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Technical Note

Solubility and Ionization Behavior of the Antiarrhythmic 4-hydroxy-*N*-phenyl-3,5-bis(1-pyrrolidinylmethyl)benzamide dihydrochloride (DuP 923)[1]

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KEY WORDS: DuP 923; 4-hydroxy-*N*-phenyl-3,5-bis(1-pyrrolidinylmethyl)benzamide dihydrochloride; solubility; ionization behavior; antiarrhythmic agent.

INTRODUCTION

DuP 923 [4-hydroxy-*N*-phenyl-3,5-bis(1-pyrrolidinylmethyl)benzamide, dihydrochloride] is a class Ic antiarrhythmic agent that is effective in controlling ventricular tachycardia and atrial flutter in dogs (1) (Fig. 1). The compound has an unexpectedly high oral bioavailability for a phenol, with a value of 86 ± 14% in dogs (2). As part of the physical chemical evaluation of DuP 923 the solubility and ionization behavior were characterized. The purpose of the studies was to provide a thorough understanding of the solution behavior of DuP 923 to facilitate formulation development and to gain insight into the reason(s) for the high oral bioavailability. The following data describe the solution behavior of the compound and provide a chemical rationale for the high oral bioavailability.

MATERIALS AND METHODS

Materials

DuP 923 was prepared in house. All solvents were HPLC grade. All other reagents were of analytical grade.

Solubility Determination

Solubility studies were carried out by placing excess DuP 923 into a suitable container with distilled water, adding varying amounts of either hydrochloric acid or sodium hydroxide to adjust the pH, 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 and that the degradation via amide hydrolysis at the extremes of pH had a minimal effect on the solubility. The suspension was passed through a 0.22-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.

Potentiometric Titration

Titrations were performed at 25°C with 0.1 *N* KOH as the titrant and initial DuP 923 concentrations of 0.001 to 0.01 *M* (Model 636 Titroprocessor, Metrohm, Heisau, Switzerland). All solutions were prepared with CO_2-free double-distilled water.

Spectrophotometric Method

The equilibria between neutral phenols and phenolate anions has been demonstrated to produce dramatic changes in the ultraviolet spectra of compounds. The method of Albert and Serjeant (3) was employed for the spectrophotometric determination of the ionization constant by following the change in absorbance at 260 nm. Acetate buffers, 0.1 *M* and $\mu = 0.10$, provided constant pH values in the range of interest, while 0.1 *N* HCl and 0.1 *M* borate, pH 9.24, permitted the spectral measurements of the phenol and phenolate. The DuP 923 concentration was maintained constant for all spectral measurements at 7.52×10^{-5} *M*. The spectral measurements were carried out on a Hewlett Packard 8451A Diode Array Spectrophotometer (Hewlett Packard, Palo Alto, CA).

^{13}C NMR

^{13}C NMR measurements were performed with a 270-MHz spectrophotometer (Model 270, IBM/Bruker Instruments, Billerica, MA). Saturated solutions of DuP 923 in 20% (v/v) D_2O containing 0.1% TMS in water were analyzed using proton decoupling and Fourier transformation.

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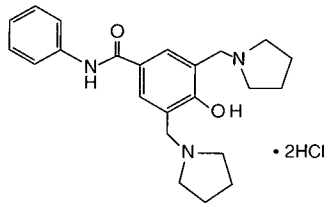

Fig. 1. Chemical structure of DuP 923.

Partition Coefficient Determination

The partitioning of DuP 923 between mutually presaturated octanol and buffer was followed by measuring the DuP 923 concentration in each phase after shaking for 1 hr. One hour provided adequate time for equilibrium to be reached and for degradation to be minimized.

Chromatographic Method

The DuP 923 concentration was measured with an isocratic reversed phase HPLC method using guaifenesin as the internal standard (4). Separation was performed on a 15-cm μ-Bondapak phenyl column (Waters Chromatography, Milford, MA) with an eluant consisting of 30% methanol in water adjusted to pH 2.2 ± 0.1 with phosphoric acid. A flow rate of 1.0 ml/min was employed (Model 870 Pump Module, DuPont Instruments, Wilmington, DE). Ultraviolet detection was used at 214 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 923:Guaifenesin peak area ratio. The standards were freshly prepared for each analysis.

RESULTS

Solubility Determinations

The solubility of DuP 923 in water (pH 2.99) was determined to be 993 mg/ml. The pH–solubility profile is given in Fig. 2. The pH–solubility profile has three distinct ionization steps because of the phenol and the pyrrolidine nitrogens.

The ionization behavior can be described with the following equilibria:

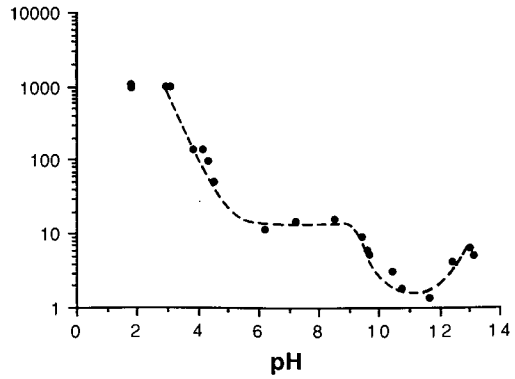

Fig. 2. Solubility of DuP 923 as a function of pH. Experimentally determined values (·) and theoretical line (——) derived with Eqs. (6) and (7), with pK_{a_1} = 4.81, pK_{a_2} = 10.13, pK_{a_3} = 12.13 and an intrinsic solubility of 1.42 mg/ml.

$$AH_3^{++} \overset{K_{a_1}}{\rightleftharpoons} AH_2^{+} + H^{+} \overset{K_{a_2}}{\rightleftharpoons} AH + H^{+} \overset{K_{a_3}}{\rightleftharpoons} A^{-} + H^{+}$$

where AH_3^{++}, AH_2^{+}, AH, and A^- represent the +2, +1, neutral, and −1 charged species of DuP 923, respectively, and K_{a_1}, K_{a_2}, and K_{a_3} are the first, second, and third ionization constants, respectively. The ionization constants are defined as

$$K_{a_1} = \frac{[AH_2^{+}][H^{+}]}{[AH_3^{++}]} \quad (1)$$

$$K_{a_2} = \frac{[AH][H^{+}]}{[AH_2^{+}]} \quad (2)$$

$$K_{a_3} = \frac{[A^{-}][H^{+}]}{[AH]} \quad (3)$$

The total solubility is defined by

$$S_T = AH_3^{++} + AH_2^{+} + AH + A^{-} \quad (4)$$

Substituting Eqs. (1), (2), and (3) into Eq. (4) yields

$$S_T = \frac{[AH][H^{++}]^2}{(K_{a_1})(K_{a_2})} + \frac{[AH][H^{+}]}{K_{a_2}} + AH + \frac{[AH]K_{a_3}}{[H^{+}]} \quad (5)$$

The data treatment assumes that the solubility of the hydrochloride or sodium salts is not exceeded and the solid phase results from the neutral charged species. The equation typically results in a V-shaped profile. However, the solubility of DuP 923 plateaus in the pH 6 to 9 range, suggesting that the solubility of the +1 form is exceeded. Since the AH and A^- species would be expected to be negligible at pH values of 9 and below, Eq. (5) was modified to Eqs. (6) and (7).

$$S_T = \frac{[AH_2^{+}][H^{+}]}{K_{a_1}} + AH_2^{+} \quad (6)$$

$$S_T = \frac{[AH][H^{+}]}{K_{a_2}} + AH + \frac{[AH]K_{a_3}}{[H^{+}]} \quad (7)$$

The data were analyzed by nonlinear regression analysis with Eq. (6) at pH 9 and below and with Eq. (7) at pH 9 and above to obtain the ionization constants of pK_{a_1} = 4.81, pK_{a_2} = 10.13, pK_{a_3} = 12.13, and AH = 1.42 mg/ml.

Potentiometric Titration

Titration of 0.01 *M* DuP 923 with 0.1 *N* KOH permitted the determination of one pK_a of 4.31 ± 0.01, after which precipitation occurred. A subsequent attempt employing an initial concentration of 0.001 *M* revealed only one ionization at 4.3 without precipitation.

Spectrophotometric Method

The absorbance of the DuP 923 was followed at 260 nm, where the neutral phenol (0.1 *N* HCl media) exhibits a maximum and the ionized phenolate has a minimum shoulder (0.1 *M* borate pH 9.24, Fig. 3). The pK_m was defined with the following equation:

$$pK_m = pH + \log \frac{A_{OBSERVED} - A_{IONIZED}}{A_{NONIONIZED} - A_{OBSERVED}} \quad (8)$$

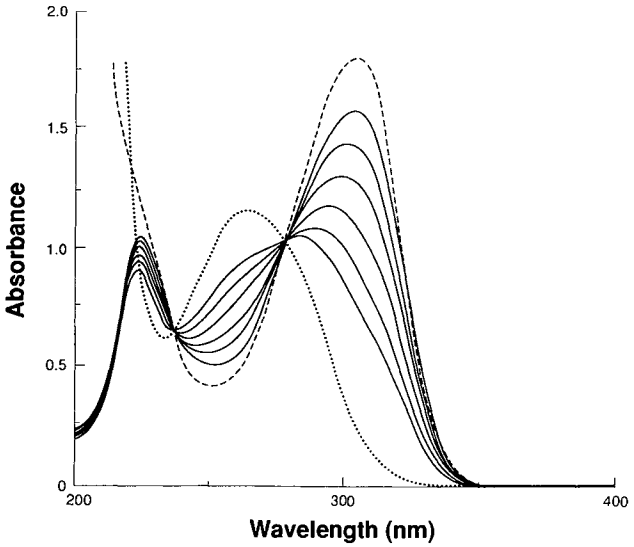

Fig. 3. The ultraviolet spectrum of DuP 923 in 0.1 *N* HCl (····) and 0.1 *M* borate, pH 9.24 (----). The solid lines are from the acetate buffer in decreasing order of absorbance at 260 nm at pH 3.9, 4.1, 4.3, 4.5, 4.7, and 4.9, respectively. The sample concentration was 7.52×10^{-5} *M*. The molar absorptivity of the neutral species in 0.1 *N* HCl was 1.54×10^{4} L mol^{-1} cm^{-1} and of the anionic species in 0.1 *M* borate, pH 9.24, is 6.40×10^{3} L mol^{-1} cm^{-1} at 260 nm.

where pK_m is the mixed-type ionization constant, $A_{OBSERVED}$ is the observed absorbance, $A_{IONIZED}$ is the absorbance of the ionized species, and $A_{NONIONIZED}$ is the absorbance of the nonionized species. Converting this mixed ionization constant to the thermodynamic ionization constant,

$$pK_a = pK_m + \frac{0.51(2Z - 1)\mu^{0.5}}{1 + \mu^{0.5}} \quad (9)$$

where Z is the charge of the acid and μ is the ionic strength. A pK_a of 4.80 was determined.

^{13}C NMR

^{13}C NMR exhibited a downfield shift in the carbon that is bonded to the phenolic hydroxyl group from 161 ppm at pH 2.68 to 164 ppm at pH 4.74 and to 169 ppm at pH 7.49 (Table I). The shift continued to 172 ppm at pH 12.3. The downfield shift is attributed to an increase in the electron density of that carbon at pH values of 4.74 and above.

Partition Coefficient Determination

The apparent octanol:water partition coefficient of DuP 923 as a function of pH is presented in Fig. 4. Recovery of

Table I. ^{13}C NMR Signal of the Carbon Bonded to the Phenolic Hydroxyl of DuP 923 as a Function of pH

pH	^{13}C NMR signal (ppm)
2.68	160.5
4.74	164.3
7.49	168.6
10.5	171.5
12.3	172.1

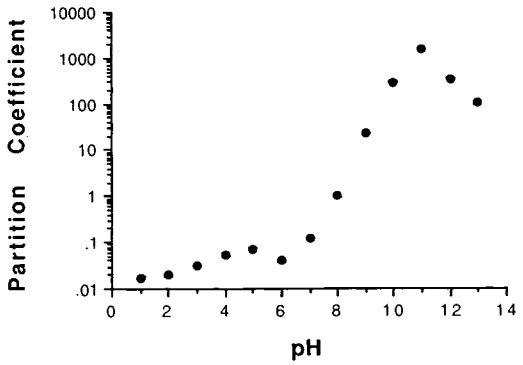

Fig. 4. Apparent partition coefficient of DuP 923 as a function of pH. The data points represent the mean ± standard deviation of five replicates with the error bars contained within the data points.

DuP 923 was 98–102% in all cases except for pH 12 and 13, where the recovery was 90 and 88%, respectively. The apparent partition coefficient remains low, less than 0.1, in acidic environments. However, as the pH increases above 7, the partition coefficient begins to increase reaching a maximum value of 1550 at pH 11, the minimum of the pH–solubility profile.

DISCUSSION

DuP 923 contains two sterically equivalent tertiary amines in pyrrolidine rings and a phenolic function. The solubility studies indicate three distinct ionization constants of 4.81, 10.13, and 12.13. *N*-Methylpyrrolidine has a pK_a of 10.46 (3). A benzylic substituent on pyrrolidine would be expected to lower the pK_a approximately 1 unit, based on the changes observed with methylamine, pK_a = 10.66, and benzylamine, pK_a = 9.34, (3). *p*-Hydroxybenzamide has a pK_a of 8.56 (3). The addition of an *o*-amino substituent, which would be expected to have a greater effect than an *o*-methylamino substituent, does not affect the pK_a of phenol (3). Examining these relatively simple structural analogues cannot account for the lowest pK_a, suggesting that a more complex mechanism must be responsible.

The spectrophotometric data suggest that the ionization at 4.8 involves the phenol, as judged by the ultraviolet spectral shift as a result of the phenol ionization and the shift of the carbon bonded to the hydroxyl group of the phenol in the ^{13}C NMR.

The partition coefficient and solubility data indicate that at pH 11 the compound becomes very lipophilic. The molecule has the ability to stabilize the phenolate species, thereby reducing the pK_a of the phenol, as demonstrated by

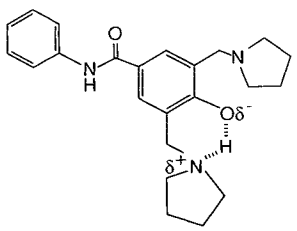

Fig. 5. DuP 923 charge delocalized structure exhibiting intramolecular hydrogen bonding.

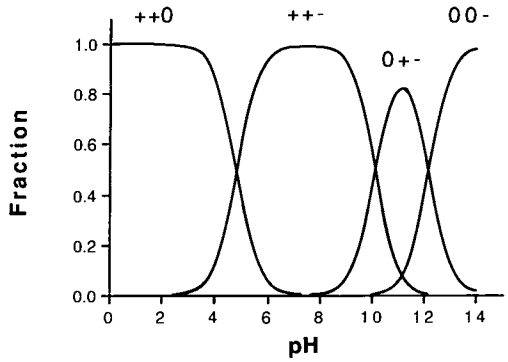

Fig. 6. Distribution diagram for DuP 923 where + +0 represents the species with both of the pyrrolidine nitrogens ionized and the phenol nonionized, + + − represents the species with both of the pyrrolidine nitrogens and the phenol ionized, 0+ − represents the zwitterionic species with one of the pyrrolidine nitrogens and the phenol ionized, and 00− represents the species with both of the pyrrolidine nitrogens nonionized and the phenol ionized.

the spectral data. The proposed structure (Fig. 5) permits the protonated pyrrolidine to intramolecularly hydrogen bond with the phenolate, forming a six-membered ring. The intramolecularly hydrogen-bonded structure stabilizes the phenolate and the protonated nitrogens resulting in a decrease in the pK_a of the phenol and an increase in the pK_a values of the nitrogens. The charge density of the molecule is reduced making it appear very lipophilic. This scheme is consistent with the ^{13}C NMR data in that the bulk of the carbon shift occurs between pH 4 and pH 7, where the molecule goes from the protonated phenol to the intramolecularly hydrogen-bonded structure. The carbon shift continues as the pH increases as a result of the nitrogens losing their protons that are forming the intramolecularly hydrogen-bonded structure. The distribution diagram for the four species presented in Fig. 6 indicates that the molecule exists predominantly as the + + − species at physiologic pH. The phenol is sterically protected by the bulky pyrrolidine groups and their ability to hydrogen bond intramolecularly with the phenolate. The structure provides a plausible explanation for the unusually high oral bioavailability exhibited by this phenol (2).

ACKNOWLEDGMENT

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