A Research Note

The Formation of N-Nitrosodimethylamine and N-Nitrodimethylamine from the Plant Growth Regulator Daminozide (Alar)

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ABSTRACT

Daminozide (Alar) was found to produce N-nitrosodimethylamine (NDMA) and N-nitrodimethylamine (NITDMA) when treated with nitrite in acidic solution. Unsymmetrical dimethylhydrazine (UDMH), the hydrolysis product of Daminozide, gave the same products. Use of 15N-nitrite and 15N-Daminozide showed that both the nitroso group of NDMA and the nitro group of NITDMA arise from the nitrite.

Daminozide (Fig. 1) is the active component in the plant growth regulator Alar® and unsymmetrical dimethylhydrazine (UDMH) is its hydrolysis product. Although Daminozide is no longer allowed for use on agricultural crops (1), there have been several recent reports on its determination in foods and related commodities (3,12). These methods quantitate Daminozide concentration after it is totally hydrolyzed to UDMH.

Sen et al. (75) reported that they found NDMA as the only product formed from the reaction of Daminozide and excess nitrite under acidic conditions; pH 1.5 to 3.4 for 2 h. The NDMA which formed could not be easily quantitated by the thin layer chromatographic method used and the method also failed to observe analogous compounds. That study was conducted before the development of the Thermal Energy Analyzer (TEA). It was with the use of the TEA that a second product from the reaction has now been found: N-nitrodimethylamine (NITDMA), also an animal carcinogen (10).

MATERIALS AND METHODS

Chemicals

All chemicals and solvents were ACS reagent grade or better. Water was distilled and purified with a Barnstead NANOpure II system. NDMA was received from the NCI Chemical Carcinogen Reference Standard Repository, Division of Cancer Etiology, NIH, Bethesda, MD. NITDMA, purity 99%, was prepared as described by Bulusu et al. (2). Daminozide and UDMH were purchased from Aldrich Chemical Co., Inc., Milwaukee, WI. Sodium 15N-nitrite, purity 99%, was purchased from Cambridge Isotope Laboratories, Woburn, MA. 15N-Daminozide, labeled on the secondary nitrogen, purity 99%, was obtained from the Unibro Chemical Co., Middlebury, CT.

Figure 1. Structures of the compounds investigated from the reaction of Daminozide and UDMH with nitrite.

Daminozide residues in and on fruits could, however, undergo other reactions in human systems. Secondary and tertiary amino drug compounds can form nitroso compounds by reaction with nitrite as well as via oxidative reaction pathways (4,5,8). For example, aminopyrine, which contains a dimethylamine moiety, as does Daminozide, reacts rapidly with acidic nitrite to produce large amounts of N-nitrosodimethylamine (NDMA) (9). Daminozide is a secondary amine and under acidic conditions similar to those in the stomach, e.g., pH 3, and in the presence of nitrite, might itself be nitrosated or produce other nitrosamines. Since nitrosamines are powerful carcinogens (70) we investigated Daminozide under acidic conditions that would favor formation of these compounds.

Sen et al. (15) reported that they found NDMA as the only product formed from the reaction of Daminozide and excess nitrite under acidic conditions; pH 1.5 to 3.4 for 2 h. The NDMA which formed could not be easily quantitated by the thin layer chromatographic method used and the method also failed to observe analogous compounds. That study was conducted before the development of the Thermal Energy Analyzer (TEA). It was with the use of the TEA that a second product from the reaction has now been found: N-nitrodimethylamine (NITDMA), also an animal carcinogen (10).
Procedures

Reaction mixtures containing Daminozide or UDMH and a large excess (70:1) of nitrite were prepared at pH 3 using a citrate and hydrochloric acid buffer (7). The reaction mixtures were allowed to stand overnight at 25°C to duplicate residence time in the stomach and the entire alimentary canal. A 10-ml portion of each reaction mixture was then extracted with three 15-ml portions of dichloromethane. The extracts were concentrated to 4 ml using a Kuderna-Danish concentrator. Two µl of each extract was analyzed by gas chromatography with either a Thermal Energy Analyzer (GC-TEA) or a quadrupole mass spectrometer (GC/MS). The instrumental conditions have been described previously (11,13).

RESULTS AND DISCUSSION

Preliminary analysis of the reaction of nitrite and Daminozide by GC-TEA showed two peaks, one for NDMA and a second, unexpected peak. When the extract was analyzed by GC/MS, two compounds were identified; the NDMA with a molecular ion at m/z 74 and the unknown compound with a molecular ion at m/z 90. The unknown compound was identified as N-nitrodimethylamine (NITDMA) using mass spectral library data (6). Fig. 1 shows the structures of all compounds in this study. Confirmation of the two compounds was made by injection of authentic NDMA and NITDMA into the GC/MS system. The peaks from the authentic compounds matched the peaks in the reaction mixture, giving identical retention times by GC-TEA, and identical retention times and mass spectra by GC/MS.

Due to the formation of two products, further investigation of the reaction was performed using 14 N-nitrite and Daminozide. This mixture was analyzed by GC/MS and the products were identified as NDMA with a molecular ion at m/z 75 and NITDMA with a molecular ion at m/z 91. In an attempt to further clarify the reaction of nitrite with Daminozide and UDMH, several other reaction mixtures were prepared with 14 N-nitrite, 15 N-nitrite, 14 N-Daminozide, and UDMH. The reaction combinations and the results are given in Table 1.

<table>
<thead>
<tr>
<th>Reactant 1</th>
<th>Reactant 2</th>
<th>Product 1</th>
<th>Product 2</th>
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<tbody>
<tr>
<td>14 N-Daminozide</td>
<td>15 N-nitrite</td>
<td>14 N-NDMA</td>
<td>15 N-NITDMA</td>
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<tr>
<td>14 N-Daminozide</td>
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<td>15 N-NITDMA</td>
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<td>14 N-UDMH</td>
<td>14 N-nitrite</td>
<td>15 N-NDMA</td>
<td>15 N-NITDMA</td>
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The data in Table 1 show that the nitroso and nitro nitrogen in NDMA and NITDMA, both come from the nitrite ion and that the N-N bond in Daminozide and UDMH is cleaved in the reaction. The yields of NDMA and NITDMA, under these conditions of excess nitrite, were 74.5% and 1.8%, respectively, from Daminozide and 48.2% and 4.2%, respectively, from UDMH.

It is well known that Daminozide rapidly decomposes in processed foods to form UDMH (3). In a recent study of heated solutions, Daminozide decomposition to UDMH was highest at pH 3.6 (14). In this study we have shown that NDMA and NITDMA can form from Daminozide and UDMH in the presence of acidic nitrite, under conditions similar to the human stomach and alimentary canal. Due to the carcinogenicity of NDMA and NITDMA, caution should be exercised in the use of Daminozide on products destined for human consumption.

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REFERENCES