acid	total product N	% N balance	% N based on NO _x
167	0	0	0	0	0	0		
64	103	121	42	3.5	12	178	58	53
42	125	182	20	3.7	13	219	58	54
19	148	320	15	3.8	13	352	79	75
10	157	415	14	4.8	15	449	95	91

TABLE 3. Carbon Balance^a on Ozonation Products of 2-ADNT (μ mol)

2-ADNT	AADNT	glycolate	acetate	formate	oxalate	glyoxylic acid	pyruvic acid	pyruv- amide	glyoxal	pyruv- aldehyde	product C	C balance, %
160	0	0	0	0	0	0	0	0	0	0		
50.8	109	0	54.2	70.0	0	127	37.5	56.7	16.6	7.2	369	48
20.4	140	0	70.4	87.9	1.0	147.6	53.4	81.6	23.4	13.2	478	49
10.3	150	0	86	109	3.0	126	62.4	77.7	21.8	13.8	500	48
2.0	158	8.2	100.8	125	8.8	96.2	80.1	65.4	17.4	11.4	505	46

^a Sample calculation: At the first point, 109 μ mol of ADNT consumed corresponding to 763 μ mol of carbon (7 × 109). The micromoles of carbon from products found = 369. Carbon balance = 396/763 × 100 = 48%.

	TABLE 4.	Carbon	Balance	on	Ozonation	Products	of	4-ADNT	(umo	I)
--	----------	--------	---------	----	-----------	----------	----	--------	------	----

4-ADNT	∆ADNT	glycolate	acetate	formate	oxalate	glyoxylic acid	pyruvic acid	oxamic aldhyde	oxamic acid	unknown 1 ^a	pyruv- aldehyde	product C balance	% C balance
167	0	0	0	0	0	0	0	0	0	0	0		
63.9	103	2.4	0	104.3	0	26.2	60.6	7.0	24	13.6	4.5	243	34
41.6	125	5.8	0	132.8	7.2	31.4	90	7.4	26	18.4	7.5	327	37
19.3	148	20	0	185	28.6	30.6	121.8	7.6	26	14.4	6.9	441	43
10.3	157	39.2	0	194.7	20.8	18.6	143	9.6	30	8.8	3.9	469	43

^a Assumes four carbon product

initial products formed in the oxidation and their initial mole ratios. Table 5 shows the result of low conversion oxidations of 2-ADNT, starting with 100 mM 2-ADNT, and evaluating products after 6%, 19%, 28%, and 45% 2-ADNT oxidation by ozone. The ozone/2-ADNT stoichiometry is close to 2. Glyoxylic and pyruvic acids were produced in a 2:1 mole ratio with no evidence for production of pyruvamide. This result suggests that ring cleavages in the vicinity of the amine group are not involved in the initial oxidative steps. The finding that both glyoxylic and pyruvic acids are produced at the same time suggests that multiple sites are involved in the initial cleavages and that some selectivity exists for particular sites. Nitrate and nitrite are also produced in a 2:1 ratio, which is controlled at least in part by the rapid oxidation of nitrite to nitrate by ozone; however, the NO_x/ADNT ratio is less than 2. Carbon and nitrogen balances in these experiments were low: only 13% of the carbon was accounted for while 50% of nitrogen (based on nitro groups) was found as NO_x^- ions

The results for 100 μ M 4-ADNT appear in Table 6 and represent product distributions after 10%, 18%, 24%, and 39% 4-ADNT oxidation by ozone. In this case, pyruvic and glyoxylic acids were the first small acids to result from 4-ADNT cleavages, and they were formed in a 2:1 ratio. A small amount of oxamic acid aldehyde was also observed, again indicating that multiple cleavage sites occur during the initial oxidation. However, the relative mole ratios indicate some selectivity for initial ring rupture. Ozone/4-ADNT ratios began to approach 2. Interestingly, nitrite/nitrate ratios were 2:1 while the opposite was observed with 2-ADNT. Also, the NO_x/4-ADNT ratio approaches 0.6 instead of 2 as observed with 2-ADNT. Nitrogen balances are 31–34%, based on nitro groups in oxidized ADNT; however, carbon balances are only 3% based on oxidized 4-ADNT.



FIGURE 4. HPLC profile of carboxylic acids generated from the ozonation of 2-ADNT (A) and 4-ADNT (B).

TABLE 5. I	Products from	o Ozonatio	on of 2-ADN	IT at Low	Conversions (μ mol)				
2-ADNT	ADNT	$\Delta 0_3$	nitrate	nitrite	glyoxylic acid	pyruvic acid	O ₃ /ADNT	NO _x /ADNT	% N in products	% C in products
100	0	0	0	0	0	0	0	0		
94	6	17.2	6.4	3.5	2.5	1.1	2.8	1.6	80	20
81	19	34	10.5	5.6	5.2	2.5	1.8	0.85	48	13
72	28	51	28.1	15.3	7.3	3.6	1.8	1.55	43	13
55	45	85	29.5	16.2	10.3	5.4	1.9	1.02	51	12

TABLE 6. Products from Ozonation of 4-ADNT at Low Conversions (µmol)

4-ADNT	AADNT	$\Delta 0_3$	nitrate	nitrite	glyoxylic acid	oxamic acid aldehyde	pyruvic acid	O ₃ /ADNT	NO _x /ADNT	% N in products	% C in products
100	0	0	0	0	0	0	0	0	0		
90	10	14	2.6	4.1	0.2	0.10	0.4	1.4	0.67	34	2.6
82	18	28	3.4	5.6	0.4	0.14	0.9	1.6	0.50	34	3.0
76	24	44	4.4	11.6	0.6	0.23	1.0	1.8	0.67	33	2.8
61	39	75	11.7	12.2	0.9	0.37	1.6	1.9	0.61	31	2.7

SCHEME 1. Pathways for Ozonation of 2-ADNT



Discussion

Mechanistic Pathway. Scheme 1 illustrates a plausible pathway for ozonation of 2-ADNT, accounting for the results of product studies at low conversions: the \sim 2:1 ozone:ADNT stoichiometry, prompt formation of selected short-chain carbonyl compounds, and NO_x ions. A similar scheme (not shown) describes the pathways for ozonation of 4-ADNT. The ¹⁵N-labeling experiments show that amino nitrogen is not involved in ozone–ADNT interactions.

Ozonation very likely involves 1,3-dipolar cycloaddition of ozone to selected double bonds of the aromatic ring to form one or more isomeric ozonides via the Criegee mechanism (13, 14). Early formation of pyruvic and glyoxylic acids, but not pyruvamide, from ozonation of 2-ADNT points to selective attack at C5 to form C6–C5 and C5–C4 ozonides (Scheme 1). Since the estimated lifetimes of aliphatic ozonides are less than a millisecond (14), the initial ADNT ozonides probably cleave to ring-opened diene dicarbonyls and nitrohydroperoxides before reacting with additional ozone

502 ENVIRONMENTAL SCIENCE & TECHNOLOGY / VOL. 34, NO. 3, 2000



FIGURE 5. Ultraviolet spectra of the 2,4-dinitrophenylhydrazones of (A) pyruvic acid, (B) pyruvaldehyde, and (C) multicarbonyl intermediate from the ozonation of 4-ADNT.

to give secondary ozonides. Cleavage of a second ozonide or diozonide would give detectable two- or three-carbon aldehydes and acids plus four- or five-carbon chain fragments, which hydrolyze by multiple routes to unidentified carbonyl intermediates. Continued ozonation results in further oxidative degradation of intermediate carbonyls and amides. Eventually, almost all of the organic nitrogen converges to the oxidatively stable nitrate ion.

A similar evaluation of the ozonation of 4-ADNT also leads to conclusion that ozone attacks the aromatic ring of 4-ADNT selectively and at sites remote from the amine carbon. Products of ring cleavage of 4-ADNT show spectral properties of polycarbonyl DNPHs. For example, Figure 5 shows the spectral profiles of the DNPHs of pyruvic acid, pyruvaldehyde, and an unknown product from the ozonation of 4-ADNT. The unknown DNPH shows the characteristic spectrum of a monocarbonyl and a conjugated dicarbonyl compound, suggesting that at least three carbonyl groups are present in the unknown product. Some possible intermediates that possess these features are shown below.



Material Balance. Element balances on products account for 30-50% of the reacted 2-ADNT nitrogen as NO_x ions at 10-40% conversion (Table 5). However, only 3-20% of carbon in ozonized 2- or 4-ADNT is identified in glyoxylic and pyruvic acids. The ¹⁵N-labeled experiments with the 2and 4-ADNT show that essentially all NO_x ions initially arise from the nitro groups, not the amino group, probably by hydrolysis of the intermediate nitrohydroperoxides (5 and 6 in Scheme 1). The significant shortfalls in carbon at low conversions means that intermediates 5 and 6 hydrolyze to form aminonitrodienoic aldehydes or acids, which remain undetected or unidentified by HPLC analysis (see above). HPLC traces of product mixtures are devoid of the longer retained peaks, with UV absorption characterizing oxidized aromatic rings such as hydroxyanilines or quinomethides that might form by electrophilic or radical ozonation (13, 15).

Carbon and nitrogen balances on oxidized ADNTs steadily improve with conversion as the intermediate carbonyls are oxidatively cleaved to shorter, more stable oxalic or pyruvic acids, and amide nitrogen in oxamic acid and pyruvamide is oxidized to nitrate (Tables 1–4). Similar results are reported for ozonation of aniline and aminobenzoic acid, where most nitrogen is accounted for as ammonia, with very little nitrate formation and glyoxal, the major organic intermediate from SCHEME 2. Proposed Reaction Pathways for Nitro Ketones



aniline, is oxidized to oxalic acid on further ozonation (15–17).

Nitrohydroperoxide intermediates **5** and **6** in Scheme 1 almost certainly hydrolyze promptly to nitro ketones and H_2O_2 and then undergo further hydrolytic or redox cleavage, producing aldehydes and nitrate or carboxylic acids and nitrite. The nitro ketone structure has not been reported to our knowledge, and its existence must be transient in water. An alternative intramolecular pathway from **5** or **6** to carboxylic acids and nitrate cannot be ruled out. Nitrite ion rapidly oxidizes to nitrate with ozone (*18*). Scheme 2 summarizes these reactions.

The nitro ketone hydrolyzes to the carboxylic acid and nitrite by pathway A. Nitrite would be oxidized to nitrate by ozone using pathway C. Pathway B is a reduction leading to an aldehyde and nitrate. Since nitrite is observed to form with aldehydes in the initial oxidation (glyoxal, pyruvaldehyde, and oxamic acid aldehyde), both pathways A and B appear to be possible for the transformation of a nitro ketone.

The treatment of 2- or 4-ADNT with ozone produces a variety of small molecules with no observable mineralization. The products, however, are readily assimilated into biologic pathways such as the tricarboxylic acid cycle (pyruvic acid) and glyoxylate cycle (glyoxylic acid) (*19*). Ozonation offers a rapid and effective technology to reduce the hazards of ADNTs in the environment. One application could be the finishing step after bioslurry treatment of 2,4,6-trinitrotoluene (TNT; *20*).

Acknowledgments

This study was funded by the U.S. Army Corps of Engineers Cold Regions Research and Engineering Laboratory, Hanover, NH, with SERDP funds from the Waterways Experiment Station, Vicksburg, MS, under Contract DACA-39-95-K-0046.

Literature Cited

- (1) Spanggord, R. J.; Yao, C. C. D.; Mill, T. *Environ. Sci. Technol.*, in press.
- (2) Criegee, R.; Wenner, G. Ann. 1949, 546, 9.
- (3) Bailey, P. S. Chem. Rev. 1958, 58, 925-1010.
- (4) Zbarskii, V. L.; Sonis, M. A.; Orlova, E. Y. *Zh. Prikladnoi Khim.* 1971, 44, 2578–2579.
- (5) Spanggord, R. J.; Clizbe, L. A. J. Labelled Compd. Radiopharm. 1998, 41, 615–621.
- (6) Tits, M.; Bruylant, A. Bull. Soc. Chim. Bel. 1948, 57, 50-64.
- (7) Claison, H.; Shadwell, K. In *Berichte Organische Chemie*; 1910; Band III, p 620.
- (8) Bader, H.; N Hoigné, J. Water Res. 1981, 15, 448-456.
- (9) Kieber, R. J.; Mopper, K. Environ. Sci. Technol. 1990, 24, 1477– 1481.
- (10) Thayer, J. R.; Huffaker, R. C. Anal. Biochem. 1980, 27, 110-119.
- (11) Glover, D. J.; Hoffsommer, J. C. J. Chromatogr. 1974, 94, 334-7.
- (12) Woodburn, K. B.; Fontaine, D. D.; Bjerke, E. L. *Environ. Toxicol. Chem.* **1989**, *8*, 769–775.
- (13) Bailey, P. Reactions of ozone with various organic functional groups. In *Water and Wastewater Treatment*; Rice, R. G., Cotruvo, J. A., Eds.; International Ozone Institute: Cleveland OH, 1975; Vol. 1, pp 101–119.
- (14) Dowideit, P.; von Sonntag, C. *Environ. Sci. Technol.* **1998**, *32*, 1112–1119.
- (15) Caprio, V.; Insola, H. Ozone Sci. Eng. 1985, 7, 169-17.
- (16) Caprio, V.; Insola, H. Inquinamento 1984, 26, 33-36.
- (17) Dore, M.; Legube, B. J. Fr. Hydrol. 1983, 14, 11-30.
- (18) Neta, P.; Huie, R. E.; Ross, A. B. J. Phys. Chem. Ref. Data 1988, 17, 1083.
- (19) Brock, T. D. *Biology of Microorganisms*, 7th ed.; Prentice Hall: Englewood Cliffs, NJ, 1994; pp 107–108, 612–613.
- (20) Griest, W. H.; Vas, A. A.; Stewart, A. J.; Ho, C.-H. Chemical and Toxicological Characterization of Slurry Reactor Biotreatment of Explosives-Contaminated Soils, Report SFIM-AEC-ET-CR-96186; Oak Ridge National Laboratory: Oak Ridge, TN, August 1998.

Received for review February 17, 1999. Revised manuscript received August 2, 1999. Accepted October 24, 1999.

ES990190H