0273-2289/94/4546—0861$03.00

Porphyrin-Catalyzed Reduction of Nitroaromatics for the Treatment of Pink Water

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ABSTRACT

Porphyrins are tetrapyrrole ring structures which are metal chelating and often found in the active sites of enzymes involved in redox reactions. We have demonstrated that porphyrins alone may act as mediators in the transfer of electrons to or from various functional groups under a range of reaction conditions. One such application is the porphyrin-catalyzed reduction of nitroaromatics to the corresponding amines. For nitroaromatic components of munition wastes such as 'pink water,'' porphyrin pretreatment can render the wastes safer to handle and more amenable to biotreatment.

Index Entries: Porphyrin; nitroaromatic; nitrotoluene; munitions waste; pink water.

INTRODUCTION

Pink water is a wastewater stream produced during loading, assembly, and packing (LAP) of munitions. Pink water contains 2,4,6-trinitrotoluene (TNT), isomers of TNT, dinitrotoluenes, and often cyclotrimethylenetrinitroamine (RDX), cyclotetramethylenetetranitroamine (HMX), wax or other additives. TNT is the major constituent and is soluble in water to about 100 mg/L (0.440 m*M*) at room temperature (*1*). Freshly made aqueous TNT solutions are colorless but upon exposure to ultraviolet light or sunlight form pink complexes. Not surprisingly, surveys have shown that

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the discharge of munitions and related chemical effluents exert deleterious effects on the receiving water biota (2). Consequently, the release of munition wastewaters into the environment can constitute a serious problem.

Currently, munition plants treat pink water by physical means with prefiltration followed by activated carbon adsorption with some use of evaporative and leaching ponds (3). Carbon adsorption can result in excellent removal of TNT and RDX. Removal of up to 99.5% of TNT at an initial concentration of 121.8 mg/L has been reported (4). However, spent carbon is difficult to regenerate by chemical means and hazardous to regenerate thermally when the concentration of explosives exceeds 8% of the total adsorbent weight (5).

It is proposed that chemical reduction of nitrobodies in pink water by reduced porphyrins may facilitate the safe treatment of pink water and result in ultimate disposal of the hazardous components. Porphyrins are naturally occurring biomolecules containing planar, tetrapyrrole ring systems that are metal chelating. In nature, porphyrins are often found at the active sites of certain enzymes involved in oxidation-reduction reactions.

Various investigators have reported porphyrin-mediated reductive dehalogenation of halogenated aliphatic hydrocarbons (*6–10*). Marks et al. (*11*) reported a detailed study of the dehalogenation of lindane by various porphyrin-metal complexes. The principal variables were porphyrin, complexed metal ion, ligand, and pH. No lindane dehalogenation activity was observed without a chelated metal ion. However, many porphyrin-metal complexes tested were also inactive. Those complexes exhibiting activity contained chelated Co^{+2}, Mo^{+3}, Ni^{+2}, Mg^{+2}, or Fe^{+2}. Co^{+2}-centered hematoporphyrin was observed to dehalogenate lindane to 3,4,5,6-tetrachlorocyclohexene at a rate of over 300 nmol/min/mg of porphyrin at pH 9.0 with dithiothreitol as the reducing agent.

Many other types of organic compounds in addition to alkyl halides are capable of reacting with reduced porphyrin-metal complexes. For example, Wade et al. (*12*) found alkyl halides, peroxides, quinones, nitro, and nitroso compounds to be active in the oxidation of Fe^{+2}-centered deuteroporphyrin at room temperature in 1:1 *N*-methylpyrrolidone:acetic acid. Recently, Schwarzenbach et al. (*13*) reported the reduction of nitrobenzenes and nitrophenols by reduced Fe^{+2}-centered porphyrins in aqueous solutions. Cysteine was the "bulk electron donor." Corresponding anilines were observed to appear in reaction mixtures at the same rate of disappearance of the nitroaromatic.

Here we show that Co^{+3}-centered porphyrins will catalyze the reduction of nitrosubstituted toluenes in aqueous solution to corresponding amino-substituted products. Carbon adsorption of amines from a porphyrin pretreated pink water may be more amenable to thermal regeneration leading to reuse of carbon and thus significantly improving the economics of pink water treatment.

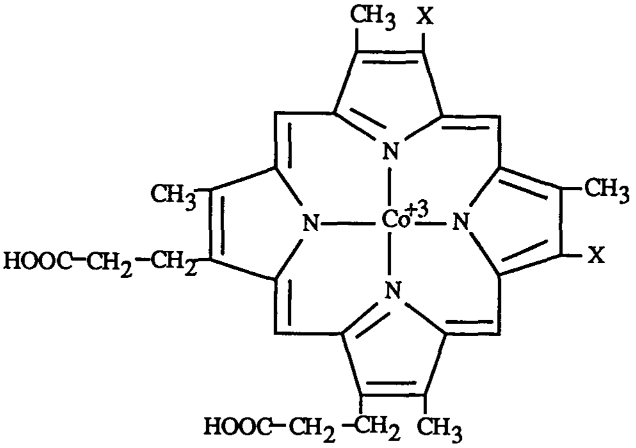

Fig. 1. Molecular structure of Co^{+3}-centered hematoporphyrin (X = -CH(OH)CH_3) and Co^{+3}-centered deuteroporphyrin IX-2,4-disulfonic acid (X = -SO_3H).

METHODS AND MATERIALS

Chemicals

2-Nitrotoluene (2NT), 4-nitrotoluene (4NT), 2,4-dinitrotoluene (2,4DNT), 2,4-diaminotoluene (2,4DAmT), 4-methyl-3-nitroaniline (4M3NA), 2-methyl-5-nitroaniline (2M5NA), 2-nitrosotoluene (2NSoT), 2-aminotoluene (2AmT), and 4-aminotoluene (4AmT) were purchased from Aldrich Chemical (Milwaukee, WI). 2,4,6-Trinitrotoluene (TNT) and standard solutions (50 or 100 mg/L) of each of the compounds listed above were obtained from Chem Service (West Chester, PA). Dithiothreitol (DTT), sodium dithionite, and sodium sulfide were obtained from Sigma Chemical Co. (St. Louis, MO). Co^{+3}-centered hematoporphyrin and Co^{+3}-centered deuteroporphyrin IX-2,4-disulfonic acid (Figure 1) were purchased from Porphyrin Products Inc. (Logan, UT). Diethylaminoethyl (DEAE)-substituted glass beads (24.2 μm mean pore diameter; 0.96 cc/g pore volume; 88.1 m^2/g surface area; 120–200 μm particle size) were purchased from Controlled Pore Glass Inc. (Fairfield, NJ).

Porphyrin Assay Conditions

Co^{+3}-centered hematoporphyrin was assayed for nitrobody reduction as follows. Stock solutions (0.308 m*M*) of Co^{+3}-centered hematoporphyrin and nitrobodies were prepared in 100 m*M* Tris/HCl buffer (pH 9.0). The concentrations of Co^{+3}-centered hematoporphyrin and Co^{+3}-centered deuteroporphyrin IX-2,4-disulfonic acid were verified by use of a calibration curve based on the α and β bands in the visible adsorption spectra

(*14*). The concentrations of nitrobodies were determined by high performance liquid chromatography (HPLC) by comparison with commercially-purchased standard solutions (*see below*). All stock solutions were stored at 4°C in dark to avoid possible photochemical reactions. When pH was a variable of interest, 100 m*M* phosphate buffer was also used for pH 4.0, 5.0, and 6.0 mixtures.

Reaction mixtures typically contained about 0.3–0.4 m*M* nitrobody, 9.7 m*M* dithiothreitol, and 50 μ*M* porphyrin at 37°C. Reactions were initiated by the addition of the porphyrin stock solution to a prepared solution of nitrobody and reducing agent in buffer. Controls were identical to reaction mixtures except for the absence of porphyrin. When nitrobody, dithiothreitol, or porphyrin concentrations were variables of interest, the concentrations ranged 0–0.16 m*M*, 0–15.0 m*M*, and 0–60 μ*M*, respectively. When sodium sulfide and sodium dithionite were tested as reducing agents, the concentrations ranged 0–23.1 m*M* and 0–14.5 m*M*, respectively.

Sampling and Analysis

Reaction mixtures and controls were sampled periodically and analyzed by HPLC using a Hewlett Packard 1090 HPLC with an 5 μ ODS-Hypersil (Hewlett Packard, Avondale, PA) column (4.6 × 100 mm). A variable wavelength diode array detector was used with the detector wavelengths set at 210 nm and 560 nm. The mobile phase was methanol:water (1:1). The injection volume was 0.5 μL, the flow rate 1.5 mL/min, and the temperature 40°C. Before analysis each sample was filtered using 0.45 μm nylon 66 syringeless filters (Genex Corporation, Gaithersburg, MD). Retention times for nitrobodies and various reduction products were as follows: 2-nitrotoluene, 3.48 min; 2-nitrosotoluene, 3.68 min; 2-hydroxyaminotoluene, 1.36 min; 2-aminotoluene, 1.53 min; 4-nitrotoluene, 3.62 min; 4-nitrosotoluene, 3.95 min; 4-aminotoluene, 2.35 min; 2,4-dinitrotoluene, 2.68 min; 2,4,6-trinitrotoluene, 2.38 min; 2,4-diaminotoluene, 1.14 min; 2,4,6-triaminotoluene, 0.79 min.

Immobilization of Porphyrin on DEAE Glass Beads

Co^{+3}-centered deuteroporphyrin IX-2,4-disulfonic acid was immobilized by adsorption to the positively charged DEAE glass beads at pH 7.0. (Sulfonated Co^{+3}-centered hematoprophyrin was unavailable). This pH was chosen to improve adsorption of the porphyrin to the glass beads. Stock solutions of Co^{+3}-centered deuteroporphyrin IX-2,4-disulfonic acid (0.312 m*M*) were prepared in 100 m*M* Tris/HCl buffer (pH 7.0) at room temperature. A bead suspension was then made by adding 1.0 g of glass beads to 200 mL of porphyrin stock solution in an Erlenmeyer flask (250 mL). After being shaken by hand for 5 min, the bead suspension was refrigerated overnight at 4°C. The amount of immobilized porphyrin per unit mass of glass beads was typically 4 mg porphyrin/g glass beads. This value was calculated from the mass of beads added, the total volume of the solution, and the initial and final porphyrin concentration in solution.

Porphyrin-Catalyzed Reduction of Nitrobodies in a Continuous Packed-Bed Reactor

Each packed-bed reactor vessel consisted of a 7-mm ID glass condensers, 120 mm long (Ace Glass Co., Vineland, NJ). Glass beads with immobilized porphyrin were packed in one reactor. A second reactor containing glass beads only (without porphyrin) was used as a control. In each reactor the bed depth was 100 mm and the packing material was held in place by glass wool inserted in both ends. Temperature was maintained at 38°C by circulating water from a constant temperature water bath through the condenser jackets. A feed reservoir (500 mL Erlenmeyer flask) containing 4-nitrotoluene (0.26 m*M*) or 2,4,6-trinitrotoluene (0.308 m*M*) and 5–10 m*M* dithiothreitol was kept chilled in an ice bath. The pH of each feed was 7.0. When 4NT was the substrate, the feed rate was maintained at 1.5 mL/h. When TNT was the substrate, however, a lower feedrate of 0.5 mL/h was also used. Feed rates of 0.5 mL/h or 1.5 mL/h were equivalent to residence times of 3.46 and 1.15 h, respectively.

Feed was drawn up through each reactor by a Masterflex (Cole-Parmer Instrument Co., Chicago, IL) peristaltic pump on the outlet side of each reactor. Reactor effluent samples were taken at the segment between the reactors and the pump. The pump tubing was Pharmed (Cole-Parmer Instrument Co.). All other tubing was PTFE Teflon (Cole-Parmer Instrument Co.). Effluents from the test and control reactors were received into graduated cylinders seperately to monitor the flowrates.

RESULTS AND DISCUSSION

Reduction of Nitrotoluenes Products of Porphyrin-Catalyzed

The optimum pH of Co^{+3}-centered hematoporphyrin-catalyzed reduction of nitrobodies was determined by comparing the rate of disappearance of 4NT from reaction mixtures at different pH values. A pH of 9.0 was determined to be optimum for these reaction conditions.

At pH 9.0 each nitrotoluene investigated was reduced to the corresponding amine by Co^{+3}-centered hematoporphyrin in the presence of dithiothreitol as a reductant. For mononitrotoluenes (2NT, 4NT), 96% of 2NT, and 94% of 4NT were converted to 2AmT and 4AmT, respectively, through nitrosotoluenes and hydroxyaminotoluenes intermediates. The pathway for the porphyrin-catalyzed reduction of the nitro group to the corresponding amino group is assumed to take three steps as follows (*15*).

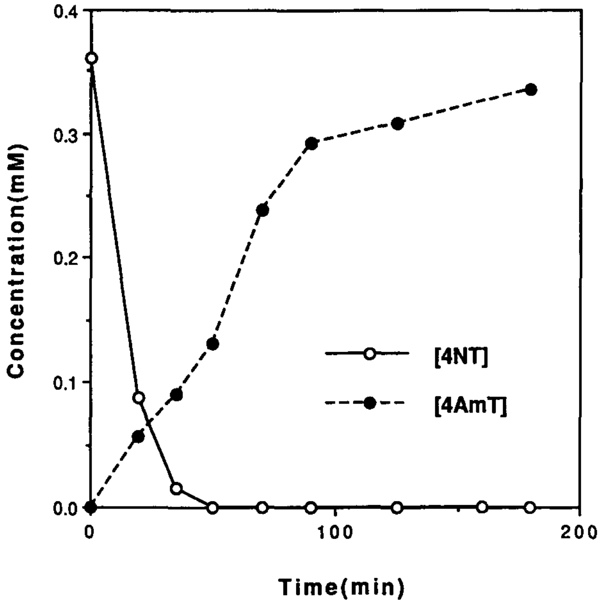

Fig. 2. Co^{+3}-centered hematoporphyrin catalyzed reduction of 4-nitrotoluene and formation of 4-aminotoluene: Temp. 38°C; pH 9.0; 9.73 m*M* dithiothreitol; 0.36 m*M* initial [4NT].

$$PhNO_2 \text{ —I—> } PhNO \text{ —II—> } PhNHOH \text{ —III—> } PhNH_2$$

Figure 2 shows the reduction of 4NT and formation of the final product (4AmT) on a molar basis. Complete conversion of nitrobodies to the corresponding amine was observed only with a tenfold excess of the reducing agent. Experimentally it was observed that 26.7 mol of dithiothreitol was required to completely reduce 1 mol of nitro group in the presence of 77 *μM* of Co^{+3}-centered hematoporphyrin.

With dinitrotoluene (2,4DNT) as substrate, intermediate reduction products 4M3NA and 2M5NA were observed to accumulate with ultimate conversion to 2,4DAmT. Trinitrotoluene (TNT) was converted to TAmT through unidentified intermediates which were presumably various combinations of nitro-, amino-, and nitroso-substituted toluenes. Complete conversion of 2,4DNT and TNT were not observed under the standard assay conditions described above. However, complete conversion to the corresponding amines with sufficient additional reducing power was observed.

In controls with mononitrotoluenes and 2,4DNT as the substrate (without porphyrin) about 15% of the nitrobodies were converted to nitrosotoluenes and unknown products in 125 min. For TNT, about 50% of the substrate was reduced to the unknown intermediates in 2 h. This conversion, however, could be limited by reducing the temperature. No aminotoluenes were detected in any controls without porphyrin.

Catalytic Nature of Porphyrin-Catalyzed Reduction

UV/visible absorbance spectra of reaction mixtures including porphyrin (Co^{+3}-centered hematoporphyrin), substrate (4NT), and reductant (dithiothreitol or dithionite) were taken when the reaction was initiated and periodically thereafter. Standard porphyrin assay conditions were used except for reduced levels of reductant. With the pure porphyrin in 100 m*M* Tris/HCl buffer solution (pH 9.0), two peaks were observed in the visible region at wavelengths of 528 and 560 nm. After the reaction was initiated, these peaks disappeared and merged into one with a maximum at 548 nm in the presence of the reducing agent. As the reaction continued, this peak again split into two peaks with maximum wavelengths of 528 and 560 nm. The cobalt ion in the porphyrin-metal complex was originally in a +3 state and Co^{+3}-centered hematoporphyrin showed two peaks at the wavelengths of 528 and 560 nm (*14*). Co^{+2}-centered hematoporphyrin, however, shows a broad peak in the visible range. Therefore, the reduced form is seen to have predominated in the reaction mixture until the reducing power (DTT or dithionite) was exhausted. At that time a significant amount of the oxidized form was again present.

These absorbance spectra are consistent with a catalytic role for the porphyrin in the reaction. The cobalt ion in Co^{+3}-centered hematoporphyrin was reduced to Co^{+2} by DTT or other reductants. The reduced Co^{+2} was then oxidized back to Co^{+3} by the nitro group. After the reducing power was exhausted, the reaction ceased, and the oxidized form, Co^{+3}, accumulated.

Kinetics of Porphyrin-Catalyzed Reduction of Mononitrotoluenes

Co^{+3}-centered hematoporphyrin-catalyzed reduction of mononitrotoluenes was found to be pseudo-first order over a substrate concentration range 0–0.15 m*M* 4NT and 0–0.15 m*M* 2NT with 12.5 μ*M* porphyrin, 9.73 m*M* dithiothreitol, and pH 9.0. It was observed that the rate constant of the reduction of 4NT (15.6 min^{-1} (m*M* porphyrin)$^{-1}$) was threefold greater than that of 2NT (4.6 min^{-1} (m*M* porphyrin)$^{-1}$) under these reaction conditions. The difference in rate may be due to steric hindrance in the ortho-substituted toluene.

Dithiothreitol exhibited saturation kinetics below 15.0 m*M* dithiothreitol with 55 μ*M* porphyrin and 0.36 m*M* 4NT at pH 9.0 and 38°C. Above 15 m*M* DDT the concentration of reductant was apparently sufficient that essentially all of the porphyrin was in the reduced form at any time and further increases in DTT concentration did not result in increased reaction rates (zero order with respect to DTT).

The reaction rate was shown to increase with increasing porphyrin concentration in the concentration range 0–60 μ*M* porphyrin with 0.26 m*M* 4NT and 9.73 m*M* DTT at pH 9.0 and 38°C. The reaction was observed to be less than first order in this concentration range.

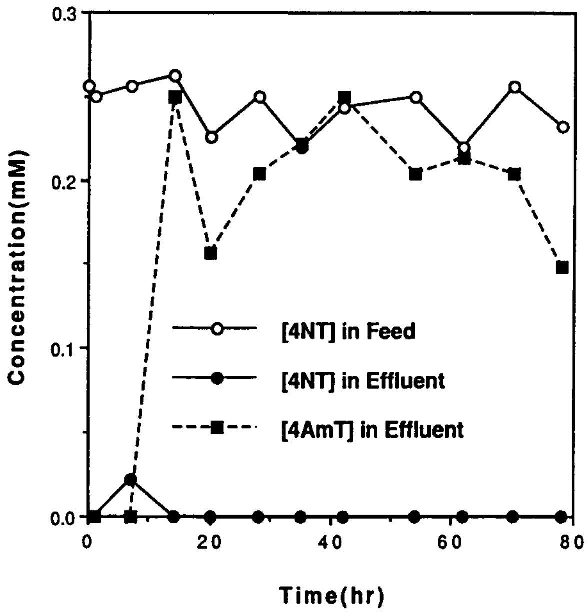

Fig. 3. Reduction of 4-nitrotoluene catalyzed by Co^{+3}-centered deuteroporphyrin IX-2,4-disulfonic acid in a packed-bed reactor containing DEAE-glass beads: Temp. 38°C; 0.256 m*M* initial [4NT]; 9.73 m*M* DTT; 1.5 mL/h flowrate. Time zero corresponds to initiation of feed.

Alternative Reductants

In porphyrin-catalyzed reduction of nitrobodies, the reducing agent obviously plays an important role since the reaction can not be sustained without a bulk reductant. Several types of reductants such as borohydride, sulfide, phosphite, hypophosphite, and sulfite were considered in addition to dithiothreitol. Of these reductants, only sodium sulfide and sodium dithionite, gave significant reduction of 2NT, 4NT, and TNT. With Co^{+3}-centered hematoporphyrin (50 μ*M*)-catalyzed reduction of 2-nitrotoluene (0.49 m*M*), the pseudo-first order rate constants were observed to be 15.6 min^{-1} (m*M* porphyrin)$^{-1}$, 3.8 min^{-1} (m*M* porphyrin)$^{-1}$, and 0.15 min^{-1} (m*M* porphyrin)$^{-1}$ for dithiothreitol (9.73 m*M*), sodium dithionite (8.13 m*M*), and sodium sulfide (23.1 m*M*) as reductants, respectively. Interestingly, with sodium sulfide no nitroso and hydroxyamino intermediates were observed.

Porphyrin-Catalyzed Reduction of Nitrobodies in a Continuous Packed-Bed Reactor

As noted above, the packed-bed reactors were operated at pH 7.0. At this pH the pseudo-first order rate constant for reduction of 4NT was about 60% of that observed at the optimum pH of 9.0 (data not shown). When

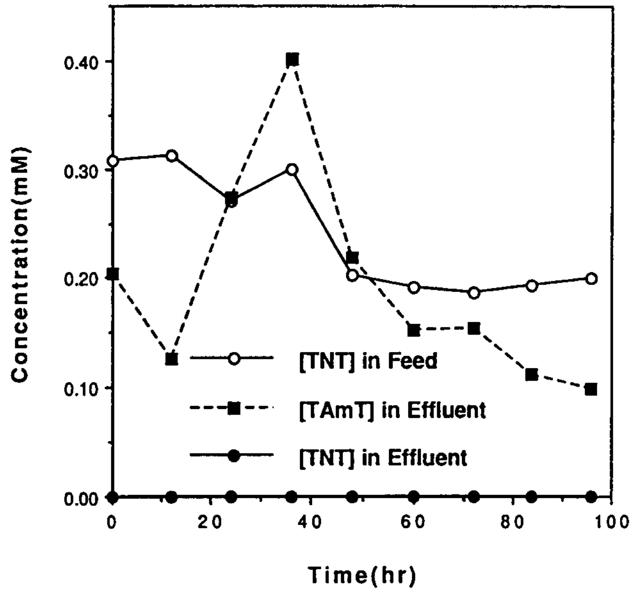

Fig. 4. Reduction of 2,4,6-trinitrotoluene catalyzed by Co^{+3}-centered deuteroporphyrin IX-2,4-disulfonic acid in a packed-bed reactor containing DEAE-glass beads: Temp. 38°C; 0.308 m*M* initial [TNT]; 6.0 m*M* DTT; 0.5 mL/h flow-rate.

4NT (0.26 m*M*) was fed (1.5 mL/h) to the packed-bed reactor, about 90% of 4NT was converted to 4AmT (Fig. 3). This conversion was maintained up to 70 h until loss of porphyrin from the column (as evidenced by loss of color from glass beads) began to significantly affect the reaction rate. No intermediates of 4NT reduction were detected in the effluent. In the control reactor with DEAE glass beads only, about 18% of 4NT disappeared, but no 4AmT was detected.

When TNT (0.308 m*M*) was fed to the packed-bed reactor at a feed rate of 0.5 mL/h, TAmT was observed to accumulate in the reactor effluent (Fig. 4). At this feedrate no intermediates of TNT reduction were detected. After 60 h at a feed rate of 0.5 mL/h the [TAmT] in the effluent was seen to decrease presumably due to loss of porphyrin from the column.

In the control reactor with a TNT feed at this same feed rate, large amounts of an apparent intermediate in TNT reduction accumulated in the effluent and more than 90% of TNT disappeared. This intermediate had the same HPLC retention time as an intermediate detected in batch tests without porphyrin described above. In the absence of porphyrin, however, no TAmT was observed.

At the higher feed rate of 1.5 mL/h, some intermediates of TNT reduction were detected in the presence of the porphyrin. However, no TAmT was observed in the effluent at this higher feed rate.

CONCLUSION

Toxic and refractory nitrobodies (2-nitrotoluene, 4-nitrotoluene, 2,4-dinitrotoluene, and 2,4,6-trinitrotoluene) were reduced to corresponding amino-substituted products by various reductants such as DTT, sodium dithionite, and sodium sulfide in a reaction catalyzed by Co^{+3}-centered porphyrins. Porphyrin-catalyzed reduction of nitrobodies has potential for use as a pretreatment for biotreatment or carbon adsorption of pink water. However, additional work is needed to optimize the process with lower cost porphyrins and bulk reductants.

ACKNOWLEDGMENTS

This work was funded by ABB Environmental Services (Portland, ME).

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