Aromatic Hydroxylation of Lidocaine and Mepivacaine in Rats and Humans

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The syntheses of the 3′-hydroxy (14) and 4′-hydroxy (9) derivatives of lidocaine (1) and the 3′-hydroxy (16) derivative of mepivacaine are described, as well as that of [^{14}C]mepivacaine (2). Approximately 60% of the dose of 2 is excreted as conjugated 16 in the rat. In humans both 16 and the 4′-hydroxy (3) derivative of mepivacaine are excreted in about equal amounts. Between 25 and 35% of the dose is excreted as conjugated 16 and 3. Both 14 and 9 are formed in humans but they represent a minor metabolic pathway of lidocaine, together accounting for less than 1% of the dose. In the rat ring hydroxylation of lidocaine occurs to a significant extent, 14 representing about 15% of the dose and 9 representing up to 5% of the dose.

The metabolism of the anilide local anaesthetics, lidocaine (1) and mepivacaine (2), has been examined by various workers, but there are inconsistencies and ambiguities in many of the reports. In particular, the importance of aromatic hydroxylation with these compounds is uncertain.[1-5] 1-Methyl-4′-hydroxy-2′,6′-pipecoloxylidide (3) has been identified as a minor metabolite of mepivacaine in rats[6,7] and in man.[8] A major metabolite in the rat, representing up to 60% of the dose, has been reported but it has not been identified.

The present investigation was undertaken to identify the major metabolite of mepivacaine in the rat, to further investigate the metabolism of mepivacaine in man, and to clarify the position with regard to ring hydroxylation of lidocaine in animals and man.

Experimental Section†

Racemic [^{14}C]pipecolic acid (5) was prepared from lysine·HCl (100 mg unlabeled) and 0.125 mCi of NEC-280L-[U-^{14}C]lysine (New England Nuclear) by the method of Hamilton.[9] The product was isolated by paper chromatography using pyridine–H_2O–*n*-BuOH (1:1:1 ascending). After extraction with MeOH, the product was recrystallized from EtOH–Et_2O. This afforded 34.4 mg (32.5%). R_f values were identical on three paper chromatographic systems with authentic 5. 2′,6′-[^{14}C]Pipecoloxylidide (4) was prepared from 5 and 2,6-xylidine by literature methods,[10] yield 81%.

[^{14}C]**Mepivacaine (2).** 4 (35 mg) was made up to 100 mg with unlabeled compound and methylated.[10] The product was isolated by tlc (silica gel GF-254) using cyclohexane–$CHCl_3$–Et_2NH (5:4:1). After extraction with Et_2O, the product was made up to 100 mg with unlabeled 2 and recrystallized from cyclohexane, mp 151–152° R_f values were identical with authentic 2 on two tlc systems. Reverse isotope dilutions from *n*-heptane and cyclohexane gave purities of 100.3% and 100.6%, respectively.

4-Acetoxy-2,6-xylidine (6). 4-Hydroxy-2,6-dimethylazobenzene[11] was acetylated with Ac_2O in pyridine, hydrogenated at 1800 lb in.$^{-2}$ at 40° for 12 hr with W_2 Raney nickel, and recrystd from EtOH, mp 95–96°.

2-Chloro-4′-acetoxy-2′,6′-acetoxylidide (7). Chloroacetyl chloride was condensed with 6[12] and recrystd from toluene, mp 152–153°. Nmr was consistent with assigned structure.

2-Diethylamino-4′-acetoxy-2′,6′-acetoxylidide (8). Et_2NH was condensed with 7,[13] mp 179–184°.

2-Diethylamino-4′-hydroxy-2′,6′-acetoxylidide (9). Base-catalyzed hydrolysis of 8 produced 9, which was recrystd from benzene–cyclohexane, mp 183–184°. Nmr was consistent with assigned structure and 9 gave a single peak on glc. *Anal.* ($C_{14}H_{22}N_2O_2$) C, H, N. The 2-dimethylamino (10) analog (mp 159–161°) (*Anal.* ($C_{12}H_{18}N_2O_2$) C, H, N) and the 2-di-*n*-propylamino (11) analog (mp 137–138°) (*Anal.* ($C_{16}H_{26}N_2O_2$) C, H, N) of 9 were prepared by cognate methods.

2-Diethylamino-3′-nitro-2′,6′-acetoxylidide (12). Lidocaine·HCl was nitrated by standard methods, and the product was purified by distillation (190° (0.5×10^{-3} mm)), mp 51–55°. Nmr was consistent with assigned structure, and 12 gave a single glc peak on OV-17 3% on Gas Chrom Q at 240°.

2-Diethylamino-3′-amino-2′,6′-acetoxylidide (13). 12 was reduced with H_2 at room temperature and atmospheric pressure over Pd/C. 13 was recrystd from toluene–EtOAc, mp 150°. Single peak on glc and nmr was consistent with assigned structure.

2-Diethylamino-3′-hydroxy-2′,6′-acetoxylidide (14). 13 was diazotized and added dropwise to boiling $CuSO_4$ (10%) solution. This was adjusted to pH 9.5 and extracted with EtOAc, and the extract was washed with 1 *N* NaOH. The NaOH solution was adjusted to pH 9.5 and extracted with EtOAc. 14 was recrystd from EtOH–benzene, mp 203–204°. Single peak on glc and nmr was consistent with assigned structure. *Anal.* ($C_{14}H_{22}N_2O_2$) C, H, N.

1-Methyl-3′-hydroxy-2′,6′-pipecoloxylidide (16) was prepared by an analogous series of reactions to those used for 14 but using mepivacaine as starting material, sublimed 150° (0.5 mm), mp 194–197°. *Anal.* ($C_{15}H_{22}N_2O_2$) C, H, N.

Determination of ^{14}C. Two scintillator solutions were used. Solution A consisted of 2,5-diphenyloxazole (PPO) (0.15%), 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene (dimethyl POPOP) (0.005%), and EtOH (10%) in thiophene-free redistilled toluene. Solution B consisted of naphthalene (6%), PPO (0.4%), dimethyl POPOP (0.02%), MeOH (10%), and ethylene glycol (2%) in purified dioxane. Solution A (10 ml) with MeOH (1.0 ml) and MeOH hyamine (10 *M*, 0.05 ml) was used for urine. Feces were assayed by drying the material at room temperature under reduced pressure over P_2O_5 for 7 days. The dried substance was ground with an equal weight of sand, and aliquots (10–25 mg) were heated with NaOH solution (1 *N*, 1 ml) for 2 hr. Solution B (10 ml) was added, and the mixture kept in darkness for 48 hr before counting to reduce chemiluminescence. CO_2 was determined by absorbing it in NaOH solution (20%). Suspending agent (400 mg)‡ was added to aliquots of NaOH solution (1 ml), followed by solution B (10 ml), and the mixt was shaken vigorously for a few seconds. The observed radioactivity of all samples was corrected for counting efficiency by the external standard method using a previously prepared calibration graph.

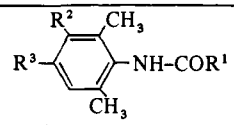
Metabolism of Mepivacaine in Rats. Initial Experiment. Three male Wistar rats (300 g) were administered [^{14}C]mepivacaine (9.20×10^5 dpm; specific activity 2.55×10^5 dpm/mg) ip in 0.6 ml of H_2O adjusted to pH 5. The rats were housed in all glass cages arranged for the separate collection of urine and feces. Samples of each were collected every 24 hr for 72 hr. CO_2 was collected from one rat using a modified glass cage.[14]

Aliquots of urine were mixed with 2 volumes of 1 *M* AcOH buffer (pH 3.8) and incubated with β-glucuronidase (1 mg) (Sigma Chemical Co. Type L1) for 24 hr at 37°. After 8 hr, a second portion of enzyme (1 mg) was added. In some runs the arylsulfatase, which is present in the enzyme preparation, was inhibited by adding phosphate (0.1 *M*) to the incubation mixture.[15] The urine, after incubation, was extracted as indicated in Table I, and the radioactivity present in the various phases was determined. The Et_2O extracts of the glucuronidase-treated urine were subjected to tlc.[6] Strips (1 cm) were suspended in MeOH (1 ml) and MeOH hyamine (0.05 ml) mixed with solution A (10 ml) and counted.

†All melting points were determined on a Kofler block and are uncorrected. Analyses, where indicated only by symbols of the elements, were within ±0.4% of theoretical values. A Packard Tricarb (Model 3314) liquid scintillation spectrometer was used to determine radioactivity. Mass spectra were obtained using an AEI MS902 double-focusing mass spectrometer operating at an ionizing potential of 70 eV at 150°. Nmr spectra were obtained on a Varian Associates Model HA-100.

‡Carb-O-Sil. Packard Instrument Co.

Table I



Compd	R^1	R^2	R^3
1	CH_2NEt_2	H	H
2	*N*-Methyl-2-piperidyl	H	H
3	*N*-Methyl-2-piperidyl	H	OH
4	2-Piperidyl	H	H
7	CH_2Cl	H	CH_3COO
8	CH_2NEt_2	H	CH_3COO
9	CH_2NEt_2	H	OH
10	CH_2NMe_2	H	OH
11	CH_2N-*n*-Pr_2	H	OH
12	CH_2NEt_2	NO_2	H
13	CH_2NEt_2	NH_2	H
14	CH_2NEt_2	OH	H
15	CH_2NHEt	H	H
16	*N*-Methyl-2-piperidyl	OH	H

Isolation of the Major [^{14}C]Metabolite of Mepivacaine. [^{14}C]-Mepivacaine·HCl (100 mg, 2.333×10^5 dpm) was administered ip to a rat (500 g) in four doses at 2-hr intervals. The 24-hr urine was treated with glucuronidase and extracted with Et_2O, which was concentrated to 2 ml. The metabolites were separated using a thick-layer plate (silica gel GF-254, 1 mm).[6] A large component, R_f 0.53, was seen under uv. This was extracted with Et_2O, which was evaporated when 9.34 mg of a white compound was obtained. This was shown to be homogeneous by tlc and glc using OV-17 3% on Gas Chrom Q.

Metabolism of Lidocaine in Rats. Three male Wistar rats (300 g) received 8.1 mg of lidocaine·HCl in solution (0.2 ml of H_2O) ip. Urines (24-hr) were assayed by glc for 1, 9, 14, and **15** both before and after glucuronidase or acid hydrolysis. Acid hydrolysis was carried out by incubating an aliquot of urine with HCl (to 2 *N*) at 115° for 45 min.

Metabolism in Humans. Three males received mepivacaine·HCl (50 mg) orally, and 24-hr urines, treated as above, were assayed by glc for **2**, **3**, **4**, and **16**. The same three males received lidocaine·HCl (100 mg) orally, and 24-hr urines, treated as above, were assayed by glc for 1, 9, 14, and 15.

Glc of 9 and 14. Urine samples (either untreated or after glucuronidase or acid treatment), NH_4Cl (2 g), and internal standard (2.0 μg, **10**) were mixed and extracted with CH_2Cl_2 (5 ml) in a 15-ml, glass-stoppered centrifuge tube on a Vortex mixer for 2 min and then centrifuged. The aqueous layer was separated and adjusted to pH 9.0 ± 0.2 with 10 *N* NaOH solution and extracted with CH_2Cl_2 (6 ml). The CH_2Cl_2 solution was evaporated to approximately 5–10 μl at 42° in a tapered tube, which was then immersed in ice to wash the sides with condensed solvent. The CH_2Cl_2 solution was treated for 90 min at 60° with 10 μl of a solution of *N*-acetylimidazole (1%) and Et_3N (2.0%) in ethylene dichloride in a microreaction tube,§ and 3 μl of solution was used for glc. Glc conditions were: Hewlett Packard 5750; flame detector; glass column 6 ft × 0.125 in. i.d.; OV-17 3% on Gas Chrom Q; oven temperature 222°; inlet and detector temperature, 250°; carrier gas N_2 at 40 ml/min. Retention times for **10**, **14**, and **9** were 7.4, 11.6, and 12.8 min, respectively, as acetate derivatives. Quantities of metabolite greater than 5 μg could be chromatographed without forming the acetate derivative. The respective retention times as free phenols were 7.6, 11.0, and 12.4 min.

Glc of 2 and 4. Urine (1 ml), internal standard (1.0 μg of bupivacaine), and NaOH (0.5 ml, 5 *N*) were mixed in a 15-ml, glass-stoppered centrifuge tube and extracted with recently distilled anaesthetic Et_2O (2 × 5 ml) as described above. The Et_2O phase was extracted with HCl (1 ml, 0.5 *N*), the acid phase basified with NaOH (0.5 ml, 5 *N*) and extracted with Et_2O (2 × 5 ml), and the Et_2O evaporated to 5–10 μl in a tube, with an elongated bubble of 20-μl capacity on the base, at 40°. The tube was immersed in ice, and 3 μl of solution was used for glc. Glc conditions were as above except for the following: OV-225 3% on Gas Chrom Q; oven temperature 218°. Retention times under these conditions were, for **2**, **4**, and bupivacaine, 6.4, 7.8, and 10.6 min, respectively.

Glc of 3 and 16. Extraction from urine and glc conditions were as described for 9 and 14 except that acetate derivatives were not prepared, the oven temperature was 240°, and 11 (10 μg) was used as internal standard. Retention times for **11**, **16**, and **3** were 7.4, 9.7, and 10.8 min, respectively.

Glc of 1 and 15. Extraction from urine and glc conditions were as described for **2** and **4**, except that dimethisoquin (**2** μg) was used as internal standard and column temperature was 195°. Retention times for 15, 1, and internal standard were 7.2, 7.9, and 11.9 min, respectively.

§ Reactivial (Pierce Chemical Co.).

Results

Isolation and Identification of the Major Metabolite of Mepivacaine in the Rat. Approximately 60% of the dose was excreted in the urine in 0–24 hr, followed by 1.5–8.1% in the next 72 hr. Feces (1–72 hr) contained 5.3–15.6%, carbon dioxide < 0.1%. Total recovery was ~80%. The urinary metabolites (0–24 hr) consisted, on the basis of ether extraction, primarily (~83%) of water-soluble compounds. Approximately 12% of the total was extracted into ether at pH 9, while ~2% was extracted at pH 1. After enzymatic hydrolysis with either glucuronidase or a glucuronidase-sulfatase preparation, approximately 54–58% of dose extracted into ether at pH 9.5. Similar results were obtained after acid hydrolysis. Tlc showed at least three components which had R_f values of 0.16 (2.2%), 0.36 (4.6%), and 0.63 (85%). It was shown using authentic compounds that the bases were neither **2** nor **4**. The results obtained are in reasonable agreement with those previously reported.[5] Both sets of results show that there is a major metabolite and that it is a conjugated hydroxymepivacaine. Relatively large quantities of low activity [^{14}C]mepivacaine were administered to rats, and the major metabolite was isolated.

Mass Spectrometry. The parent ion at *m/e* 262 showed an increase of 16 amu over **2**, providing further conformation that the metabolite was an oxidation product. The presence of peaks at *m/e* 98 (base), 96, and 70, which were also present in the spectrum of **2**, indicates that the oxygen atom was not contained in the piperidine ring. This was confirmed by high-resolution mass spectrometry on the ion *m/e* 98 which was shown to have the composition $C_6H_{12}N$.# This conclusion was supported by the fact that the ion *m/e* 136 had the composition $C_8H_{10}NO$# indicating that the oxygen was present in the aromatic portion of the molecule. Ions at *m/e* 108 and *m/e* 107 correspond to the loss of CO and HCO, respectively, from *m/e* 136 which is characteristic of phenols. The absence of peaks at *m/e* 244 ($M^+ - 18$) or 118 (136 − 18) indicates that the oxygen function is not an alcohol and hence does not arise from oxidation of an aromatic methyl group. The mass spectrum indicates that the metabolite is **2**, hydroxylated on the aromatic moiety.

Nmr. Nmr of the metabolite showed two features which were not observed with **2**. Two singlets at 2.50 (3 H) and 2.56 ppm (3 H) and two doublets at 7.30 and 7.52 ppm (2 H) coupled with 8 Hz. Assignments of these peaks are given in Figure 1, and they are consistent with the aromatic moiety of the metabolite being 3-hydroxy-2,6-xylidine. The major metabolite of mepivacaine is **16** in the rat. This was confirmed by a mixture of metabolite and authentic synthetic **16** producing a single peak on glc.

Excretion of Ring-Hydroxylated Metabolites. The percentages of the administered doses of **1** and **2** excreted as 3′-hydroxy or 4′-hydroxy metabolites are given in Table II along with those of **1**, **2**, **4**, and **15**.

#$C_6H_{12}N$. Calcd, 98.0970; found, 98.0966 ± 0.001. $C_8H_{10}ON$. Calcd, 136.0763; found, 136.0762 ± 0.001.

Table II. Metabolites of Lidocaine and Mepivacaine, Administered to Rats (ip) and Humans (oral), Recovered in 24-Hr Urine

		Percentage of dose administered in 24-hr urine					
				β-Glucuronidase hydrolysis		Acid hydrolysis	
Species	Compound and dose[a]	Unchanged compound	Monodealkylated metabolite	3'-Hydroxy metabolite	4'-Hydroxy metabolite	3'-Hydroxy metabolite	4'-Hydroxy metabolite
Man	Lidocaine						
L. M.	100	1.30	3.1	0	0	Trace (0.01-0.2) of both 3' and 4' isomers	
G. M.	100	0.5	2.1	0	0		
P. M.	100	0.8	3.1	0	0		
Rat							
1	27	0.8	0.7	0.6	0	13.0	3.7
2	27	0.4	0.8	0.9	0	16.3	5.0
3	27	0.5	0.3	0.5	0	5.9	3.0
Man	Mepivacaine						
L. M.	50	1.2	0.7	15.2	11.5	19.0	6.5
G. M.	50	1.1	1.8	20.5	14.5	19.8	7.0
P. M.	50	0.4	0.5	15.4	10.4	21.4	7.2
Rat							
1	13.5	Trace	Trace	55.8	0		
2	13.5			59.0	0		
3	13.5			63.0	0		

[a] Dose in man given in mg, in rat in mg/kg.

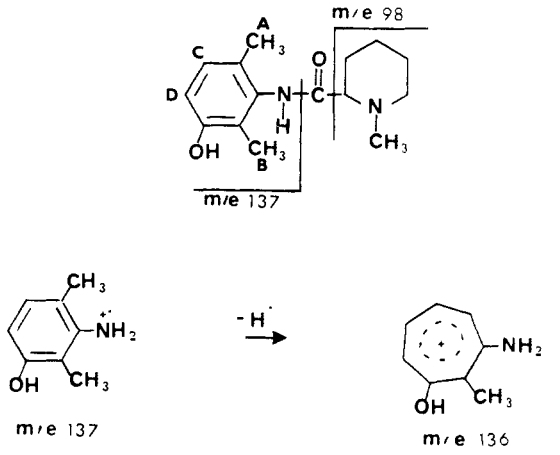


Figure 1. Nmr assignments and abbreviated scheme of mass fragmentation pattern of mepivacaine metabolite 1-methyl-3'-hydroxy-2',6'-pipecoloxylidide (16). A = 2.50 ppm, B = 2.56 ppm, C = 7.3 ppm, D = 7.52 ppm, J_{CD} = 8 Hz.

Discussion

Metabolism of Mepivacaine. The results obtained in the rat show similarities and differences from the work of Hansson, *et al.*[6] The major point of agreement is that there is a metabolite which represents about 50% of the dose. Mass spectrometry, nmr, and glc with authentic compound show that the compound is **16**. Other points which emerge, are that Hansson, *et al.*,[6] reported that 17% of the dose was excreted by the rat unchanged whereas we only find traces of unchanged drug. It was also suggested[6] on indirect evidence that **4** could be a metabolite. We have detected it in urine of both humans and rats. It was also reported[6] that 5% of the dose was excreted as unconjugated **3** in the rat but we were unable to detect it even though the method used could separate authentic **3** or **16** and was capable of detecting <0.001% of the dose.

The metabolism of mepivacaine in man is different from that in the rat in that about equal quantities of **16** and **3** were found in the 24-hr urine and the total amount of ring-hydroxylated mepivacaine represented about 25% of the dose as opposed to about 60% in the rat. Maes, *et al.*,[8] reported only **3** in human urine. However, we have found that using their glc conditions it is not possible to adequately separate **16** and **3**.

β-Glucuronidase and acid hydrolysis of conjugates of **16** and **3** produced by man gave essentially the same results, which suggest that they are excreted as glucuronides.

Metabolism of Lidocaine. Ring hydroxylation in both 3' and 4' positions represents minor metabolic pathways in humans. Phenols were found after acid hydrolysis but not after glucuronidase, which suggests the possibility of etherea sulfate conjugation. It has been reported[1,2] that administration of lidocaine led to an increase in urinary sulfate in man. In the rat β-glucuronidase hydrolysis produced **14** only, but acid hydrolysis produced significant amounts of **14** and also **9**. This indicates that conjugates other than glucuronide were present, possibly ethereal sulfate. Even though aromatic hydroxylation of lidocaine, as such, is a minor metabolic pathway it has recently been shown that 4-hydroxy-2,6-xylidine accounts for more than half the dose in humans.[16]

The difference in the pattern of results obtained with lidocaine and mepivacaine reflects the relative rates at which the aliphatic amino moieties can be metabolized in the two compounds. The diethylamino group is metabolically more labile than the *N*-methylpiperidino group.

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Hydrophobic Bonding of Sulfonamide Drugs with Serum Albumin†

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The adsorption constants of various sulfonamide drugs with bovine serum albumin (BSA) at various pH points are analyzed with free energy related physicochemical parameters taking into account the correction for ionization of the drugs. The pH dependence of the adsorption constant is discussed in terms of the pH-dependent hydrogen ion dissociation equilibrium of BSA. It is postulated that the adsorption equilibrium is determined by the hydrophobicity of drugs and occurs through the binding of the neutral drug molecule with the hydrophobic fraction of the protein surface, the variation of which is dependent on the state of the dissociation equilibrium of basic groups on the BSA molecule.

The binding of sulfonamide drugs to serum albumin has been considered to be important for their chemotherapeutic values, such as *in vivo* antibacterial activity and the duration of action.[1] Only unbound free drug is active against bacteria,[2] susceptible to metabolism,[3] and excreted from the kidney.[4] There have been a number of efforts to elucidate the nature of the drug-protein binding force from physicochemical as well as pharmacological points of view.[2,5-8] While the view that most of the energy of binding of sulfonamide drugs is due to hydrophobic forces is receiving increasing support[9,10] some experimental evidence still seems to remain suggesting an important role of electrostatic force between the sulfonamide anion and the positive charge on the protein surface.[5,8]

We have been analyzing the bacteriostatic activity,[11] serum protein binding,[11] and the rate of elimination[12] of N^1-substituted sulfonamides in terms of their physicochemical properties with the use of free energy related substituent parameters such as $\Delta \log K_A$ for the electron-withdrawing factor and π for the hydrophobic character of the N^1 substituent. K_A is the dissociation constant for the N^1 hydrogen and π is defined as $\Delta \log P$.[13] P is the partition coefficient of drugs determined with an *i*-BuOH-H_2O system. For $\Delta \log K_A$ and $\Delta \log P$, the $\log K_A$ and $\log P$ values of unsubstituted sulfanilamide are taken as the point of reference. Taking into account the effect of dissociation at the physiological pH, our analyses show that the hydrophobic character of the N^1 substituent plays extremely important roles in the behavior of these drugs, especially in the serum protein binding[11] and the rate of elimination.[12] The purpose of the work in this paper is to obtain further insight into the role of hydrophobic force in the serum albumin binding and to further support our previous analyses, in particular, of the rate of elimination.

Experimental Section

The protein binding of sulfonamide drugs has been known to obey the Langmuir's adsorption isotherm as shown in eq 1,[5,7,8]

$$KC_F = \frac{r}{n - r} \tag{1}$$

where K is the binding constant, C_F is the equilibrium concentration of the unbound free drug, n is the maximum number of binding sites, and r is the number of sites occupied by the bound drug per single molecule of the protein.

In fact, the drugs exist as an equilibrium mixture of neutral and ionized forms and the degree of ionization varies according to dissociation constants in a series of sulfonamides at a certain pH. If the drugs are bound only as either the neutral or ionized form, eq 1 is modified to either eq 2 or 3 so that experimentally determined

$$K_n C_F(1 - \alpha) = \frac{r}{n - r} \tag{2}$$

$$K_i C_F \alpha = \frac{r}{n - r} \tag{3}$$

$$K_n = \frac{K}{1 - \alpha} \tag{4}$$

$$K_i = \frac{K}{\alpha} \tag{5}$$

apparent constant can be corrected as shown in eq 4 and 5, where K_n and K_i are constants for the neutral and ionized forms and α is the degree of ionization of a drug. We assume that the logarithmic values of binding constant, K_n and K_i, can be described by free energy related parameters as shown in eq 6 and 7, where

$$\log K_n = \log K + \log\left(\frac{K_A + [H^+]}{[H^+]}\right) = a\pi + b\Delta \log K_A + c \tag{6}$$

$$\log K_i = \log K + \log\left(\frac{K_A + [H^+]}{K_A}\right) = a'\pi + b'\Delta \log K_A + c' \tag{7}$$

$[H^+]$ is the hydrogen ion concentration of the medium and a, a', b, b', c, and c' are constants.

If both the neutral and ionized forms of drugs are involved in binding simultaneously with their respective equilibrium constants, K_n' and K_i', the adsorption isotherms for the two forms are described as shown in eq 8 and 9 where r_n and r_i are the number of sites binding neutral and ionized forms. Taking the ratio of eq 8 to eq 9, eq 10

$$K_n' C_F(1 - \alpha) = \frac{r_n}{n - r_i - r_n} \tag{8}$$

$$K_i' C_F \alpha = \frac{r_i}{n - r_i - r_n} \tag{9}$$

$$\frac{K_n'}{K_i'}\frac{(1 - \alpha)}{(\alpha)} = \frac{r_n}{r_i} \tag{10}$$

is derived. If the bound drug molecule is also in a dissociation equilibrium on the protein surface, the ratio of r_n to r_i is $(1 - \alpha)/\alpha$

†Studies on Structure-Activity Relationships. 5.