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# Physical Chemical Properties of Alpha Styryl Carbinol Antifungal Agents

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## INTRODUCTION

The prevalence of fungal infections has risen sharply due in part to increases in the patient population at risk. The immunocompromised patient is the major target of fungal infections. The compromised immune system is generally a result of another pathophysiologic state such as cancer or acquired immunodeficiency syndrome. Thus, the need for systemically active antifungal agents such as the azole antifungals has increased. The class includes clotrimazole, miconazole, ketoconazole, itraconazole, and fluconazole (1).

Alpha styryl carbinol antifungal agents represent a novel type of triazole antifungal agent (2). The structures of DuP 860 and DuP 991 are shown in Fig. 1. The triazoles are active against a variety of fungal organisms. They are active orally against *Aspergillis* and *Candida* species in animal models. As part of the development of these compounds, their various physicochemical properties were evaluated and are described herein. The thermal properties, solubility, ionization behavior, and solid-state and solution stability are presented.

## MATERIALS AND METHODS

### Materials

All compounds were prepared in house. These included the free bases X-7801 and A-9991, their methane sulfonate salts DuP 860 and DuP 991, and X-9484, the HCl salt of X-7801. All solvents were HPLC grade. All other reagents were of analytical grade.

### Chromatographic Method

The DuP 860 and DuP 991 concentrations were measured with an isocratic reversed-phase HPLC method using butylparaben as the internal standard. Separation was performed on a 15-cm Novapak C18 column (Waters Chromatography, Milford, MA) with an eluant consisting of methanol:water (53:47) for DuP 860 and methanol:00.1 $M$ acetate buffer, pH 4 (60:40) for DuP 991. Flow rates of 1.5 and 1.0 mL/min were employed for DuP 860 and 991, respectively (Model 870 Pump Module, DuPont Instruments, Wilmington, DE). Ultraviolet detection was used at 230 nm with AUFS 0.1 (Spectroflow Model 773, Kratos, Ramsey, NJ). Chromatograms were recorded on a Hewlett Packard 3392A integrator (Avondale, PA) with programmed calculation of the peak area ratios. Sample concentrations were determined from a standard curve based on the DuP 860: or DuP 991:butylparaben peak area ratio. The standards were freshly prepared for each analysis.

### Thermal and Solid-State Stability Studies

Differential scanning calorimetry (Model 1090, Du Pont Instruments) was performed for X-7801 and X-9484 by placing 10 mg of drug substance in a closed pan and heating at 10°C/min. The solid-state stability of DuP 860 was examined by placing aliquots of drug substance at 22°C with 5% (w/w) water, 60°C, 60°C with 5% (w/w) water, 600 f-candle at 25°C, and 600 f-candle at 25°C with 5% (w/w) water. Drug substance was stored in airtight glass vials. The percentage drug remaining at 1, 3, and 8 weeks was determined by a specific HPLC assay.

The solid-state stability of X-9484 was assessed by storing drug substance in open petri dishes at various temperatures. Samples were analyzed for chloride ion content by titration (Model 636 Titroprocessor, Metrohm) with 0.01 $M$ silver nitrate solution. Results were adjusted by subtracting the quantity of water originally present in the sample as determined by Karl-Fischer titration (Brinkman 652 KF - Coulometer).

### Solubility Determination

Solubility studies were carried out by placing excess drug substance into a suitable container with distilled water, adding varying amounts of either hydrochloric acid or sodium hydroxide to adjust the pH, or in a mixed cosolvent system and rotating end-to-end for 24 hr at room temperature (22°C). Preliminary experiments indicated that 24 hr provided sufficient time to reach equilibrium. The suspension was passed through a 0.45-μm filter, with the first portion discarded to ensure saturation of the filter. An aliquot of the filtrate was diluted and analyzed chromatographically and the remainder of the filtrate was employed for pH determination in the aqueous solutions.

### Solution Stability

The solution stability of the alpha styryl carbinols was examined at initial concentrations of 1 mg/mL in propylene glycol and polyethylene glycol 400 at 60°C. In addition, the effect of nitrogen flushing and the addition (all at a level of 0.01%, w/v) of the antioxidants citric acid, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA) was examined at 60°C. The percentage remaining from the initial values was determined at various time intervals with an HPLC assay.

## RESULTS AND DISCUSSION

### Solid-State Stability

Early in the development process a hydrochloride of

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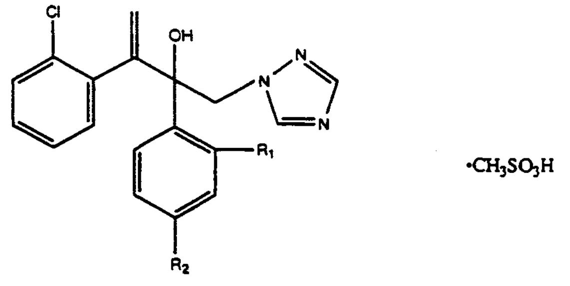

| | $R_1$ | $R_2$ |
|---|---|---|
| DuP 860 | H | Cl |
| DuP 991 | F | F |

Fig. 1. Chemical structure of DuP 860 and DuP 991.

X-7801 was contemplated, X-9484. Because of the relatively weak basicity of this compound, there was the potential for the evolution of HCl upon storage. This potential was investigated by storing aliquots of X-9484 drug substance in open dishes at various temperatures and then analyzing for chloride content. It was apparent from the results of these studies that at high temperatures this salt was unstable. A 12.7% loss of chloride ion was observed after storage at 60°C for 6 hr, a 4.8% loss at 80°C for 30 min, and an almost-complete loss (95.2%) at 140°C for 30 min. The tendency for HCl loss for this salt was also noted during thermal analysis. DSC thermograms of unheated X-9484 samples produced an endotherm at 189.2°C, which corresponds to the melting of X-9484 (confirmed by hot stage microscopy), followed by decomposition endotherms at 208.5 and 215.1°C. In addition, there was a broad endotherm present at 148.7°C (an endotherm corresponding with pure X-7801), indicating the presence of drug substance in which the HCl salt was liberated. This may have occurred as a result of the heating of the DSC sample. DSC analysis of a sample previously heated to 140°C for 30 min confirmed the chloride titration results, which indicated a virtual complete conversion to the free base at this condition, as a single endotherm was seen at 152.6°C, indicating the presence of X-7801.

Solid-state stability studies conducted for DuP 860 showed no loss of parent compound after 3 weeks of storage at 22°C with 5% (w/w) water, 60°C, 60°C with 5% (w/w) water, 600 f-candle at 25°C, and 600 f-candle at 25°C with 5% (w/w) water.

## Solubility

Aqueous solubility studies employed A-9991 and X-7801, the free bases of DuP 991 and DuP 860, respectively. Under equilibrium conditions, the free base provided equilibrium solubility values as a function of pH without the potential interference from the methane sulfonate counter ion. The solubility of A-9991 in water (pH 8.2) was determined to be 7.0 μg/mL and the solubility of X-7801 in water was 1.59 μg/mL (pH 6.8). For both compounds, the pH–solubility profile (Fig. 2) has one distinct ionization step. The ionization behavior can be described by the following equilibrium:

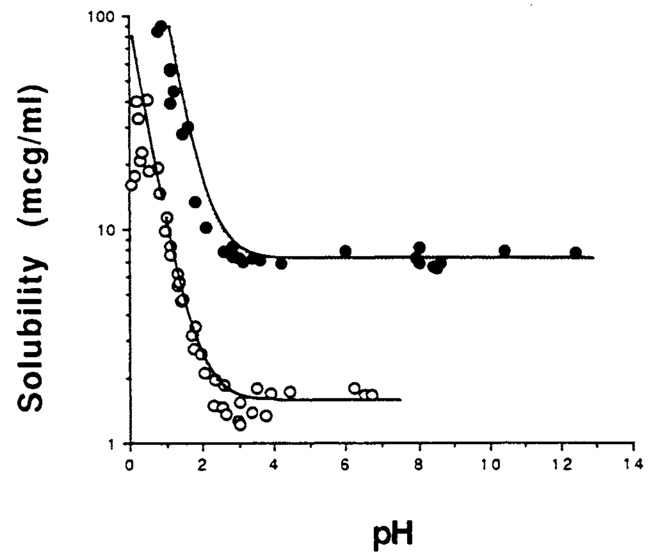

Fig. 2. Solubility of DuP 860 (○) and DuP 991 (●) as a function of pH. Experimentally determined data points and theoretical line (——) derived with Eq. (4) with p$K_a$'s for DuP 860 and DuP 991 of 1.79 and 2.15, respectively, and intrinsic solubilities for DuP 860 and DuP 991 of 1.59 and 7.0 μg/mL, respectively.

$$AH^+ \overset{K_a}{\rightleftharpoons} A + H^+$$

where $AH^+$ is the protonated base, A is the free base, $H^+$ is the hydrogen ion, and $K_a$ is the ionization constant. The ionization behavior can be described by the following well-established equation (8):

$$\log(ST/[A] - 1) = pK_a - pH \qquad (1)$$

where $ST$ is the total solubility of the drug. The data were analyzed by linear regression analysis with Eq. (1) to obtain ionization constants for DuP 860 and DuP 991 of 1.79 and 2.15, respectively.

The solubility of DuP 860, X-7801, and DuP 991 was determined in the water-miscible cosolvents glycerin, propylene glycol, and polyethylene glycol 400. The log solubility versus percentage cosolvent for DuP 860 and X-7801 is shown in Fig. 3. For DuP 860 in both propylene glycol and polyethylene glycol 400, a log-linear increase in solubility was observed as the fraction of cosolvent was increased, and solubility reached a maximum value at 80 and 90% cosolvent for propylene glycol and polyethylene glycol 400, respectively. DuP 860 dissolved in glycerin exhibited a log-linear increase in solubility across the entire cosolvent range. For the free base (X-7801) a log-linear increase in solubility was observed with increasing cosolvent fraction for all cosolvents, with the exception of glycerin, where a slight decrease in solubility occurred at 25% cosolvent. When the solubilities of DuP 860 and X-7801 in the various cosolvents were compared, for both polyethylene glycol 400 and propylene glycol the salt initially had a slightly higher solubility than that of the free base, but at higher cosolvent fractions this order was reversed. However, in glycerin the salt exhibited a substantially greater solubility across the entire range of cosolvent fractions. This behavior can be explained by the fact that glycerin exhibits a greater polarity than the other

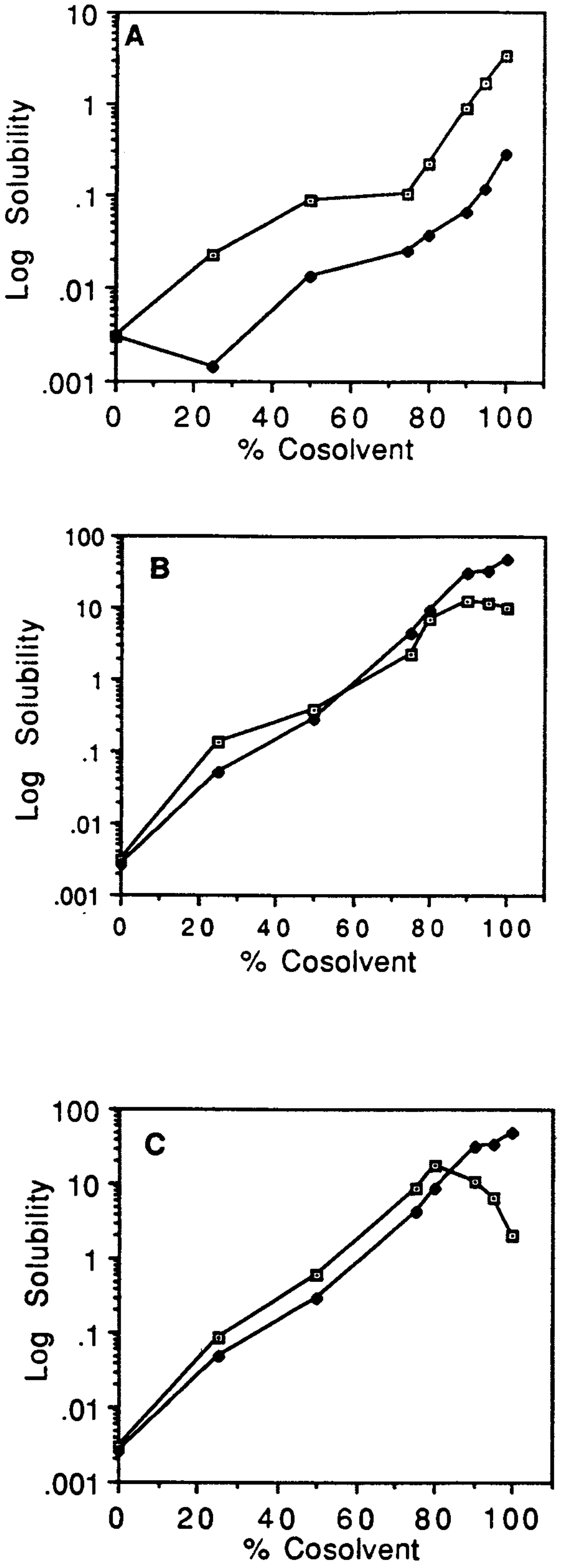

Fig. 3. The log solubility versus percentage cosolvent for DuP 860 (open squares) and X-7801 (filled diamonds) in (A) glycerin/water, (B) propylene glycol/water, and (C) polyethylene glycol 400/water mixtures.

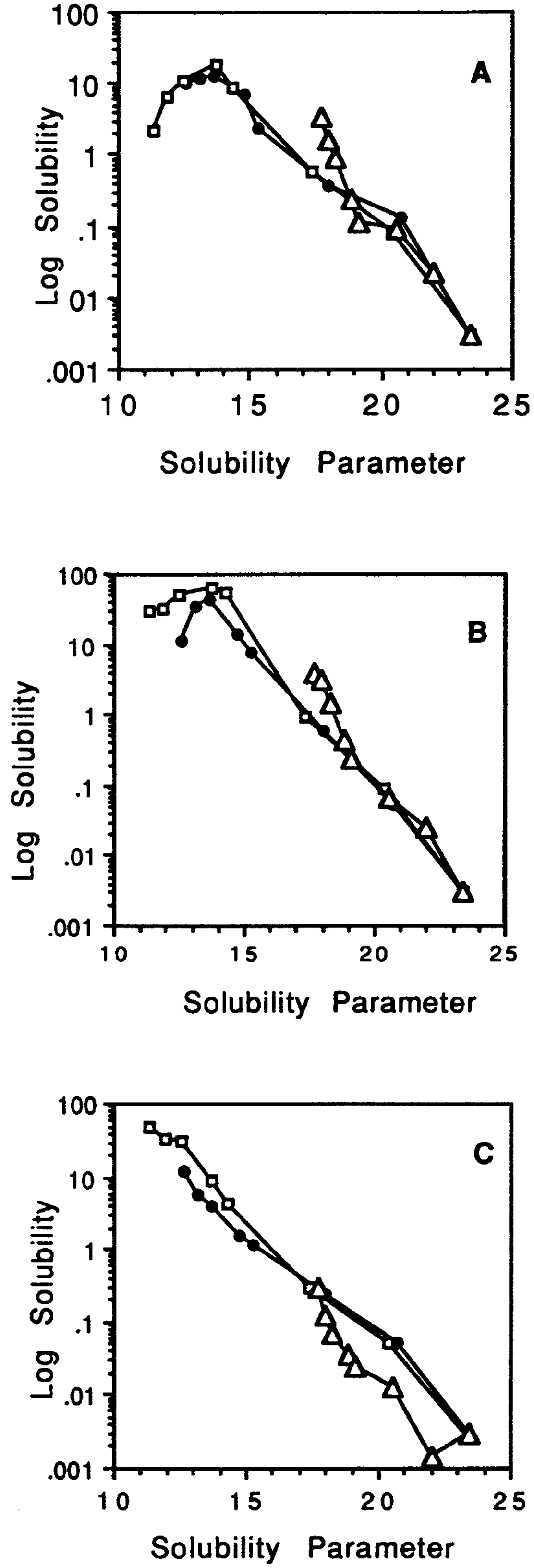

Fig. 4. The log solubility as a function of the solubility parameter for (A) DuP 860, (B) DuP 991, and (C) X-7801 dissolved in glycerin (open triangles), propylene glycol (filled circles), and polyethylene glycol 400 (open squares) cosolvent/water mixtures.

two solvents do, and it is this increased polarity which provides a favorable environment for dissolution of the more polar salt at all cosolvent fractions.

The solubility parameter is defined as the square root of the cohesive energy density and can be considered to be a measure of the total polarity of the substance (3). Solubility parameters were obtained from the literature (3) for the neat cosolvents and were 23.4 for water, 17.7 for glycerin, 11.3 for polyethylene glycol 400, and 12.6 for propylene glycol. For water/cosolvent mixtures, the solubility parameters were calculated based on linear combinations of the neat cosolvent and water values (3). In Fig. 4A, a plot of log solubility versus solubility parameter for DuP 860 in the various cosolvent mixtures revealed that the plots for propylene glycol and polyethylene glycol 400 closely paralleled one another, and a maximum in the plots was seen at solubility parameter values of 13.68 and 13.72 for propylene glycol and polyethylene glycol 400, respectively. Glycerin yielded a distinct linear pattern which was dissimilar to those of the other two cosolvents in the region corresponding to high cosolvent concentrations (75–100% cosolvent), but the plot more closely followed those of the other cosolvents at lower cosolvent concentrations. An almost identical behavior was noted for DuP 991 log solubility versus solubility parameter plots (Fig. 4B). The maximum solubility in both propylene glycol and polyethylene glycol 400 was reached at the same solubility parameter values as those which were observed for DuP 860. The similarity of the propylene glycol and polyethylene glycol 400 plots and their dissimilarity with the glycerin plot may be explained by the fact that the solubility parameters for polyethylene glycol 400 and propylene glycol (11.3 and 12.6, respectively) are similar, whereas the more polar glycerin has a much higher solubility parameter (17.7). Figure 4C is a plot of log solubility versus solubility parameter for the free base of DuP 860 in the various cosolvents. As was the case for the methane sulfonate salts, the propylene glycol and polyethylene 400 plots paralleled one another, while glycerin once again yielded a distinctly different profile.

### Solution Stability

In the cosolvents propylene glycol and polyethylene glycol 400, after storage of the samples for 2 weeks at 60°C, a 51.0 and 31.3% loss of DuP 991 was observed, respectively. As evidenced by HPLC analysis, the principal route of degradation was via oxidation of the styryl portion of the molecule to an epoxide. The oxidation was consistent with the formation of peroxides in the glycols (4–7). The solution stability was improved dramatically with nitrogen flushing (no loss of drug in propylene glycol and 5.5% loss in PEG-400 after 2 weeks at 60°C). Butylated hydroxytoluene provided protection in propylene glycol (2.8% loss of drug), while citric acid and butylated hydroxyanisole provided marginal stability improvements (29.7 and 11.7% loss with citric acid and BHA, respectively, after 2 weeks at 60°C). It was unclear whether the antioxidant protection was a result of decreased peroxide formation or of protection of the DuP 991 from the peroxides after their formation.

In summary, the physical chemical properties of two alpha styryl carbinol antifungal agents and their corresponding methane sulfonate salts were evaluated. The compounds are characterized by poor aqueous solubility, ranging from 3 to 20 μg/mL as a function of pH. The ionization of the compounds depends upon a triazole ring that is a very weak base, with a $pK_a$ ranging from 1.7 to 2.2. Their solubility was enhanced with the cosolvents propylene glycol, polyethylene glycol 400, and glycerin. However, in the cosolvent systems, oxidative degradation to the corresponding epoxide was observed. For the salts, while polyethylene glycol 400 and propylene glycol cosolvent systems yielded log solubility vs solubility parameter plots which were superimposable, glycerin yielded a profile which was distinctly different.

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