Table V—Formulation Content Determined by UV Spectrophotometry and Differential Pulse Polarography

Tablet	Concentration by UV, mg	Concentration by Differential Pulse Polarography, mg
Orindazole		
1	501.9	507.0
2	518.2	519.2
3	498.5	502.4
Mean ± *SD*	506.4 ± 11.3	509.5 ± 9.2
Isoniazid		
1	93.56	92.50
2	97.69	96.25
3	96.75	95.88
Mean ± *SD*	96.0 ± 1.77	94.9 ± 1.26

CONCLUSION

Polarographic measurement of drug dissolution and formulation content of four different drug formulations demonstrated the utility of polarography to provide such data with high accuracy and precision. The technique provides for measurement of compounds with varied chemical structures contained in formulations covering a wide range of concentrations and in the presence of excipients. The tapered dropping mercury electrode served as an excellent sensor for noninvasive measurements, thereby precluding the use of flowcells, transfer lines, and pumps as well as simplifying the calculation of data. Invasive measurements were also made by incorporating the dropping mercury electrode into a specially designed flowcell to obtain continuously sampled polarographic data.

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Functional Group Contribution of Bile Salt Molecules to Partitioning of a Quaternary Ammonium *N*,*N*-Dimethyl Derivative of Propranolol

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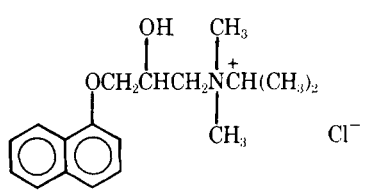
Abstract □ A quaternary ammonium *N*,*N*-dimethyl derivative of propranolol was extracted from pH 7.4 phosphate buffer into 1-octanol as ion-pairs with 12 different bile salts. The binding number, n, and the extraction constant, K_e, were determined. To obtain group contribution values of the bile salt molecule from the ion-pair extraction data, multiple linear regression analysis by the Free–Wilson technique was applied. The results showed that the fundamental premise of the functional group's contribution to the ion-pair extraction is valid. The functional groups of counterions contribute to the partitioning of the ammonium compound independently and additively in this system.

Keyphrases □ Bile salt molecules—functional group contribution to ion-pair partitioning of quaternary ammonium derivative of propranolol □ Quaternary ammonium salts—*N*,*N*-dimethyl derivative of propranolol, ion-pair partitioning, functional group contribution of bile salt molecules □ Ion-pair partitioning—*N*,*N*-dimethyl derivative of propranolol, functional group contribution of bile salt molecules

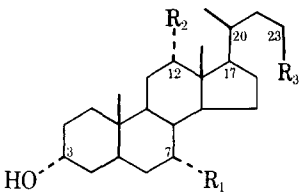
The effect of the structure of the pairing ion on quaternary ammonium compounds has received considerable attention (1). Partitioning of organic salts or complexes was reported to be influenced by the molecular weight of the organic ions, the branching effect of the aliphatic amine cations, and the organic solvent (2, 3).

A comprehensive group contribution study based on partition coefficient measurement was presented (4). From the consistency of the thermodynamic data, it was concluded that ion-pair extraction equilibria provide a feasible method for determining group contribution. The use, with some limitations, of ion-pair extraction data to obtain group contribution values was demonstrated (5).

The *N*,*N*-dimethyl derivative of propranolol chloride, *N*,*N*-dimethyl-1-isopropylamino-3-(1-naphthyloxy)-2-propanol chloride (I), has antiarrhythmic activity without significant β-blocking and local anesthetic effects (6). In general, quaternary ammonium compounds are absorbed poorly from the GI tract because of their cationic nature (7). This poor absorption might be circumvented if the cationic nature of the onium head could be masked by ion-pair formation. The present investigation studied the interaction between I and bile salt molecules by the group



I



II–XIII: R_1 = R_2 = H or OH, R_3 = COONa, $CONHCH_2COONa$, or $CONHCH_2CH_2SO_3Na$

contribution approach, because group contribution data of the bile salt molecules (II) might be used to design a system to enhance the bioavailability of I.

EXPERIMENTAL

Material—Twelve different bile salts[1] and I[2] were used as received. Grade 1 (99% pure) 1-octanol[3] was the organic phase in the partitioning studies.

Partition Studies—Octanol saturated with pH 7.4 phosphate buffer and pH 7.4 phosphate buffer saturated with octanol were used. The bile salts and I were dissolved in pH 7.4 phosphate buffer (0.1 *M*). The I concentration was kept constant at 10^{-4} *M*, with only the bile salt concentration being varied between 10^{-4} and 10^{-3} *M*. A 10-ml aliquot of each aqueous buffer solution containing I, the bile salt, and octanol was placed in a screw-capped test tube and vigorously agitated for 2 min[4].

The aqueous layer was withdrawn by syringe and centrifuged at 1000 rpm for 20 min. The I concentration in the aqueous phase before and after partitioning was measured spectrophotometrically at λ_{max} 292 nm, and the apparent distribution constant of I was then calculated.

RESULTS AND DISCUSSION

Extraction Constant—The extraction constant was derived using the assumptions that the bile salt existed as the ion-pair with the quaternary ammonium ion in the organic phase, the cation and anion were completely dissociated in the aqueous phase (8, 9), and the concentration of the free anion of the bile salt in the organic phase was negligible compared with that of the free anion in the aqueous phase. The extraction of quaternary cation into the organic phase may be represented by Schemes I and II:

$$[A^+]_{aq} + n[B^-]_{aq} \overset{K_e}{\rightleftharpoons} [AB_n]_{org}$$

Scheme I

$$[A^+]_{aq} \overset{K_0}{\rightleftharpoons} [A^+]_{org}$$

Scheme II

where $[A^+]_{aq}$ represents the quaternary cation in the aqueous phase, $[A^+]_{org}$ is the quaternary cation in the organic phase, $[B^-]_{aq}$ is the anion in the aqueous phase, $[AB_n]_{org}$ is the ion-pair in the organic phase, n is the binding number of the anion against 1 mole of the quaternary cation, K_0 is the extraction constant for partitioning of the quaternary cation into the organic phase, and K_e is the extraction constant for partitioning of the ion-pair into the organic phase.

The apparent distribution constant of the quaternary ammonium compound, D_Q, measured experimentally, is defined as:

$$D_Q = \frac{[A_t]_{org}}{[A^+]_{aq}} = \frac{[AB_n]_{org} + [A^+]_{org}}{[A^+]_{aq}} \qquad \text{(Eq. 1)}$$

where the total concentration of A in the organic phase, $[A_t]_{org}$, is the sum of $[AB_n]_{org}$ and $[A^+]_{org}$.

The mathematical expression for the extraction constant, K_e, is:

$$K_e = \frac{[AB_n]_{org}}{[A^+]_{aq}[B^-]^n_{aq}} \qquad \text{(Eq. 2)}$$

[1] A grade, Calbiochem, San Diego, CA 92112.
[2] Provided by Chemical Development Group, Searle Laboratories.
[3] Aldrich Chemical Co., Milwaukee, WI 53233.
[4] Two minutes of vigorous agitation with a Vortex G mixer (Scientific Products, McGaw Park, Ill.) yielded better extraction of I than overnight shaking with a wrist-action mechanical shaker (model S-500, Kraft Apparatus, Mineola, N.Y.).

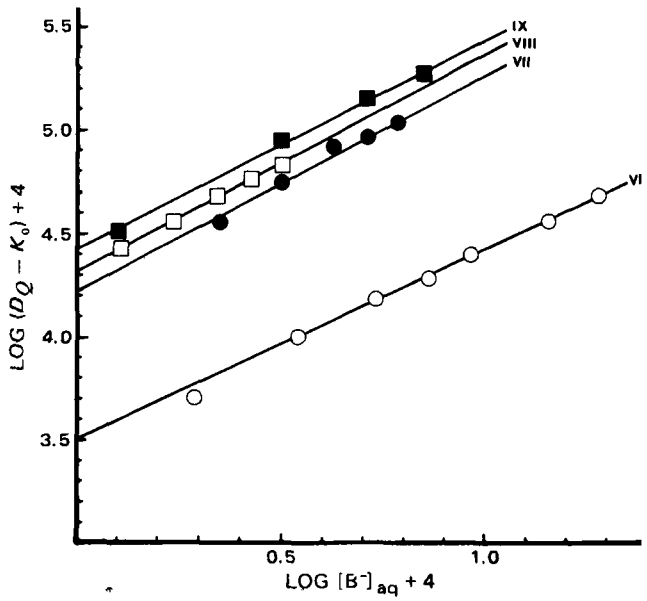


Figure 1—*Log-log plots of the distribtuion ratio of the ion-pair in the organic phase and of I in the aqueous phase, ($D_Q - K_0$), to the concentration of the glycocholic acid derivatives in the aqueous phase, $[B^-]_{aq}$. Key: VI, glycocholate; VII, glycochenodeoxycholate; VIII, glycodeoxycholate; and IX, glycolithocholate.*

Equation 2 may be expressed in the logarithmic form as:

$$\log K_e = \log \frac{[AB_n]_{org}}{[A^+]_{aq}} - n \log [B^-]_{aq} \qquad \text{(Eq. 3)}$$

Combining Eqs. 3 and 1 and rearranging give:

$$\log (D_Q - K_0) = \log K_e + n \log [B^-]_{aq} \qquad \text{(Eq. 4)}$$

The binding number, n, of the anion and the extraction constant, K_e, can be estimated from the slope of the line and antilog of the intercept value obtained by plotting $\log (D_Q - K_0)$ *versus* $\log [B^-]_{aq}$. The plots of some data are presented in Figs. 1 and 2, and n and K_e values are listed in Table I.

All systems followed a good linear relationship, as defined by Eq. 4,

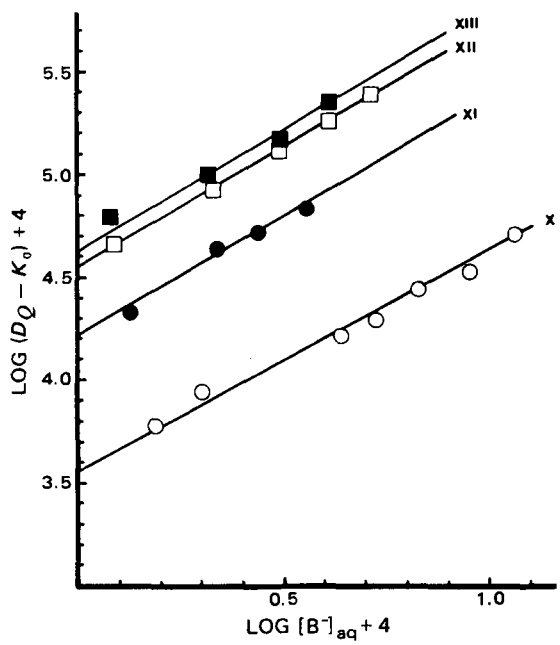


Figure 2—*Log-log plots of the distribution ratio of the ion-pair in the organic phase and of I in the aqueous phase, ($D_Q - K_0$), to the concentration of the taurocholic acid derivatives in the aqueous phase, $[B^-]_{aq}$. Key: X, taurocholate; XI, taurochenodeoxycholate; XII, taurodeoxycholate; and XIII, taurolithocholate.*

Table I—Structures of Bile Salts, Ion-Pair Extraction Constant, K_e, and Binding Number, n

Number	Bile Salt	R_1	R_2	R_3	K_e	n
II	Cholate	OH	OH	COO^-	1.375×10^3	0.97
III	Chenodeoxycholate	OH	H	COO^-	1.652×10^3	0.91
IV	Deoxycholate	H	OH	COO^-	1.862×10^3	0.98
V	Lithocholate	H	H	COO^-	1.742×10^3	0.94
VI	Glycocholate	OH	OH	$CONHCH_2COO^-$	2.716×10^3	0.98
VII	Glycochenodeoxycholate	OH	H	$CONHCH_2COO^-$	1.694×10^4	1.06
VIII	Glycodeoxycholate	H	OH	$CONHCH_2COO^-$	2.143×10^4	1.01
IX	Glycolithocholate	H	H	$CONHCH_2COO^-$	2.570×10^4	1.02
X	Taurocholate	OH	OH	$CONH(CH_2)_2SO_3^-$	3.972×10^3	0.98
XI	Taurochenodeoxycholate	OH	H	$CONH(CH_2)_2SO_3^-$	1.660×10^4	0.94
XII	Taurodeoxycholate	H	OH	$CONH(CH_2)_2SO_3^-$	3.672×10^4	1.13
XIII	Taurolithocholate	H	H	$CONH(CH_2)_2SO_3^-$	4.813×10^4	1.01

and these slopes, n, were close to unity. Therefore, the initial assumptions are valid. The interaction between I and bile salt is a 1:1 ion-pair formation, so any contribution from 1:2 or 1:3 binding in the aqueous or organic phase would be negligible. Since micellization of bile salts in an aqueous solution can occur (9), the bile salt concentration in this study was far below the critical micelle concentration to avoid micelle formation.

Functional Group Contribution—The mathematical model developed by Free and Wilson (10) to study structure–activity relationships is applicable to analogous series of compounds with corresponding activity data. The Free–Wilson model is based on the assumption that each substituent makes an additive and constant contribution to the activity and is expressed by:

$$\text{activity (linear or log values)} = \mu + \sum_{ij} G_{ij}X_{ij} \qquad \text{(Eq. 5)}$$

where μ is the overall average of activity of the parent skeleton and G_{ij} is the activity contribution of the substituent X_i in position j ($X_{ij} = 1$ if the substituent X_i is in position j; otherwise, $X_{ij} = 0$).

A procedure similar to the Free–Wilson method was used to determine the group contribution of all respective substituents of the bile salts by ion-pair formation with the quaternary ammonium compound. All activities were expressed as log K_e, and μ was assigned the value of the parent molecule. As a limiting condition, the group contribution for a hydrogen substituent at any position was assigned a value of zero.

Table II—Structural Matrix and Log Extraction Constant of the Ion-Pair for Observed and Calculated Values

Bile Salt[a]	R_1, OH	R_2, OH	R_3[b] A	B	C	Log K_e Observed	Calculated[c]
II	1	1	1	0	0	3.175	2.959
III	1	0	1	0	0	3.218	3.164
IV	0	1	1	0	0	3.270	3.289
V	0	0	1	0	0	3.241	3.493
VI	1	1	0	1	0	3.434	3.834
VII	1	0	0	1	0	4.229	4.039
VIII	0	1	0	1	0	4.331	4.163
IX	0	0	0	1	0	4.410	4.368
X	1	1	0	0	1	3.599	3.998
XI	1	0	0	0	1	4.220	4.202
XII	0	1	0	0	1	4.565	4.327
XIII	0	0	0	0	1	4.684	4.532

[a] Each bile salt number corresponds to those given in Table I. [b] A, B, and C at R_3 were COO^-, $CONHCH_2COO^-$, and $COHN(CH_2)_2SO_3^-$, respectively. [c] Calculated by multiple linear regression analysis.

Table III—Group Contribution of Substituents and Overall Average Activity of Parent Skeleton, μ, Calculated by Free–Wilson Technique

Substituent	Group Contribution
7α-OH	−0.329
12α-OH	−0.205
23-COO^-	0.117
23-$CONHCH_2COO^-$	0.992
23-$CONH(CH_2)SO_3^-$	1.156
μ	3.376

The hydrogens of the groups at the 7- (R_1) and 12- (R_2) positions were not included in the matrix. However, the hydrogens at the 23-position (R_3) were entered as log K_e = 0, 0, 0, and 0, and the sum of the group contribution for A, B, C, and H at R_3 was set at zero. In this way, the group contribution values of A, B, and C became positive contribution values because the formation of the ion-pair between the negatively charged R_3 and the positively charged quaternary ammonium head should enhance the partitioning of I into the organic phase.

It was necessary to calculate the contribution values for the five functional groups and the parent compound. The 17 simultaneous equations with seven unknowns were solved by multiple linear regression analysis. The 12 bile salts and their log K_e values are presented in Table II, and functional group contributions are shown in Table III. The results of statistical analysis showed that the multiple correlation coefficient of the regression was 0.994 and the standard deviation was 0.27. The F test proved significant at a 99% confidence level, and 98% of the variance was explained.

Comparison of the functional group contribution values (Table III) shows that the significant activity enhancement groups are conjugated glycine (B) and taurine (C) groups, which increase the activity by a factor of 10 over a single carboxylate ion (A). This result suggests that the terminal positive quaternary nitrogen of I is more effectively shielded by a conjugated glycine or conjugated taurine moiety to give a neutral species by electrostatic interaction than is a simple carboxylate ion.

The activity-lowering group seems to be the hydroxyl group at the 7α- and 12α-positions. When both 7α-OH and 12α-OH are present, the partitioning activity is substantially reduced, probably because of the steric hindrance effect of 7α-OH and 12α-OH on the hydrophobic interaction between the steroid skeleton of bile salts and the aromatic portion of I, in addition to the hydrogen bonding effect of the hydroxyl group.

These results show that the functional groups contribute to the total activity almost independently and additively in this system. This approach utilizes an extrathermodynamic property that has linear free energy relationships. Thus, the total free energy change of the process is the sum of the independent contributions from the functional groups in this system. These findings also suggest that ion-pair extraction varying with the functional groups of counterion molecules can be applicable to the group contribution approach to enhance the bioavailability of a quaternary ammonium compound.

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