Solubility and Ionization Characteristics of Doxepin and Desmethyldoxepin

KORAL EMBIL and GEORGE TOROSIAN[x]

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Abstract □ The thermodynamic pKa values for doxepin and its metabolite desmethyldoxepin were determined by the solubility method to be 8.96 and 9.75, respectively at 25°. The intrinsic solubilities for doxepin and desmethyldoxepin were linearly dependent upon ionic strength. The intrinsic solubilities at zero ionic strength and 25° were determined to be 1.13×10^{-4} *M* for doxepin and 3.95×10^{-4} *M* for desmethyldoxepin. The solubility experiment was repeated at different temperatures and a constant ionic strength of 0.167 *M*. The change in enthalpy (6.71 kcal/mole) and entropy (−4.16 cal/mole °K) of solution for doxepin was determined from a van't Hoff plot for this nonideal system. The apparent partition coefficient between hexane and water for the doxepin free base was determined to be 13,615 at an ionic strength of 0.067 *M*.

Keyphrases □ Doxepin—solubility and ionization characteristics □ Desmethyldoxepin—solubility and ionization characteristics □ Solubility—of doxepin and desmethyldoxepin, ionization characteristics □ Ionization—of doxepin and desmethyldoxepin, solubility

Doxepin hydrochloride is one of a class of psychotherapeutic agents known as dibenzoxepin tricyclic compounds. To further understand its *in vivo* behavior, the fundamental physicochemical properties of doxepin and one of its metabolites, desmethyldoxepin, were studied. These properties may partially explain the ability of the drug to cross membranes and accumulate and/or distribute in various tissues. The ease of extraction of the drug from biological samples, even at pH values at which the protonated species predominate, may be explained by the magnitude of the partition coefficient and the intrinsic solubility of the free base.

The apparent ionization constants for similar tricyclic compounds were determined earlier using the solubility method (1). Similarly, the solubility method was also used to obtain an apparent pKa value for phenytoin (2).

In this paper, apparent pKa values were determined for doxepin and desmethyldoxepin at different ionic strengths and were used to calculate the thermodynamic pKa values for each compound. In addition, the intrinsic solubilities at zero ionic strength were also determined.

EXPERIMENTAL

Materials—Doxepin and desmethyldoxepin were gifts[1]. Sodium chloride, sodium hydroxide, sodium carbonate, hydrochloric acid, and hexane were all reagent grade and used as obtained.

Determination of pKa—The intrinsic or saturated solubility of doxepin and desmethyldoxepin at different pH values was determined and plotted using the following (3, 4):

$$\mathrm{pH} = \mathrm{pKa} - \log\left(\frac{S}{S_0} - 1\right) \qquad \text{(Eq. 1)}$$

where S_0 is the intrinsic solubility of the free base, and S is total solubility and includes both protonated and unprotonated forms of the drug.

The experiment was carried out by determining the solubility of the compound at several pH values. Ten 15-ml culture tubes with polytef[2]-lined screw caps were silylated[3] and maintained at 25° to minimize the separation of the drug as an oil. Into each pair of tubes, 1 ml of 0.05 carbonate buffer (pH 9.0–10.8) was added.

The solubility for each compound was determined at four to six different pH values at constant ionic strength. The intrinsic solubility (S_0) was obtained by measuring the amount of drug in solution at 25° after the pH was adjusted to 12.6 with sodium hydroxide. This pH provided a ratio in excess of 1000:1 in favor of the unprotonated species. Five milliliters of stock solution (1.0×10^{-3} *M* of doxepin hydrochloride or 4.5×10^{-3} *M* desmethyldoxepin hydrochloride) was added to all tubes, approximating a 10-fold excess of drug as the free base.

The tubes were capped, sealed with paraffin film[4], and equilibrated in a water bath at 25° for 5 hr and centrifuged[5] at 25° and 3000 rpm for 30 min. The supernate was then transferred into a clean culture tube to minimize the redispersion of excess drug present as an oil. The solutions that did not exhibit a Tyndall effect were assayed spectrophotometri-

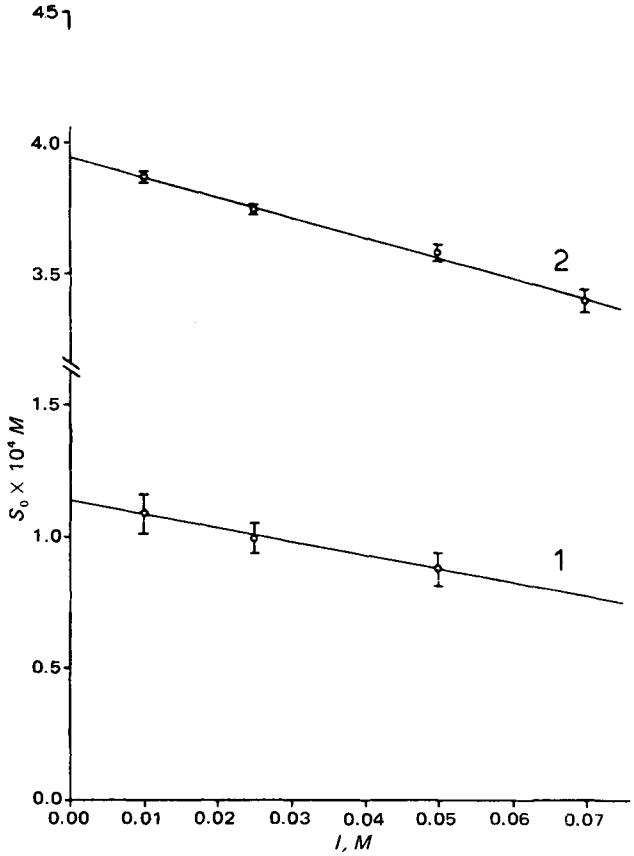

Figure 1—*Plot of intrinsic solubility* (S_0) versus *ionic strength* (I). *Line 1 represents the intrinsic solubility of doxepin at increasing ionic strengths. Each point is an average of five to seven determinations, and the solid line is obtained by least-squares regression. Line 2 represents desmethyldoxepin, each point is the average of two determinations, and the solid line is obtained by least-squares regression. Error bars indicate one standard deviation around the mean.*

[1] Pennwalt Corp., Rochester, NY 14623.

[2] Teflon.

[3] Silyl 8, Pierce Chemical Co., Rockford, Ill.

[4] Parafilm, American Can Co., Dixie/Marathon, Greenwich, CT 06830.

[5] Universal model UV International Equipment Co., Needham Heights, Mass.

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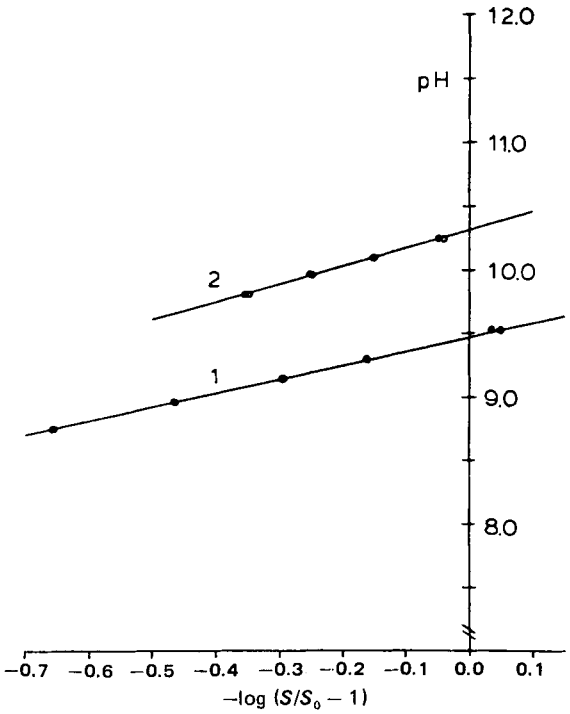

Figure 2—*Typical plot of pH* versus $-log(S/S_0 - 1)$ *for doxepin (Line 1) and desmethyldoxepin (Line 2), obtained at an ionic strength of 0.05 M. Each point represents at least two observations. The solid line in each case represents a least-squares regression through the data points.*

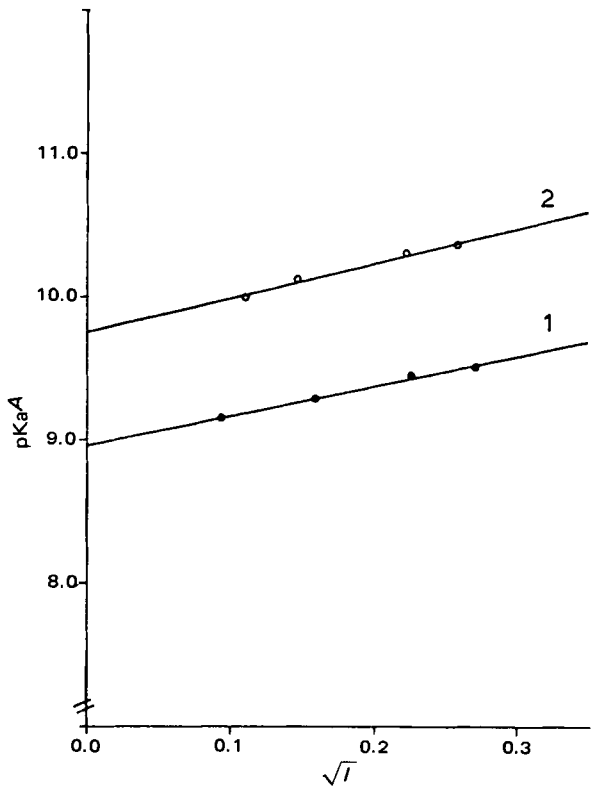

Figure 3—*Plot of apparent pKa* versus $\sqrt{I}$. *Line 1 represents doxepin and line 2 represents desmethyldoxepin. The solid lines are obtained by least-squares regression.*

cally[6] at 292 nm and their pH values were recorded[7]. The absorbances were then converted into concentrations using a standard curve.

Determination of Thermodynamic Parameters—The van't Hoff plot was obtained by determining the intrinsic solubility of doxepin at different temperatures, but at constant ionic strength and plotting the natural log of the mole fractions (X_2) *versus* the reciprocal of the absolute temperatures. The thermodynamic parameters ΔH and ΔS, the change in ethalpy and entropy, respectively, were obtained from the slope and intercept, (5):

$$\ln X_2 = \frac{\Delta S}{R} - \frac{\Delta H}{R}\left(\frac{1}{T}\right) \qquad \text{(Eq. 2)}$$

where R is the gas constant and T is the absolute temperature.

The procedure to determine these parameters was identical to that for the pKa determination, except that 16 tubes were used for each temperature (5, 10, 13, 18, and 25°), and sufficient sodium hydroxide was used to adjust the pH to a constant 12.6. The solutions were brought to temperature (to minimize pressure changes inside the tubes) prior to capping. The tubes were then equilibrated at the selected temperature. After equilibration, the tubes were transferred immediately into a refrigerated centrifuge and spun at 7000 rpm for 40 min at a constant temperature.

The supernate of replicate tubes were combined, transferred into a clean tube and centrifuged[8] at 7000 rpm for an additional 1 hr. The resultant supernate (10 ml) was then spectrophotometrically analyzed as described previously. The data obtained from the doxepin solubilities were converted to mole fractions using the appropriate specific gravity of the solution in question. Specific gravities were determined with a 25-ml pycnometer.

In all cases, the differences in weight per milliliter between the samples and distilled water was less than 1%; hence, the thermodynamic values were obtained from these data without the correction for volume changes upon mixing.

Determination of Partition Coefficient—The conditions were identical to those used in the pKa determination except that four 20-ml tubes were used, each containing 15 ml of doxepin stock solution (7.5×10^{-2} *M*), sufficient sodium hydroxide to obtain a constant pH of 12.6, and 0.5 ml of hexane. After equilibration, the tubes were centrifuged[4] at 2500 rpm for 20 min, and both phases were assayed spectrophotometrically at 292 nm for doxepin content. Saturation of both phases by doxepin was confirmed by repeating the assays at various time intervals during the equilibration period of 5 hr and observing that the drug concentration in both phases remained constant. Excess free base of doxepin was clearly visible in the container forming a separate phase of oil droplets within the aqueous layer. The drug concentration in each phase was calculated, adjusted for volume differences, and used to determine the apparent partition coefficient.

RESULTS AND DISCUSSION

Figure 1 shows the linear relationship of the intrinsic solubility of doxepin and desmethyldoxepin free base obtained at the corresponding ionic strengths. The reduction of the intrinsic solubility with increasing ionic strength may be due to a salting-out effect. The intrinsic solubility at zero ionic strength and 25° was determined from the intercept of the least-squares regression to be 1.13×10^{-4} *M* with a standard error of $\pm 3.12 \times 10^{-6}$ *M* for doxepin and 3.95×10^{-4} *M* with a standard error of $\pm 1.75 \times 10^{-6}$ *M* for desmethyldoxepin.

Tricyclic antidepressants have a high surface activity and it was speculated (1) that they may form micelles. It is therefore possible that the true solubilities may be slightly lower than those indicated. However, discontinuities in the spectrophotometric standard curves which would suggest association, have not been observed over the solubility range. In addition, no Tyndall effect has been detected visually. Therefore, associated species or micelles do not appear to be formed at the concentrations studied for doxepin and desmethyldoxepin.

Figure 2 shows a typical plot of $-\log(S/S_0 - 1)$ *versus* pH (Eq. 1) for doxepin and desmethyldoxepin at an ionic strength of 0.05 *M*. For each compound, the apparent pKa was obtained from the intercept of the least-squares regression line over a range of ionic strengths. For each determination of the apparent pKa, the intrinsic solubility was used at the appropriate ionic strength required. At low ionic strengths of <0.01 *M*, the equation relating pKa to ionic strength (I) is described by (6):

$$pKa^T = pKa^A - 0.505\sqrt{I} \qquad \text{(Eq. 3)}$$

where pKa^T is thermodynamic pKa and pKa^A is apparent pKa.

[6] Beckman DB-GT spectrophotometer connected to a 25.4-cm Beckman recorder.

[7] Beckman Century SS-1 pH meter connected to a model 39030 combination electrode.

[8] Sorvall superspeed RC2-B refrigerated centrifuge fitted with a SS-34 rotor. Sorvall Inc., Newtown, CT 06470.

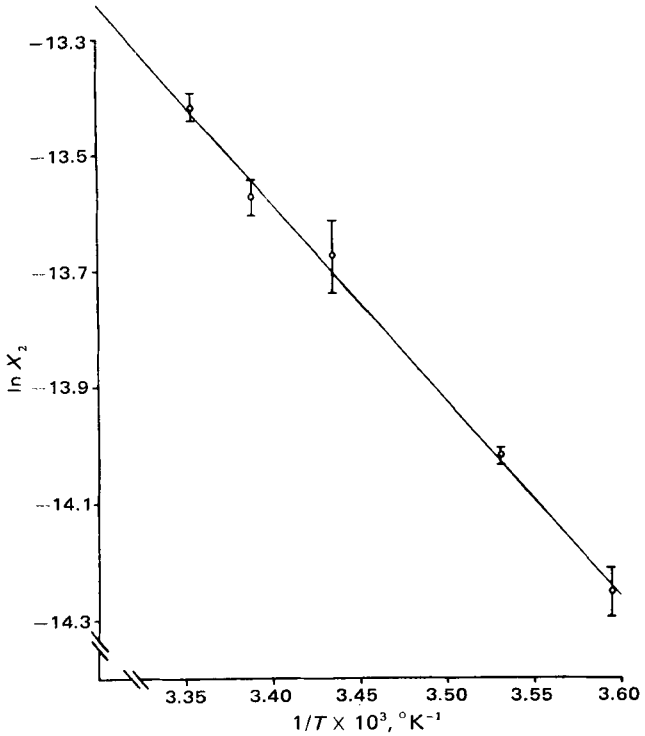

Figure 4—*The van't Hoff plot of ln* X_2 versus 1/T, *in degrees K, for doxepin at an ionic strength of 0.167* M. *Error bars indicate one standard error around the mean with each point representing four to eight determinations.*

Figure 3 shows the least-squares regression line representing the relationship between the apparent pKa values and the square root of the ionic strength of the system at which they were determined (Eq. 3). The y-intercept yields a pKa^T of doxepin at zero ionic strength of 8.96 with a standard error of ±0.05. This value substantially differs from the value of 8.0 (7) but is similar to the apparent pKa of other tricyclics with a similar alkyl side chain, such as chlorpromazine and imipramine whose apparent pKa values are 9.3 and 9.5, respectively (1).

For desmethyldoxepin, a pKa^T value of 9.75 with a standard error of ±0.05 was obtained from the intercept of the least-squares regression line. This value is in the same range as other demethylated tricyclic compounds such as desipramine, whose apparent pKa is 10.2 (1). The increase in the pKa of desmethyldoxepin over that of doxepin may be due to the loss of a methyl group and its replacement by a hydrogen atom on the amine side chain. Hydrogen bonding between the solvent water and the hydrogen atom on the secondary amine of desmethyldoxepin displaces the hydrogen from the nitrogen, causing the nitrogen to be more electron rich, hence, increasing its basic character. This increased basicity leads to a weaker conjugate acid and an increase in pKa (8).

The linearity of the van't Hoff plot ($r^2 = 0.979$) indicates that the number of water molecules necessary for solvation is temperature independent in the range tested. The slope and the intercept of the van't Hoff plot yielded the thermodynamic values for the change in entropy and enthalpy of solution (Fig. 4). The change in enthalpy of solution was 6.71 with a standard error of ±0.19 kcal/mole, a positive value indicating that the system absorbed heat from its surroundings as would be expected from a nonpolar molecule with low aqueous solubility. The change in entropy of solution was −4.16 with a standard error of ±0.64 cal/mole °K.

The apparent partition coefficient for doxepin in hexane–water was determined at pH 12.6 and was 13,615: 1 with a standard deviation of ±360. The high value of the partition coefficient in hexane–water indicates the large lipid solubility of doxepin. The log of the partition coefficient was 4.13 and compares well with other tricyclic psychotherapeutic agents such as imipramine and chlorpromazine whose log octanol–water partition coefficients are 4.62 and 5.32, respectively (9). Preliminary experiments were able to extract 90% of 6.25×10^{-4} *M* doxepin contained in 30 ml of water with a single 3-ml sample of hexane by vortexing for 5 min at pH 7.5. This is 1.5 units below its pKa and the base should be substantially protonated; yet the extraction at a somewhat unfavorable pH took place with ease, apparently due to its large partition coefficient.

However, only 75% of 6.33×10^{-4} *M* desmethyldoxepin was extracted at pH 8.7, 1.3 units below its pKa but otherwise similar conditions. Although the partition coefficient of desmethyldoxepin was not determined in hexane–water, we speculate that it cannot be as large as that of doxepin. Based on our calculations, using a direct proportionality of 90% doxepin with a partition coefficient of 13,615:1 to 75% desmethyldoxepin extracted under similar conditions, the partition coefficient of desmethyldoxepin for hexane–water was estimated to be about 10,000:1.

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