Daminozide: Oxidation by Photochemically Generated Singlet Oxygen to Dimethylnitrosamine and Succinic Anhydride

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Daminozide [Alar, succinic acid mono(2,2-dimethylhydrazide)] is oxidized by photochemically generated singlet oxygen, with rose bengal as a sensitizing agent in methanol-d_4, to yield equimolar amounts of N,N-dimethylnitrosamine (DMN) and succinic anhydride (1.8%/h up to 11% yield) as the only products detected by ^{1}H and ^{13}C NMR. The reaction is efficiently inhibited by 2,5-dimethylfuran as a competitor for or sodium azide as a quencher of singlet oxygen. Humic acid, similar to that found in natural and waste waters, and a red pigment isolated from apple peel also sensitize the photooxidation of daminozide to produce DMN and succinic anhydride. The applicability of these findings in model systems to environmental degradation remains to be determined.

Daminozide [Alar, succinic acid mono(2,2-dimethylhydrazide)] is a plant growth regulator used primarily on apples. The succinyl and N-methyl moieties each covalently bind to both liver DNA and protein of treated mice and to human hemoglobin in vitro via hydrogen peroxide oxidation. Oxidation by 3-chloroperoxybenzoic acid, sodium hypochlorite, or hydrogen peroxide is very rapid and occurs almost exclusively at the dimethyl nitrogen to produce the acylating species succinic anhydride (SAN) and a methylating species, probably a diazo hydroxide. Traces of N,N-dimethylnitrosamine (DMN) are also formed under these conditions, probably via oxidation at the acyl nitrogen (Brown and Casida, 1988).

The sensitivity of daminozide to various oxidants suggests that photochemical oxidation may contribute to its environmental degradation. Singlet oxygen is responsible for some environmental oxidations occurring in air and natural and waste waters and on soil surfaces in sunlight (Kahn et al., 1967; Zepp et al., 1977; Wolff et al., 1981; Gohre and Miller, 1983; Haag and Holgné, 1986). The insecticide disulfoton is oxidized by photochemically generated singlet oxygen (Draper et al., 1976; Zepp et al., 1981). Although most nitrogen-containing compounds such as amines quench singlet oxygen and the excited states of singlet oxygen photosensitizers (e.g., rose bengal or methylene blue) (Davidson and Trethewey, 1977), 3- and 4-aminobiphenyl are converted to the corresponding nitrobiphenyls by eosin-sensitized photooxidation (Spee et al., 1983) and a wide variety of N,N-dimethylhydrazones are oxidized with singlet oxygen to yield the corresponding ketone (Friedrich et al., 1977). The present study considers the products formed on reaction of daminozide with photochemically generated singlet oxygen (Figure 1).

MATERIALS AND METHODS

Chemicals. Daminozide (99%), 2,5-dimethylfuran (DMF) (99%), rose bengal (93%), and humic acid (technical sodium salt) were obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI).

Spectroscopy. NMR (Bruker WM 300) spectra for solutions in methanol-d_4 were obtained at 300 MHz (^{1}H) or 75 MHz (^{13}C), and chemical shifts are reported (ppm) downfield from tetramethylsilane. When the reactions are monitored by ^{1}H NMR, emphasis is given to the regions of the primary oxidation products, i.e. 2.9–3.85 ppm for daminozide and 1.35–1.6 and 5.75–6.1 ppm for DMF. A Perkin-Elmer 576 ST spectrophotometer was used to measure the absorbance (A) of photosensitizing pigments directly in the NMR solvents after the samples were transferred from NMR tubes to quartz cuvettes.

Pigments from Fruit Skins. Fresh apple peels (ripe Red Delicious) were homogenized in methanol-2 N aqueous hydrochloric acid (500:1), and the filtrate was evaporated at reduced pressure to produce a bright red solid. Chromatography on a Florisil column with ethyl acetate-2-propanol-formic acid (50:50:1) followed by thin-layer chromatography (TLC, silica gel 60 F-254) with 1-propanol-methylene chloride-water-formic acid (65:30:4:4) produced two major red bands at R_f 0.45 and 0.60, probably anthocyanins. Tomato skin pigments were purified according to Baraldi (1971) by TLC, developing with hexane-methylene chloride (4:1). The two major red-orange bands (R_f 0.80 and 0.85) (probably lycophyll and lycopene; free from chlorophyll) were combined, leaving behind a top yellow band (R_f 0.95), presumably β-carotene.
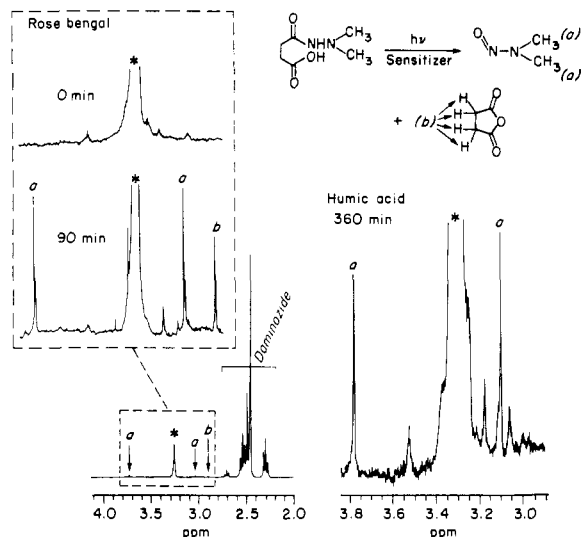
Photooxygenations. Photooxygenations of daminozide (125 mM) or DMF (230 mM) were carried out in 5-mm Pyrex NMR tubes, irradiating with an incandescent lamp (60 W) 20 cm from the sample. Photosensitizers were rose bengal (A = 2.0 at 560 nm) or pigments from apple and tomato skins (various concentrations) in methanol-d_4 (0.5 mL) or humic acid (sodium salt) (A = 1.25 at 370 nm) in methanol-d_4-D$_2$O (2:1, 0.5 mL), with or without sodium azide (62 mM). The concentration of rose bengal or humic acid was adjusted to absorb nearly all (95–99%) of the incident light. Oxygen was bubbled through the solutions at 1 mL/min such that no appreciable loss of solvent occurred during the experiment. The relatively high concentration of substrate (daminozide or DMF) required for NMR analysis provides saturated or zero-order reaction conditions; i.e., the products are formed at a constant rate over time, as experimentally verified in the present case. The rate-limiting factor in these reactions is presumed to be the concentration of singlet oxygen. Irradiation times were 5–380 min depending upon the relative reaction rate, which was much faster for DMF than for daminozide. The reaction products were monitored by ^{1}H and ^{13}C NMR directly and repeatedly (approximately every 5–15 min) without workup or extraction during the course of the photooxygenation. They were confirmed and quantified at the end of the experiment by adding authentic standards of DMN (100 μg) and SAN (1.3 mg) followed by integration.

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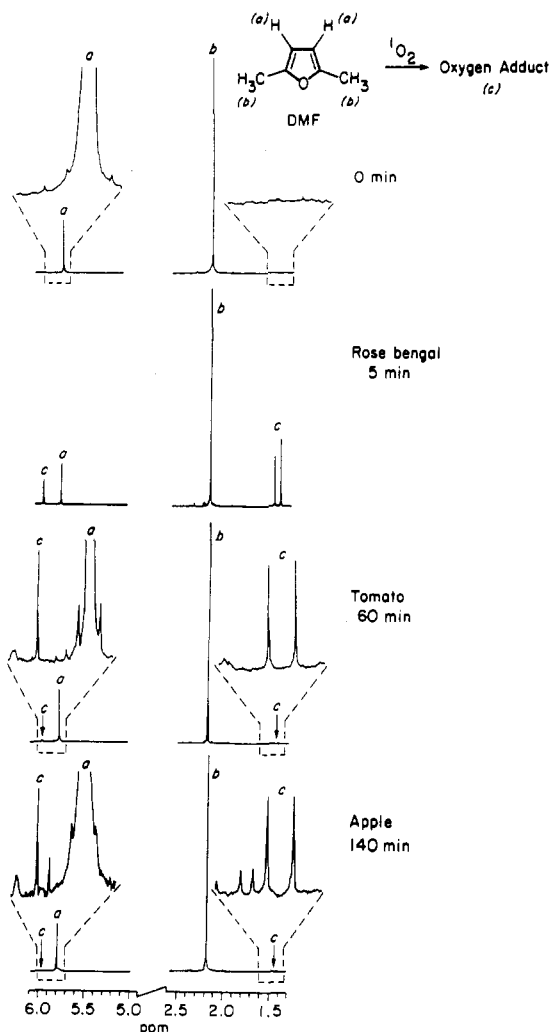
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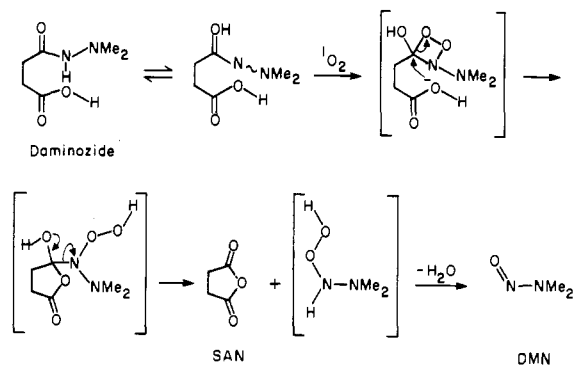
Figure 1. Proposed pathway for the oxidation of daminozide by singlet oxygen to form succinic anhydride (SAN) and *N,N*-dimethylnitrosamine (DMN).

Figure 2. Partial ^{1}H NMR spectra of photooxygenated mixtures of daminozide with rose bengal (in methanol-d_4) at 0 and 90 min or with humic acid (in methanol-d_4–D_2O, 2:1) at 360 min. Signals in the region of 2.9–3.85 ppm are expanded to emphasize peaks associated with dimethylnitrosamine (a) produced with either pigment and succinic anhydride (b) formed with rose bengal. The residual methanol solvent peak is indicated by *.

Figure 3. Partial ^{1}H NMR spectra of photooxygenated mixtures of 2,5-dimethylfuran in methanol-d_4 (peaks a and b) with rose bengal at 0 and 5 min or with pigments from apple or tomato peels at 60 and 140 min, respectively. Signals in the regions of 1.35–1.6 and 5.75–6.1 ppm are expanded in three cases to emphasize peaks (c) associated with the DMF–singlet oxygen adduct.

RESULTS

Daminozide in methanol-d_4 photolyzed with rose bengal in the presence of oxygen yielded equimolar amounts of DMN and SAN (0.8% each at 30 min and 3.0% each at 90-min irradiation, 0.03%/min) (Figure 2) [DMN: ^{1}H NMR, 3.77 (s, 3 H), 3.08 (s, 3 H); ^{13}C NMR, 41.5 (s), 34.1 (s). SAN: ^{1}H NMR, 2.93 (s, 4 H)]. SAN disappeared after 24 h in the dark at room temperature in methanol-d_4. The photooxygenation rate is approximately linear up to 380 min (yielding 10.9% DMN, 0.03%/min, 177-fold more slowly than DMF under the same conditions). Treatment with oxygen with or without irradiation but without rose bengal showed no detectable oxidation products after 60 mn. Photooxygenation of daminozide with rose bengal in the presence of DMF for 30 min yielded the oxygen adduct of DMF (48%) and only a trace of DMN and SAN. Similarly the presence of sodium azide completely inhibited DMN or SAN formation over 45-min irradiation. No detectable oxygen adducts were formed upon oxygenation for 55 min in the absence of pigments or at 0 min under any conditions. Photooxygenation of DMF [^{1}H NMR: 5.80 (s, 2 H), 2.18 (s, 6 H)] in methanol-d_4 for 5–10 min with rose bengal gave a single oxygen adduct [^{1}H NMR: 5.99 (s, 2 H), 1.49 (s, 3 H), 1.42 (s, 3 H)] (final yield 53% in 10 min) (Figure 3). This photooxygenation of DMF provided the standard reference rate (5.3%/min) under these zero-order reaction conditions. Addition of sodium azide at the start of the rose bengal sensitized photolysis reduced the formation of the oxygen adduct to 6% over 10 min (0.6%/min).

Photooxygenation of diaminozide with humic acid yielded 0.1% DMN after 360-min irradiation (Figure 2) (0.0003%/min, 19 000-fold more slowly than DMF under the same conditions). Photooxidation in a dilute solution of the R_f 0.60 apple pigment yielded traces of DMN and SAN after 245-min irradiation, which were not detectable after only 105 min. Daminoazide with tomato pigment yielded no detectable oxidation products after up to 130-min irradiation.

Photooxygenation with apple and tomato pigments (purified by column chromatography and diluted to $A <$ 0.05 at 530 or 439 nm, respectively, in methanol-d_4) yielded a trace of the DMF oxygen adduct after 40 min and 1.7% after 140 min with apple and 1.8% after 60 min with tomato (Figure 3). TLC-purified fruit skin pigment sensitizers at high concentration ($A >$ 2 at 530 and 439 nm for apple and tomato, respectively) yielded only a trace of the DMF oxygen adduct with apple, and none was detectable with tomato after 30 min. Dilution to $A <$ 0.05 restored activity as singlet oxygen sensitizers but only to the efficiencies observed for the crude pigment mixtures. Pho-

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tolysis of DMF with humic acid in methanol-d_4–D_2O (2:1) yielded 1.6% of the oxygen adduct after 40 min (0.040%/min).

DISCUSSION

Figure 1 shows a proposed mechanism by which singlet oxygen can oxidize daminozide involving a 2 + 2 cyclo-addition intermediate followed by cyclization to form SAN and an *N*-peroxide leaving group that dehydrates to yield DMN. The significance of the hydrazone tautomer of daminozide is suggested by its ^1H NMR spectrum (Figure 2, region between 2.3–2.8), indicating the existence of two geometric isomers in unequal abundance.

Naturally occurring pigments such as porphyrin derivatives and hypericin can act as photosensitizers to induce photooxygenations of many organic substrates via the generation of singlet oxygen (Maillard et al., 1980; Yang et al., 1986). Although carotenoids are considered to function as protective agents by quenching singlet oxygen (Krinsky and Deneke, 1982), we find that dilute solutions of certain carotenoids also function as singlet oxygen photosensitizers. Many pigments act as both singlet oxygen photosensitizers and quenchers (Tanielian, 1986); e.g., retinal, with many of the structural features of the carotenoids, is a singlet oxygen photosensitizer and also a weak quencher of singlet oxygen generated by protoporphyrin (Krasnovsky and Kagan, 1979). Thus, singlet oxygen might be formed in sunlight by natural pigment sensitizers in fruits and act to oxidize daminozide to DMN and SAN.

Singlet oxygen reactions are known to be significant for degradation of organic compounds in air, water, and soil. Singlet oxygen in photochemical smog reacts with olefins in the gas phase or in heterogeneous systems (Khan et al., 1967). Humic acid is proposed as a model photosensitizer for estimating potential environmental degradation of chemicals in natural and waste waters by singlet oxygen (Zepp et al., 1981; Wolff et al., 1981). DMF has been used as a standard probe to measure singlet oxygen oxidation rates in natural waters and has been estimated to have a half–life of approximately 8 h (Haag and Holgné, 1986). Daminozide is oxidized much more slowly (19 000-fold) than DMF under the conditions of our experiments. Thus, the applicability of the present findings on daminozide oxidation to form DMN in model systems to its environmental degradation remains to be determined.

ACKNOWLEDGMENT

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