Photodegradation of Benzydamine: Phototoxicity of an Isolated Photoproduct on Erythrocytes

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Abstract □ Benzydamine hydrochloride (Tantum, **1**) is a photoallergic and phototoxic anti-inflammatory and analgesic agent. This drug is photolabile under aerobic and anaerobic conditions. Irradiation of a methanol solution of benzydamine under oxygen or argon at 300 nm affords 5-hydroxybenzydamine (**2**) and 2-β-dimethylaminopropyl-1-benzylindalolin-3-one (**3**) as the main isolated and spectroscopically identified photoproducts. A radical intermediate was evidenced by thiobarbituric acid that was used as a radical sonde, as well as by the dimerization of cysteine. Erythrocyte lysis phososensitized by **1**, **2**, and **3** was investigated.

Benzydamine hydrochloride is a nonsteroidal anti-inflammatory drug with analgesic properties that is widely used in topical formulations, as well as in suppositories, tablets, and intramuscular injections. This compound is capable of initiating adverse light-induced biological effects. Some cases of photodermatitis from this drug have been reported.[1–4] Recently, cases of photoallergic reactions triggered by benzydamine (Tantum) have also been reported.[5] The possibility that photoproducts of the drug were involved in the photosensitivity or photoallergic or phototoxic reactions was studied by us. It is presumed that there must be a relationship between photochemical behavior and phototoxicity. In this context, very little is known as yet about the interrelation between the photochemistry and phototoxicity of benzydamine. This has prompted us to examine the photolysis of benzydamine (**1**). These studies led to the isolation and characterization by spectroscopic means of the two main photoproducts of **1**; that is, 5-hydroxybenzydamine (**2**) and 2-β-dimethylaminopropyl-1-benzylindalolin-3-one (**3**). The formation of **2** probably involves the reaction of a radical intermediate. The latter could be detected when the irradiation was carried out in the presence of either 2-thiobarbituric acid or cysteine used as radical scavengers (glutathione was similarly used by Costanzo et al.[6]). The present work also reports the phototoxic effects of **1** and its photolysis products on erythrocytes and shows that **3** is a particularly effective photohemolytic agent.[6,7]

Experimental Section

Benzydamine (**1**) was extracted from Tantum (Elmor Laboratory) with a soxhlet extractor with dichloromethane and recrystallized from the same solvent. The purity was 99.2% as determined by ^{1}H NMR spectroscopy and UV-visible (UV-vis) spectrometry. All solvents were analytical or spectro-grade. 2-Thiobarbituric acid and cysteine were commercially obtained from Aldrich. Photolysis of **1** was carried out in methanol solution (1.50 mmol in 50 mL) at 20 °C for 6 h with a quarz immersion photoreactor (applied photophysics part no. 3230 + 3307) and a Rayonet photochemical chamber reactor model RPR-100 equipped with 16 fluorescent lamps (RPR, 300 nm). Either oxygen or argon was bubbled through the reaction mixture throughout the entire irradiation process. The photodegradation reaction was followed with a Perkin-Elmer 559 UV-vis spectrophotometer as well as by thin-layer chromatography. After irradiation was stopped, the solvent was evaporated under reduced pressure (14 Torr), and the residue was chromatographed on a silica gel (230 mesh) column. The elution was carried out with solvent mixtures (hexane:dichloromethane, 1:3, v/v). The structures of the isolated products were elucidated by ^{1}H NMR and ^{13}C NMR (Brucker Aspect 3000; 300 and 100 MHz respectively) and by IR (Nicolet DX V 5.07) and mass spectra (Carlo Erba/Kratos MS 25 RFA) in connection with a Carlo Erba chromatograph equipped with a 25-m capillary (5% phenyl methyl silicone). 5-Hydroxybenzydamine (**2**) and 2-β-dimethylaminopropyl-1-benzylindalolin-3-one (**3**) were identical in all respects with authentic samples synthesized in this laboratory according to the methods of Palazzo et al.[8] and Schmutz et al.[9] In a separate experiment, to detect the probable formation of a radical intermediate, **1** (0.5 mmol in 50 mL of H_2O) was irradiated under the same conditions described above in the presence of equimolar quantities of either 2-thiobarbituric acid or cysteine as radical scavengers.

An erythrocyte suspension from three different samples of freshly obtained human erythrocytes was prepared by washing them four times with a 10-fold volume of a pH 7.4 phosphate-buffered saline solution (0.01 M phosphate buffer and 0.135 M NaCl), centrifuging the cells each time at 2500 × *g* for 15 min, and carefully removing the supernatant. For the photohemolysis experiments, erythrocytes were diluted in phosphate-buffered saline solution containing **1**, **2**, or **3** so that the resultant suspension had an optical density (OD) of 0.4–0.8 at 650 nm. An OD value of 0.5 corresponded to 3.3×10^6 cell · ml^{-1}.

The hemolysis rate was determined by measuring the decreasing OD at 650 nm because the OD is proportional to the number of intact erythrocytes.[10]

Compound **1** and the isolated photoproducts **2** and **3** were dissolved in the erythrocyte solution and irradiated at concentrations of 20 to 80 μg · ml^{-1} under aerobic (oxygen) and anaerobic (argon) conditions in a Rayonet photochemical reactor equipped with 16 phosphor lamps with an emission maximum at 300 nm and with lamps with an emission maximum at 400 nm, for periods ranging between 10 and 100 min.

Photoproduct **2** (0.375 mmol; mp, 166–168 °C) had the following spectroscopic data: IR (KBr): 3500–3200, 2956, 1617, 1530, 1495, 1454, 1363, 1330, 1189, 743, 702, and 680 cm^{-1}; ^{1}H NMR($CDCl_3$, 300 MHz): δ 7.34 (m, 1H, aromatic-H), 6.78 (m, 7H, aromatic-H), 6.55 (m, 1H, aromatic-H), 4.74 (s, 2H, N-CH_2-Ph), 4.67 (s, 1H, -OH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155.06 (s, N=C−OH), 141.43 (s, aromatic-C), 137.11 (s, aromatic-C), 127.85 (d, aromatic-CH), 127.16 (s, aromatic-C), 126.89 (d, aromatic-CH), 126.31 (d, aromatic-CH), 126.20 (d, aromatic-CH), 119.62 (d, aromatic-CH), 119.40 (d, aromatic-CH), 118.99 (d, aromatic-CH), 111.40 (d, aromatic-CH), 108.17 (d, aromatic-CH), 65.53 (t, N-CH_2-Ph); MS: *m/e* (%) 224 (26, M^+), 207 (18, M^+-17), 91 (40), 43 (100).

Photoproduct **3** (1.125 mmol; mp, 132–134 °C) had the following spectroscopic data: IR (KBr): 3005, 3000, 2990, 2510, 2436, 2373, 1650, 1490, 1450, 1360, 1200, 1189, 742, 704, and 690 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.63 (m, 1H, aromatic-H), 7.19 (m, 7H, aromatic-H), 6.98 (m, 1H, aromatic-H), 5.31 (s, 2H, N-CH_2-Ph), 4.41 (t, J = 6.0 Hz, 2H, N-CH_2-), 2.94 (t, J = 7.7 Hz, 2H, -CH_2-N(CH_3)$_2$), 2.58 (s, 6H, -N(CH_3)$_2$), 2.20 (m, 2H, -CH_2-); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 205.89 (s, -C=O), 154.69 (s, aromatic-C), 140.73 (s, aromatic-C), 136.70 (d, aromatic-CH), 127.85 (d, aromatic-CH), 127.64 (d, aromatic-CH), 127.16 (d, aromatic-CH), 126.57 (d, aromatic-CH), 126.27 (d, aromatic-CH), 119.01 (d, aromatic-CH), 118.39 (d, aromatic-CH), 111.96 (d, aromatic-CH), 108.14 (d, aromatic-CH), 65.62 (t, N-CH_2-Ph), 54.62 (t, N-CH_2-), 51.24 (t, -CH_2-N(CH_3)$_2$), 42.79 (q, N(CH_3)$_2$), 24.69 (t, -CH_2-); MS: *m/e* (%) 309 (5, M^+), 264 (8, M^+-N(CH_3)$_2$), 222 (100, M^+-· $(CH_2)_3$-N(CH_3)$_2$), 91 (60), 85 (90, CH_2=CH-CH_2-N^+ · (CH_3)$_2$), 86 (65, $^+(CH_2)_3N(CH_3)_2$), 58 (80, $CH_2=N^+$ · (CH_3)$_2$).

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Results and Discussion

The photolysis of 1 (absorption maxima at 218 and 306 nm in methanol) was carried out under UV light (300 nm), and its gradual decrease was followed by monitoring the disappearance of these bands at 20-min intervals. The result for a methanolic solution (1×10^{-4} M) of 1 is shown in Figure 1. No difference in the photoproduct yields or in the velocity of the reaction was found in the photolysis under O_2 or under argon.

To confirm unambiguously the proposed structures for the photoproducts, independent syntheses of authentic samples were carried out from available reagents.[8,9] The compounds thus obtained exhibited spectra indistinguishable from those corresponding to the isolated photoproducts and also from those of the isolated and identified metabolites (2) from rabbit and rat urine.[11,12]

The photolysis reaction of 1 is shown in Scheme I. The major photoproduct of 1 was 3 (yield, 75%). The formation of photoproduct 2 (yield, 25%) is compatible with an initial excitation of 1 after light absorption, followed by a cleavage of the O-C bond (O-photodealkylation), which would give a radical intermediate (Scheme I) that via hydrogen abstraction could give rise to this compound. The formation of the photoproduct 3 is compatible with a rearrangement of 1 after an initial excitation.

An indication of the free radical formation was given by the capacity of 1 to induce dimerization both of 2-thiobarbituric acid, under the same conditions of irradiation as detected by UV-vis spectrophotometry at 532 nm, and of cysteine, detected by mass spectroscopy of the precipitated cystine product.

Our investigation has indicated that 3 induced photohemolysis after 20 min of irradiation (λ = 400 nm) at any of the concentrations tested under either aerobic or anaerobic conditions. Nevertheless, oxygen markedly enhances lysis. Typical experiments are illustrated in Figure 2. No lysis was observed either when cells were irradiated for 20 min in the absence of drugs or photoproducts or when they were incubated for 2 h in the dark at 37 °C with drug at 100 μg/mL. Furthermore, no photosensitization of erythrocyte lysis by 1 or 2 was observed after 20 min of irradiation under the above conditions. After this time, it is possible that photohemolysis be observed, but probably because of the formation of 3 during this process.

The possibility that singlet oxygen was produced during the irradiation of 3 and 5 in the erythrocyte solution was examined. In fact, these photoproducts indeed produced singlet oxygen as was shown when photolysis of both compounds was carried out in the presence of 2,5-dimethylfuran as a singlet oxygen scavenger.[6,13]

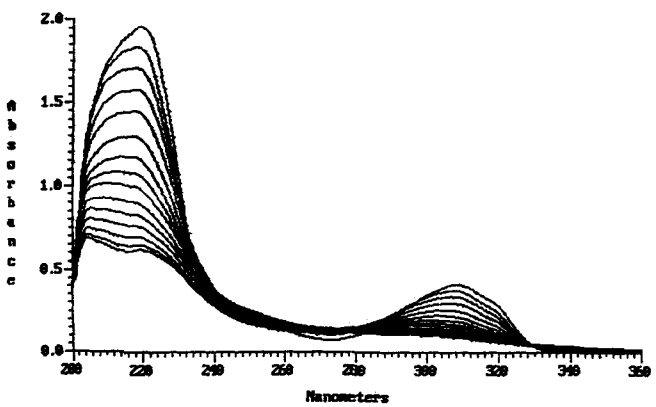
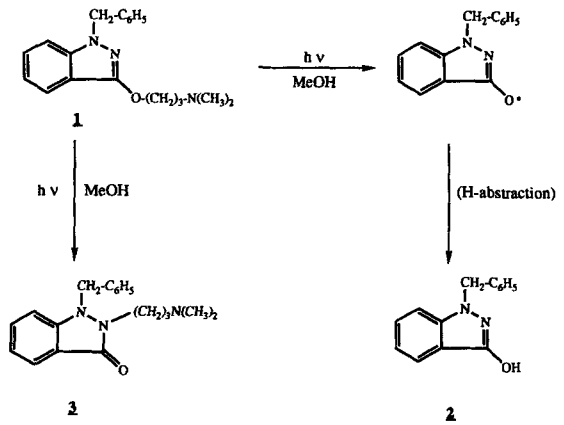


Figure 1—UV-vis monitoring of benzydamine (1) at regular time intervals (20 min).



Scheme I—Proposed mechanism for the photodegradation of benzydamine (1).

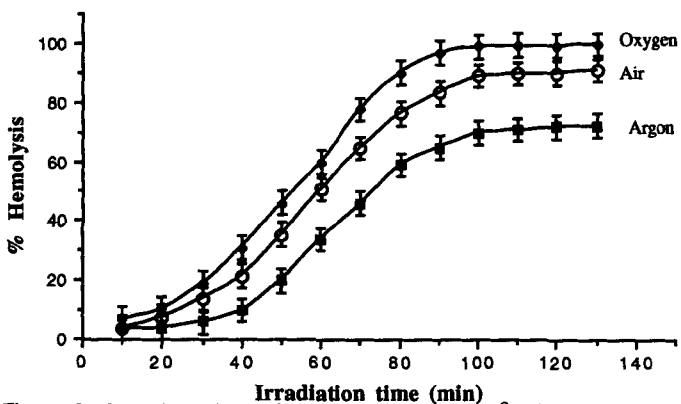


Figure 2—Photohemolysis of erythrocytes (3.3×10^6 cells/mL), photoproduct 3 (30μg/mL) in the presence of oxygen (●), air (○), and argon (■).

It may be inferred that the phototoxicity mechanism for 1 most probably involves reactions of a free radical intermediate, stable photoproducts, or singlet oxygen with cellular components. Further studies are needed on the development of devices to discriminate between effects due to stable photoproducts and to short-lived intermediates and the use of cultures and co-cultures of different human cells mimicking the human skin.

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