g/100 ml. Separate solubility studies at concentrations above the surfactant's CMC did not reveal any change in the solubility of soluble chemicals used in this investigation. The concentration of the surfactant in the tablets probably was enough to cause an increase in their porosities and, consequently, to minimize the dependence of tablet tortuosities on the ratios of the soluble components of the tablets.

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Binding of Spirolactones to Human Plasma Proteins

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Abstract □ The lipophilicity and plasma binding of 16 spirolactones and 4 hydroxy acid analogs were determined. Mathematical expressions were derived to correlate quantitatively the extent of plasma binding to the lipophilicity of the drugs. The nonspecific binding of these spirolactones and their hydroxy acid analogs was also analyzed using purified serum albumin. A computer program was developed to examine the mechanism of drug–serum protein interactions. One class of binding sites was observed for the range of concentrations used. The number of binding sites and the equilibrium binding constant were computed and were sensitive to substitution at the C-6 and C-7 positions. Hydrolysis of the C-17 lactone ring in spirolactones to form hydroxy acid analogs resulted in a decrease in the lipophilicity and, hence, the equilibrium constant for binding.

Keyphrases □ Spirolactones—lipophilicity and plasma protein binding determined and correlated quantitatively □ Lipophilicity—spirolactones, correlated quantitatively with plasma protein binding □ Plasma protein binding—spirolactones, quantitatively correlated with lipophilicity □ Binding, plasma protein—spirolactones, quantitatively correlated with lipophilicity

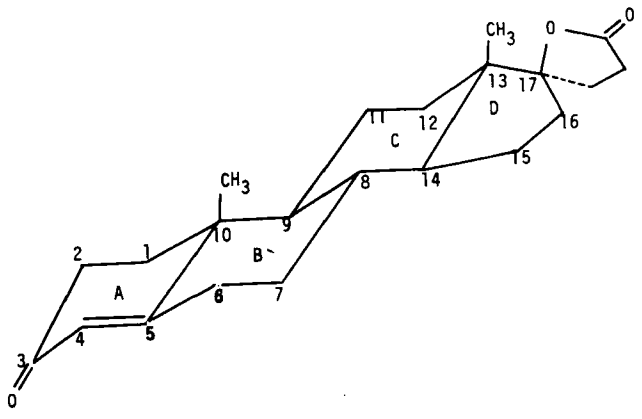
Spironolactone[1] has been used clinically to manage refractory edema and found to have the greatest selectivity of the competitive antagonists of aldosterone (1, 2).

The interaction of organic molecules with plasma proteins has been recognized for many years as an important parameter of tissue permeation and clinical efficacy of a drug (3, 4). Recently, this laboratory demonstrated that modifying the molecular structure of disopyramide significantly influenced both the antiarrhythmic activity (5) and the extent of plasma protein–drug interactions (6). Quantitative correlation was observed between the extent of drug–plasma protein interactions and the physicochemical parameters of disopyramide derivatives (6–8). Furthermore, a linear correlation was established between the extent of plasma protein binding and the frontier electron density, estimated by molecular orbital calculations, on the alkyl side chain of trichomonicidal metronidazole[2] derivatives (9).

Biopharmaceutical studies of spironolactone and its derivatives revealed that they were extensively bound to human plasma and that the strength of the plasma binding was sensitive to structural variation. The results are reported and analyzed in this paper.

EXPERIMENTAL

The procedure for measuring the lipophilicity and the membrane ultrafiltration technique for determining the binding of spirolactones to plasma protein and serum albumin[3] (1.4056×10^{-4} *M*) are essen-



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[1] Searle Laboratories, Division of G.D. Searle & Co., Chicago, IL 60680

[2] Flagyl, Searle Laboratories, Division of G.D. Searle & Co., Chicago, IL 60680

[3] Fraction V, fatty acid poor, Nutritional Biochemical Corp., Cleveland, Ohio.

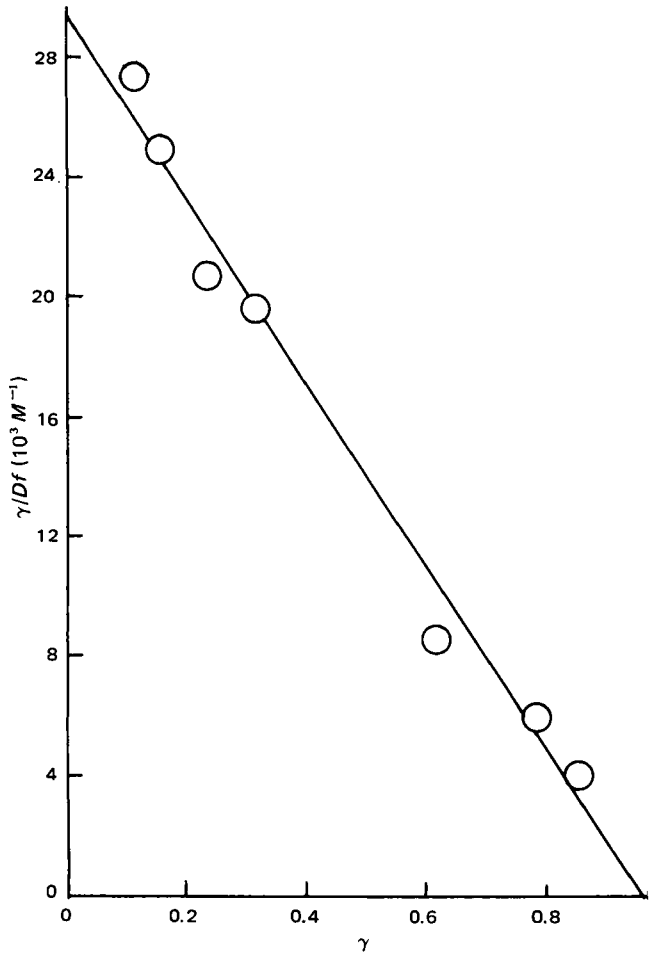


Figure 1—*Linear Scatchard plot for III, spirolactone derivative (Table I), using a representative set of data measured at pH 7.4 with a drug concentration range of 1.6–40 × 10^{-5} M and a human serum albumin concentration of 3.514 × 10^{-4} M. Key:* ○, *experimental observations; and —, computer-fitted theoretical line following Eq. 1. From the intercept and slope, the values of* n̄ *and* K *were calculated to be 0.986 and 30,457.6* M^{-1}, *respectively.*

tially the same as reported (6, 8) previously, except that 20% (v/v) 1,3-butylene glycol and 4% methanol were added to the phosphate buffer system as cosolvents to enhance the aqueous solubility of spirolactones (10). The addition of this cosolvent pair was compatible with plasma proteins and yielded no precipitation of plasma proteinaceous material.

RESULTS AND DISCUSSION

Mechanisms of Spirolactone–Plasma Protein Interaction—The mechanisms of the interaction between spirolactones and plasma proteins were analyzed using purified human serum albumin, the protein constituent in plasma responsible for most drug–plasma interactions. The binding of spirolactones to human serum albumin was observed to follow a linear Scatchard (11) relationship (Fig. 1) when the binding data were plotted in the following manner:

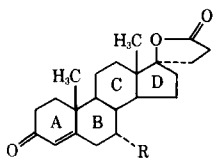
$$\frac{\gamma}{Df} = \bar{n}K - \gamma K \qquad \text{(Eq. 1)}$$

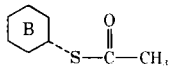
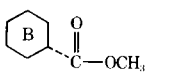
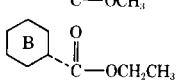
where γ is the ratio of the concentration of drug bound, Db, to a protein over the total concentration of serum protein, P, used; Df is the concentration of free (unbound) drug at equilibrium; $\bar{n}$ is the number of binding sites in a binding class available for drug–protein interactions; and K is the equilibrium binding constant. A computer program was developed to estimate the value of the binding ratio, γ, for each drug concentration investigated from the observed Df measured at equilibrium. The data obtained were then plotted following Eq. 1, and the magnitudes of $\bar{n}$ and K also were computed. Figure 1 is a typical example of this computer printout.

Both the number of binding sites, $\bar{n}$, and the equilibrium binding constant, K, for the spirolactones were observed to be influenced by structural modification of the B ring (Table I), although they all bind with only one class of binding sites in human serum albumin. This structural dependency was also illustrated in the corresponding hydroxy acid analogs with molecular structures similar to those of spirolactones, except that the lactone ring at the 17-position was hydrolyzed to form a potassium salt (Table II). Apparently, the hydrolysis process results in no significant variation in the number of binding sites, $\bar{n}$, but a substantial reduction (1.4–4.3-fold) in the strength, K, of the drug–serum protein interaction (Tables I and II).

For example, the hydrolysis of Compounds III–VII did not reduce the value of $\bar{n}$, but the magnitude of K was decreased more than twofold, from 3.09 × 10^4 to 1.29 × 10^4 M^{-1}. The effect of hydrolysis on the strength of the drug–protein interaction, K, was attributable, as expected, from the following relationship (Eq. 2) derived previously

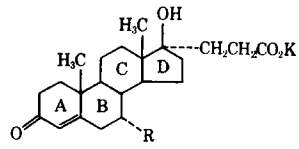
Table I—Effect of Structural Modification on Binding Characteristics of Spirolactone Derivatives to Human Serum Albumin

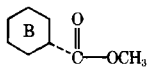
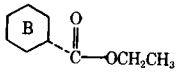


Compound	R	Human Serum Albumin Binding Characteristics[a] $\bar{n}$	K, 10^4 M^{-1}	$\bar{n}K$, 10^4 M^{-1}
I (spironolactone)		0.47	4.23	2.00
II[b]		0.94	3.64	3.41
III[c]		0.99	3.09	3.05
IV[d]		0.35	1.57	0.55
V[e]		0.65	2.08	1.36

[a] Each value is the mean of three determinations. [b] SC-9376. [c] SC-23133. [d] SC-25152. [e] SC-26163.

Table II—Effect of Structural Modification on the Binding Characteristics of Hydroxy Acid Analogs to Human Serum Albumin



Compound	R	Human Serum Albumin Binding Characteristics[a] $\bar{n}$	K, $10^4\ M^{-1}$	$\bar{n}K$, $10^4\ M^{-1}$	Relative[b] K
VI[c] (canrenoate potassium)		0.84	2.64	2.22	1.4
VII[d] (prorenoate potassium)		1.03	1.29	1.32	2.4
VIII[e] (mexrenoate potassium)		0.30	0.52	0.16	3.0
IX[f]		0.47	0.49	0.23	4.3

[a] Each value is the mean of three determinations. [b] Ratio of K (spirolactone) to K (hydroxy acid analog). [c] SC-14266. [d] SC-23992. [e] SC-26714. [f] SC-27719.

Table III—Correlation of the Strength (K) of Drug–Protein Interaction with the Lipophilicity, Log (p.c.), of Spirolactones and Hydroxy Acid Analogs

Compound	Log K[a]	Log (p.c.)[b]
I	4.626	2.777
II	4.561	2.680
III	4.490	2.536
IV	4.197	1.941
V	4.318	2.543
VI	4.422	0.449
VII	4.109	0.665
VIII	3.714	−0.379
IX	3.690	−0.220

[a] Each value is the mean of three determinations. [b] Determined in 1-octanol–pH 7.4 phosphate buffer. Each value is the mean of six determinations.

(6), to the reduction in the lipophilicity, log (p.c.), of drugs (Table III) due to the hydrolysis of 17-lactone:

$$\log K = \frac{\mu_0^0 - \mu_p^0}{2.303RT} + \log (\text{p.c.}) \qquad \text{(Eq. 2)}$$

where (p.c.) is the partition coefficient of the drug measured in a 1-octanol–pH 7.4 phosphate buffer system, and μ_p^0 and μ_0^0 are the standard chemical potentials for a drug species bound to a protein molecule and in an organic solvent system, respectively (6).

Equation 2 indicates that, for the interaction of spirolactones and their hydroxy acid analogs to serum protein, K is directly proportional to the magnitude of log (p.c.), the lipophilicity of the drug molecules. The validity of Eq. 2 in expressing the linear correlation between the strength of the drug–protein interaction (log K) and the lipophilicity of spirolactones and their hydroxy acid analogs was verified by submitting the data in Table III to a multiple regression analysis. The result of the correlation of log K with log (p.c.) is described by the following relationship:

$$\log K = 3.914 + 0.223 \log (\text{p.c.}) \qquad \text{(Eq. 3)}$$

n	r	s^2
9	0.845	0.030

The relatively high multiple correlation coefficient ($r = 0.845$) and the low residual variance ($s^2 = 0.030$) indicated that the strength of serum protein binding of spirolactones and their hydroxy acid analogs was highly dependent on the lipophilicity of the drugs. This observation agreed with results reported earlier for disopyramide derivatives (6).

Binding of Spirolactones to Plasma Proteins—To investigate further the lipophilicity dependency of spirolactones and the validity

Table IV—Lipophilicity, Log (p.c.), and Extent of Plasma Binding, Log (Db/Df), of Spirolactones (Structure I)

Compound	Structural Modification	Log (p.c.)[l]	Fraction of Drug Bound, %	Log (Db/Df)[m]
I	7α-$SCOCH_3$	2.777	94.21	1.211
X[a]	7β-$SCOCH_3$	2.262	92.08	1.065
XI[b]	—	2.978	92.91	1.117
II	6,7-Ene	2.680	98.22	1.742
XII[c]	9α-F,11β-OH	2.404	74.81	0.473
XIII[d]	9,11β-Oxide	1.962	74.22	0.459
XIV[e]	9,11α-Oxide	1.889	80.90	0.627
XV[f]	6-Spirocyclopropyl	3.149	89.69	0.940
III	6,7β-Methylene	2.536	90.59	0.984
XVI[g]	6,7α-Methylene	3.083	88.93	0.905
IV	7α-$COOCH_3$	1.941	73.52	0.444
XVII[h]	7α-COOH	−0.520	19.10	−0.627
V	7α-$COOC_2H_5$	2.543	82.66	0.678
XVIII[i]	7α-$CO_2CH(CH_3)_2$	2.818	93.66	1.170
XIX[j]	7α-CN	1.666	76.76	0.519
XX[k]	7β-$COOCH_3$	2.181	81.09	0.632

[a] SC-11940. [b] SC-5233. [c] SC-9837. [d] SC-9848. [e] SC-12950. [f] SC-17127. [g] SC-24441. [h] SC-25914. [i] SC-26304. [j] SC-26493. [k] SC-26704. [l] Each value is the mean of six determinations. [m] Each value is the mean of three determinations. The Db and Df values are the concentrations of drug bound and unbound to plasma protein at equilibrium, respectively. Data of this column were calculated from the fractions of drug bound shown in the fourth column of this table.

of Eq. 2 in defining the effect of lipophilicity on the extent and strength of binding of spirolactones to plasma proteins, more spirolactones with various structural modifications were examined (Table IV). Apparently both the lipophilicity and the fraction of spirolactone bound were sensitive to the structural modifications in the neighborhood of the B and C rings of the spirolactone molecule.

The combination of Eqs. 1 and 2 resulted in Eq. 4, a simplified mathematical expression (6) for the linear relationship between the extent of plasma binding, log (*Db/Df*), and the lipophilicity, log (p.c.), of a series of drugs:

$$\log \frac{Db}{Df} = \text{intercept} + \log (\text{p.c.}) \qquad \text{(Eq. 4)}$$

where:

$$\text{intercept} = \text{constant} + \frac{\mu_0{}^0 - \mu_p{}^0}{2.303RT} \qquad \text{(Eq. 5)}$$

The development of Eq. 4 permitted the calculation of the extent of plasma binding (*Db/Df*) directly from a binding measurement without requiring a Scatchard analysis to compute the magnitude of the equilibrium binding constant, *K*. The application of Eq. 4 in place of Eq. 2 should not only save a substantial workload when dealing with a large number of drugs but still preserve the equivalent theoretical validity (6).

The validity of Eq. 4 was demonstrated by submitting the data in Table IV to a multiple regression analysis. The correlation of log (*Db/Df*) with log (p.c.) is expressed by the following relationship:

$$\log \left(\frac{Db}{Df}\right) = -0.352 + 0.497 \log (\text{p.c.}) \qquad \text{(Eq. 6)}$$

n	r	s^2
16	0.846	0.069

Again, both the multiple correlation coefficient ($r = 0.846$) and the residual variance ($s^2 = 0.069$) demonstrate that the degree of plasma binding of spirolactones is apparently correlated to their lipophilicity. The results of Eqs. 3 and 6 suggest that the interactions of spirolactones with serum albumin and plasma protein are both nonspecific in nature. This observation agrees with results reported earlier for disopyramide derivatives (6) and other drugs (12).

In summary, the results demonstrated that the interactions of spirolactones and their hydroxy acid analogs with human plasma proteins are nonspecific in nature; any modification in their molecular structures that leads to a higher lipophilicity will result in an enhanced plasma binding.

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