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Effect of pKb on Lipophilic Binding of Disopyramide Derivatives to Human Plasma

YIE W. CHIEN ˣ, MICHAEL J. AKERS *, and PETER K. YONAN

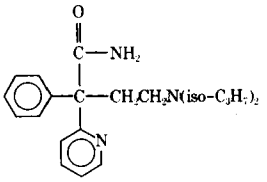
Abstract □ The extent of plasma binding, the partition coefficient, and the pKb of 13 disopyramide derivatives were determined. The structural variation on the diisopropylaminoethyl group of disopyramide molecules influenced these physical parameters to varying degrees. Results demonstrated that the extent of interaction between drugs and human plasma was a linear function of their lipophilicity and inversely proportional to the magnitude of the pKb value.

Keyphrases □ Disopyramide derivatives—determination of plasma binding, partition coefficient and pKb, relationship between pKb and binding □ Lipophilic binding of 13 disopyramide derivatives to human plasma—relationship to pKb □ Plasma binding, 13 disopyramide derivatives—determination, relationship to pKb □ Partition coefficients, 13 disopyramide derivatives—determination

Previously, it was reported (1) that the extent of plasma binding for 20 disopyramide derivatives was linearly related to their lipophilicity, log (p.c.), as defined by the following relationship:

$$\log (D_b/D_f) = \log (D_b/D_f)_0 + \log (\text{p.c.}) \qquad \text{(Eq. 1)}$$

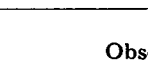
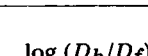
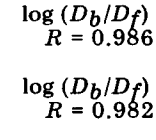
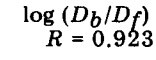
Table I—Linear Relationship between the Extent of Plasma Binding and the Lipophilicity of Disopyramide Derivatives with the Structural Variation of their Amide, Pyridyl, or Phenyl Moieties



Structural Variation on	n^a	Observed Relationships[b]
	10	$\log (D_b/D_f) = -0.303 + 0.473 \log$ (p.c.) $R = 0.986$ $s^2 = 0.0034$
	7	$\log (D_b/D_f) = -0.369 + 0.641 \log$ (p.c.) $R = 0.982$ $s^2 = 0.0059$
	6	$\log (D_b/D_f) = -0.476 + 0.734 \log$ (p.c.) $R = 0.923$ $s^2 = 0.0303$

[a] n = number of derivatives used in the analysis. [b] R = correlation coefficient; s^2 = residual variance.

Table II—Disopyramide Derivatives Series (I)

Compound[a]	R	$\log (D_b/D_f) + \log (K_b + [H^+])$	log (p.c.)	pKb
I	$-N(iso-C_3H_7)_2$	1.219	−0.181	8.36
II	$-N(n-C_3H_7)_2$	0.826	0.905	9.50
III		0.747	−0.842	9.92
IV		2.680	−0.553	5.40
V		0.999	0.560	8.50
VI		1.190	0.689	8.86
VII		0.495	−0.373	9.95

[a] SC-13957, SC-13268, SC-24566, SC-13251, SC-13212, SC-13482, and SC-27829, respectively.

where log (D_b/D_f) was theoretically derived and related to the equilibrium constant for drug–plasma interactions; D_b and D_f are the concentrations of drug bound to plasma protein and freely existing in the solution phase, respectively; and log $(D_b/D_f)_0$ is the intercept of log (D_b/D_f) *versus* log (p.c.) plots.

It was observed that the structural variation of the amide, pyridyl, or phenyl moieties resulted in varying degrees of influence on the extent of plasma binding and the lipophilicity of disopyramide derivatives (Table I). It also was found that the omission of the diisopropylaminoethyl group produced no change in the degree of plasma binding while the lipophilicity was enhanced more than 10-fold (fraction of drug bound remained at 27.3% although the partition coefficient in the *n*-octanol–phosphate buffer system was increased from 0.66 to 6.87). This observation was rationalized by the fact that the diisopropylaminoethyl group (with a pKb of 8.36) was protonated at the physiological pH of 7.4 and did not interact with the plasma protein itself.

For a better understanding of the behavior of the diisopropylaminoethyl group during the interaction of the other three moieties of disopyramide molecules with plasma protein, the studies were extended to cover a new series of disopyramide derivatives with structural variation on the diisopropylaminoethyl moiety only while the other three functional groups were held constant. These results are reported in this paper.

EXPERIMENTAL

Binding of disopyramide derivatives to human plasma and partitioning studies in the system of *n*-octanol–pH 7.4 phosphate buffer were carried out in the same manner as described earlier (1).

Potentiometric pH titration[1] was employed for the determination of pK values. This method is highly reproducible (pK ± 0.03 unit) for acids or bases having pK values between 2.5 and 11 (2). Apparent pK's were determined by the Parke and Davis method (3) instead of by the conventional half-neutralization method because the solubility of the compounds was low and the inflection of the titration curves was not very sharp.

An aqueous solution containing 2.5×10^{-4} *M* compound was titrated with standardized 0.1 *N* hydrochloric acid. An identical solution containing no compound was subsequently titrated, and the difference between these two pH *versus* titrant volume titration curves was calculated. The apparent pKb value was then measured as the pH at the point of inflection of the "pH-different titrant volume curve." Determinations of pKb values were run in duplicate for each compound. No pKb was observed for the compound without the diisopropylaminoethyl group.

RESULTS AND DISCUSSION

In solution, disopyramide derivative molecules (D) exist as an equilibrium mixture of neutral and protonated species (Scheme I):

$$D + H_3O^+ \rightleftharpoons HD^+ + H_2O$$

Scheme I

The degree of protonation (σ) of the diisopropylaminoethyl group varies according to its basicity (K_b) in a series of disopyramide derivatives and the pH profile of the drug solution:

$$\sigma = \frac{[H^+]}{K_b + [H^+]} \qquad \text{(Eq. 2)}$$

If drug molecules are bound to plasma protein only as the neutral form, the equilibrium constant (K_n) for the interaction (4) between the neutral species of disopyramide derivatives and plasma protein is related to the overall equilibrium binding constant (K) as follows:

$$K_n = \frac{K}{1 - \sigma} = \frac{K}{K_b/K_b + [H^+]} \qquad \text{(Eq. 3)}$$

or:

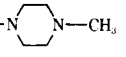
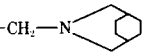
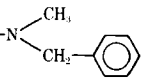
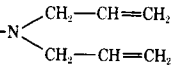
$$\log K_n = \log K - \log K_b + \log (K_b + [H^+]) \qquad \text{(Eq. 4)}$$

As analyzed previously (1), the equilibrium constant, K_n, for the interaction between the neutral species of drug and plasma protein was linearly related to its lipophilicity [as represented by log (p.c.)]:

$$\log K_n = \frac{\mu_0^\circ - \mu_p^\circ}{2.303RT} + \log \text{(p.c.)} \qquad \text{(Eq. 5)}$$

[1] Copenhagen Radiometer, London Co., Westlake, Ohio.

Table III—Disopyramide Derivatives Series (II)

Compound[a]	R	log (D_b/D_f) + log $(K_b$ + $[H^+])$	log (p.c.)	pKb
VIII		1.203	0.083	6.86
IX		0.808	0.501	7.81
X		3.894	0.874	4.74
XI		4.234	0.956	4.68
XII		3.079	1.122	6.90
XIII		1.617	0.877	8.11

[a] SC-13127, SC-13173, SC-13209, SC-13733, SC-13489, and SC-13486, respectively.

where $\mu_0°$ and $\mu_p°$ are the standard chemical potentials for a drug species existing in the organic phase and bound to a protein molecule, respectively.

Substituting Eq. 5 for the log K_n term in Eq. 4 gives:

$$\log K + \log (K_b + [H^+]) = \frac{\mu_0° - \mu_p°}{2.303RT} + \log (\text{p.c.}) - \text{pKb} \quad \text{(Eq. 6)}$$

The Scatchard relationship (5) may be expressed alternatively as:

$$K = \frac{D_b}{D_f} \times \frac{1}{\bar{n}p} \quad \text{(Eq. 7)}$$

since $\bar{n} \gg \bar{v}$. Therefore:

$$\log (D_b/D_f) + \log (K_b + [H^+]) = \log (D_b/D_f)_0 + \log (\text{p.c.}) - \text{pKb} \quad \text{(Eq. 8)}$$

where:

$$\log (D_b/D_f)_0 = \frac{\mu_0° - \mu_p°}{2.303RT} + \log \bar{n}p \quad \text{(Eq. 9)}$$

The terms $\bar{n}$ and p were defined earlier (1) as the total number of binding sites on a protein molecule and the total concentration of plasma protein in the bloodstream, respectively.

Equation 8 predicts that the extent of plasma binding is dependent on both the lipophilicity, log (p.c.), and the pKb of disopyramide derivatives. When the pH of a drug–plasma mixture is maintained at the physiological pH of 7.4, the higher the lipophilicity of drug the greater is the extent of the drug–plasma interaction. On the other hand, as the magnitude of pKb value of diisopropylaminoethyl group increases, the degree of plasma binding decreases.

Table IV—Comparison between the Observed and the Calculated Fraction of Disopyramide Derivatives Bound to Plasma Protein in Table II

Compound[a]	Fraction of Drug Bound, %[b]		
	Observed	Calculated	
		Eq. 10	Eq. 12
I	27.30	30.74	32.89
II	14.35	13.11	9.56
III	12.26	8.91	5.48
IV	10.43	8.67	27.04
V	18.81	22.16	29.10
VI	27.4	28.22	20.47
VII	7.25	9.33	5.26

[a] SC-13957, SC-13268, SC-24566, SC-13251, SC-13212, SC-13482, and SC-27829, respectively. [b] Fraction of drug bound (%) = $[D_b/(D_b + D_f)] \times 100$.

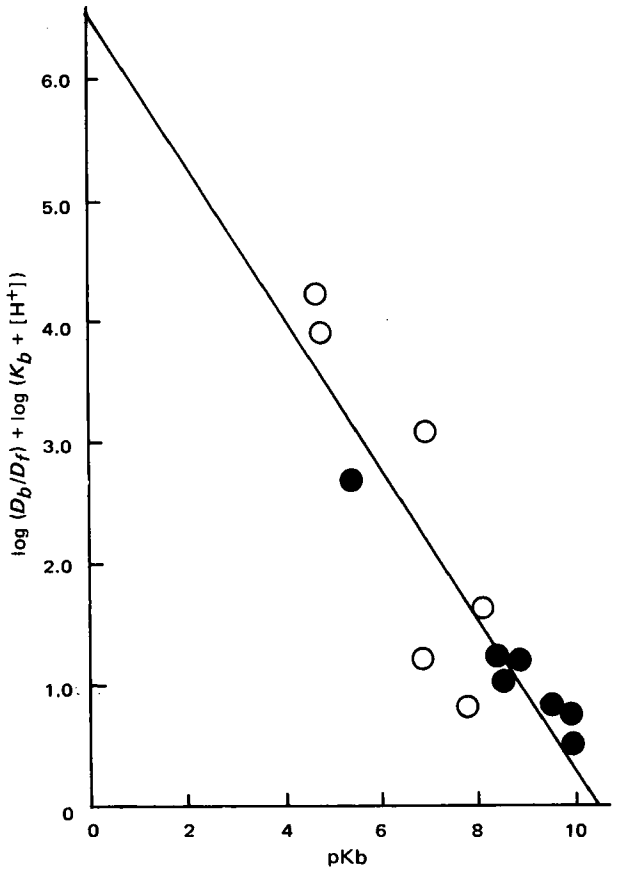


Figure 1—*Linear plot between log* (D_b/D_f) + log $(K_b + [H^+])$ *and pKb as defined by Eq. 12. All terms are defined in the text. The open circles are the data points from Table III, and the solid circles are the data points from Table II.*

The values of log (D_b/D_f) + log $(K_b + [H^+])$, log (p.c.), and pKb tabulated in Tables II and III were submitted separately to multiple regression analysis. Two relationships were obtained:

$$\log (D_b/D_f) + \log (K_b + [H^+]) = 5.035 - 0.448(\pm 0.039)\text{pKb} \quad \text{(Eq. 10)}$$

n	R	s^2
7	0.982	0.016

$$\log (D_b/D_f) + \log (K_b + [H^+]) = 5.835 + 1.857(\pm 0.29) \log (\text{p.c.}) - 0.726(\pm 0.074)\text{pKb} \quad \text{(Eq. 11)}$$

n	R	s^2
6	0.992	0.028

Both the multiple correlation coefficient (R) and the residual variance (s^2) demonstrate that the degree of plasma binding of disopyramide derivatives is highly related to their pKb values and/or their lipophilicity. An F test indicates that both Eq. 10 [$F_{1,5}$ = 134.4; $F_{1,5}$ (when α = 0.01) is 16.26] and Eq. 11 [$F_{2,3}$ = 94.16; $F_{2,3}$ (when α = 0.01) is 30.80] are highly significant statistically.

By using Eqs. 10 and 11, estimated β values (fraction of drug bound) for the disopyramide derivatives were computed and tabulated in the third column of Tables IV and V together with the observed β values (second column).

Equations 10 and 11 demonstrated that structural variation on the diisopropylaminoethyl group resulted in two subgroups whose

Table V—Comparison between the Observed and the Calculated Fraction of Disopyramide Derivatives Bound to Plasma Protein in Table III

Compound[a]	Fraction of Drug Bound, %[b]		
	Observed	Calculated	
		Eq. 11	Eq. 12
VIII	8.23	5.46	50.92
IX	10.48	18.59	46.31
X	30.03	36.41	17.35
XI	45.03	43.91	16.61
XII	87.85	83.12	51.20
XIII	46.35	44.17	39.15

[a] SC-13127, SC-13173, SC-13209, SC-13733, SC-13489, and SC-13486, respectively. [b] Fraction of drug bound (%) = $[D_b/(D_b + D_f)] \times 100$.

plasma binding was dependent, in a different degree, on the magnitude of pKb and lipophilicity as demonstrated by the difference in their slopes (−0.448 and −0.726, respectively).

If the influence of lipophilicity, log (p.c.), is neglected and only the pKb values are related to the extent of plasma binding for these two subgroups of disopyramide derivatives, the importance of pKb can be clearly shown. The linear relationship (Fig. 1) is defined by the following expression:

$$\log (D_b/D_f) + \log (K_b + [H^+]) = 6.528 - 0.621(\pm 0.090)\text{pKb} \quad \text{(Eq. 12)}$$

n	R	s^2
13	0.901	0.279

Because of the neglect of the lipophilicity term in Eq. 12, the data points from Table III were noticeably displaced from the slope (Fig. 1). The deviation of the calculated from the observed values was significantly greater for the chemicals in Table III (comparing column 2 with column 4 in Table V) than for the chemicals in Table II (comparing column 2 with column 4 in Table IV). The results demonstrate that the series of disopyramide derivatives in Table II will be better expressed by Eq. 10 and that the extent of plasma binding is linearly correlated with the magnitude of pKb with a slope of −0.448. On the other hand, the extent of plasma binding for the disopyramide derivatives in Table III is a linear function of their lipophilicity and inversely proportional to the magnitudes of pKb. This relationship is best defined by Eq. 11.

In conclusion, the extent of interaction between plasma protein and disopyramide derivatives is significantly influenced by the magnitude of the pKb of the diisopropylaminoethyl group.

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Preparation, Isolation, and Identification of 4-Dedimethylamino-11-methoxyanhydrotetracycline

L. J. STOEL *, E. C. NEWMAN ‡, G. L. ASLESON, and C. W. FRANK x

Abstract □ The reaction of diazomethane with 4-dedimethylaminoanhydrotetracycline produced a number of mono-, di-, and trimethylated products. The isolation of one monomethylated product (4-dedimethylamino-11-methoxyanhydrotetracycline) and its subsequent identification utilizing mass spectral and NMR data are described.

Keyphrases □ 4-Dedimethylamino-11-methoxyanhydrotetracycline—isolation and identification after diazomethane methylation of 4-dedimethylaminoanhydrotetracycline □ Methylation of 4-dedimethylaminoanhydrotetracycline with diazomethane—isolation and identification of one reaction product □ Mass spectrometry—identification, 4-dedimethylamino-11-methoxyanhydrotetracycline

During investigations of metal-ion complexation with tetracyclines, it was necessary to prepare, isolate, and identify tetracyclines with blocked or removed functional groups. One method employed was the preparation of methyl ethers of the acidic hydroxy proton sites of anhydrotetracycline by reaction with diazomethane. While preparation of the methyl ethers by this method was relatively simple, the difficulty remained in separating and identifying the reaction products. This paper describes the isolation and structural identification of one product of the methylation reaction.

EXPERIMENTAL

NMR spectra were obtained using high-resolution NMR spectrometers[1]. The chemical shift data were measured relative to tetramethylsilane as an internal standard and reported as parts per million. All NMR spectra were recorded using d_6-dimethyl sulfoxide as the solvent. The mass spectra were obtained on a medium

[1] Varian A-60 and Varian HA-100.