CH_3OH). Anal. ($C_{23}H_{31}N_2O_7SI$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6β-(2-iodoacetamido)morphinan Methanesulfonate (5b·CH_3SO_3H). A solution of **8b** free base (0.38 g, 1.1 mmol) in 2-propanol (20 mL) and THF (40 mL) and a solution of *N*-(2-iodoacetoxy)-succinimide (0.34 g, 1.2 mmol) in THF (20 mL) were each cooled to −20 °C and then mixed. The resulting solution was stored at −10 °C for 16 h and then at room temperature for 40 h. A solution of CH_3SO_3H in MeOH was added to pH 1.2, toluene (5 mL) was added, and all solvent was removed at reduced pressure. The residue was stirred for 40 h with acetone, and the solid obtained upon filtration was recrystallized from EtOH–toluene: yield of two crops of **5b**·CH_3SO_3H was 0.49 g (73%); decomposes without melting at >200 °C; EIMS (70 eV), *m/e* 510 (M^+); R_f 0.65 (EMA, 80:20:2); $[\alpha]^{25}_D$ −101° (*c* 1.0, CH_3OH). Anal. ($C_{23}H_{31}N_2O_7SI$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6α-isothiocyanatomorphinan Hydrochloride (6a·HCl). A mixture of **8a**·2HOAc (0.37 g, 0.8 mmol) and $NaHCO_3$ (0.42 g, 5 mmol) was dissolved in 15 mL H_2O–THF (2:1), and a solution of thiophosgene (0.15 g of an 85% solution in CCl_4, 1.1 mmol) in THF (10 mL) was added dropwise with stirring at 0 °C. The resulting two-phase solution was stirred to 25 °C overnight, and Et_2O was added. The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$. The combined organic extracts were evaporated, and the residue was taken up in MeOH–CH_3CN and acidified to pH 1.8 with HCl. Concentration of the solution yielded 0.29 g (85%) of **6a**·HCl, which was crystallized from MeOH–*i*-PrOH: mp 230 °C dec; $[\alpha]^{25}_D$ −265° (*c* 1.0, CH_3OH); R_f 0.72 (EMA, 95:5:2); EIMS (CH_3SO_3H salt, 70 eV), *m/e* 384 (M^+); IR 2080 (−N=C=S stretch) cm^{-1}. Anal. ($C_{21}H_{25}N_2O_3SCl·{}^2/_3H_2O$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6β-isothiocyanatomorphinan Hydrochloride (6b·HCl). A mixture of **8b**·2HCl (1.25 g, 3.0 mmol) and $NaHCO_3$ (1.68 g, 20 mmol) was dissolved in H_2O (20 mL) and THF (8 mL). A solution of thiophosgene (0.49 g of an 85% solution in CCl_4, 3.6 mmol) in THF (20 mL) was added dropwise with rapid stirring at 0 °C. The resulting two-layer solution was stirred to 25 °C overnight, Et_2O was added, and the organic layer was separated. The aqueous layer was then extracted twice with $CHCl_3$. The combined organic extracts were evaporated, and the residue was chromatographed rapidly on silica gel ($CHCl_3$–acetone–Et_3N, 90:10:1). All fast-moving material was converted into its HCl salt, yielding 0.93 g (74%) of **6b**·HCl. Recrystallization from CH_3CN afforded analytically pure material: decomposes without melting at 300 °C; $[\alpha]^{25}_D$ −289° (*c* 1.2, MeOH); R_f 0.75 (EMA, 80:20:2), 0.32 ($CHCl_3$–Et_3N, 100:1); EIMS (CH_3SO_3H salt, 70 eV) 384 (M^+); IR 2065 (−N=C=S stretch) cm^{-1}. Anal. ($C_{21}H_{25}N_2O_3SCl$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6α-propanamidomorphinan (7a). A solution of **8a**·2HOAc (0.46 g, 1 mmol) in 50% aqueous THF (30 mL) was brought to pH 10 with 2 N NaOH. A solution of CH_3CH_2COCl (0.32 g, 3.5 mmol) in THF (10 mL) was added dropwise with stirring at 10 °C, keeping the pH between 10 and 11 by frequent addition of 2 N NaOH. After the addition was complete, the solution was maintained at pH 10.5 for 40 h, diluted with water, concentrated, and extracted with $CHCl_3$. The residue obtained upon evaporation of the combined organic extracts was taken up in MeOH, and HCl was added to pH 1. Ethanol and toluene were added, and all solvent was evaporated at reduced pressure. The residue was triturated with acetone to yield 0.33 g (76%) of the HCl salt. The HCl salt was converted to the free base and crystallized from aqueous MeOH: mp 220–221 °C; EIMS (70 eV), *m/e* 398 (M^+); R_f 0.56 (EMA, 90:10:2); $[\alpha]^{25}_D$ −225° (*c* 0.5 CH_3CN–$CHCl_3$, 1:1). Anal. ($C_{23}H_{30}N_2O_4$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6β-propanamidomorphinan (7b). The propionylation was conducted with **8b**·2HOAc by using the above conditions for **7a**. The salt **7b**·HCl (0.312 g, 72%) was converted to the free base and crystallized from 75% aqueous EtOH: mp 233–234 °C; EIMS (70 eV), *m/e* 398 (M^+); R_f 0.30 (EMA, 95:5:2); $[\alpha]^{25}_D$ −168° (*c* 0.5, CH_3CN–$CHCl_3$, 1:1). Anal. ($C_{23}H_{30}N_2O_4$) C, H, N.

6β-(*cis*-3-Carboxyacrylamido)-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan (9). (a) A solution of **8b** base (1.4 g, 4.1 mmol) in 20 mL of CH_2Cl_2–THF (3:2) was mixed with a solution of maleic anydride (0.417 g, 4.25 mmol) in THF (12 mL) at 0 °C and then stirred to room temperature overnight. The resulting slurry was filtered, and the product was washed with Et_2O, yielding 1.82 g (100%) of **9**.

(b) To a solution of **8a**·2HCl·0.75H_2O (2.0 g, 4.66 mmol) and Et_3N (0.980 g, 9.7 mmol) in CH_2Cl_2 (30 mL), stirred over 3Å sieves for 1 h, was added a solution of maleic anhydride (0.476 g, 4.85 mmol) in THF (30 mL) at 0 °C. The resulting mixture was allowed to stir at room temperature overnight, coarse filtered to remove the sieves, and then fine filtered. The solid (3.2 g) was taken up in 80 mL of MeOH–EtOH (8:2), heated to 60 °C with stirring, cooled, and filtered to yield **9** (19.8 g, 96%): mp >270 °C; EIMS (70 eV), *m/e* 440 (M^+). Anal. ($C_{24}H_{28}N_2O_6·H_2O$) C, H, N.

Acknowledgment. This research was supported by the National Institute on Drug Abuse (DA 01533). We thank Masako Ikeda, Victoria Darrow and Mary Schwartz for the capable technical assistance in performing the biological testing.

Ion-Sensitive Electrode Potentiometry of Organic Anions: Application to Quantitative Structure–Activity Relationships

Roland F. Hirsch* and Elliot M. Rachlin

Department of Chemistry, Seton Hall University, South Orange, New Jersey 07079. *Received August 9, 1982*

Ion-sensitive electrode potentiometry is proposed for determination of substituent constants for structural modifications of organic acids. A liquid membrane anion-sensitive electrode responds reproducibly to a wide range of carboxylate and sulfonate ions. Fragment constants for the addition of a methylene group to aromatic and aliphatic acids are −2.4 and −3.3 ± 0.15 kJ/mol, respectively. Agreement is observed between these constants and those determined by other techniques, including partitioning studies in biphasic systems, suggesting the use of potentiometry for quantitative structure–activity relationship studies. Furthermore, the electrode measurements correlate with biological effects resulting from hydrophobic interactions.

Quantitative structure–activity relationships (QSAR) have become increasingly useful in interpreting data in the fields of physical organic chemistry, biological chemistry, and new drug development. Several examples can be found for the use of these QSAR's in the study of the activity of currently available drugs.[1,2] In some cases modification of a drug may be desired to increase or decrease its potency. Where lipophilicity is the determining factor, the effect may be adjusted through the addition of one or more groups.[3] Substituent constants such as those

(1) Quinn, F. R.; Beisler, J. A. *J. Med. Chem.* **1981**, *24*, 251–256.

(2) Hansch, C.; Leo, A.; Schmidt, C.; Jow, P. Y. C. *J. Med. Chem.* **1980**, *23*, 1095–1101.

(3) James, R.; Glen, J. B. *J. Med. Chem.* **1981**, *23*, 1350–1357.

0022-2623/83/1826-1235$01.50/0

Table I. Fragment Constants for the Addition of a Methylene Group to Carboxylic and Sulfonic Acids

compd 1	compd 2	$-\Delta(\Delta G)$, kJ/mol
A. Linear Aliphatic Acids		
acetate	formate	1.3
propanoate	acetate	2.8
butanoate	propanoate	2.2
pentanoate	butanoate	3.3
hexanoate	pentanoate	3.3
heptanoate	hexanoate	3.3
octanoate	heptanoate	3.2
B. Alicyclic Carboxylic Acids		
cyclohexaneacetate	cyclohexaneformate	2.7
cyclohexanepropanoate	cyclohexaneacetate	4.0
cyclohexanebutanoate	cyclohexanepropanoate	3.0
cyclopentaneacetate	cyclopentaneformate	2.4
cyclopentanepropanoate	cyclopentaneacetate	3.9
cyclohexaneformate	cyclopentaneformate	2.4
cyclohexaneacetate	cyclopentaneacetate	2.7
cyclohexanepropanoate	cyclopentanepropanoate	2.9
C. Benzoic Acid Series		
p-toluate	benzoate	2.4
p-ethylbenzoate	*p*-toluate	2.4
p-*n*-propylbenzoate	*p*-ethylbenzoate	2.7
p-butylbenzoate	*p*-*n*-propylbenzoate	2.7
D. Phenylacetate Series		
phenylacetate	benzoate	−3.3
3-phenylpropanoate	phenylacetate	2.4
4-phenylbutanoate	3-phenylpropanoate	2.4
5-phenylpentanoate	4-phenylbutanoate	2.1
E. Linear Sulfonic Acids		
pentanesulfonate	butanesulfonate	3.0
hexanesulfonate	pentanesulfonate	3.1
heptanesulfonate	hexanesulfonate	3.2
octanesulfonate	heptanesulfonate	2.8

suggested by Hansch aid in the choice of these substituents, since the effect is often some function of their magnitude.[4] Furthermore, combined hydrophobic, electronic, and steric effects have been shown to correlate well with biological activity.[5,6]

There is now a need for new techniques of characterization of substituent effects, especially for ionic molecules that can only be studied by the conventional partition technique if a high-molecular-weight ionic extractant is included in the organic extracting solvent. We report on the successful application of liquid membrane potentiometric electrodes for this purpose.

Olderman has demonstrated the existence of a qualitative relationship between the molecular structure of substituted benzoate and phenylacetate ions and the response of a liquid membrane ion-selective electrode to them.[7] The order of response depended on the size of the substituent, charge delocalization, and steric interactions.

The present study defines quantitatively the structure–electrode response relationship. The use of commercially available potentiometric probes for the study of ion–solvent interactions is demonstrated. The results are compared with those obtained from octanol–water partition data. Relationships of the electrode responses with biological activity are then discussed.

Results and Discussion

Aliphatic Compounds. A series of straight-chain carboxylic acid anions starting with formate and ending with octanoate was studied (Table I, part A). The average value of the free energy increment for the addition of a methylene group to butanoate and the larger alkanoic acids was −3.3 kJ/mol. The straight-chain sulfonic acids gave an average value of −3.0 kJ/mol per added methylene group. These are in reasonable agreement with the value reported by Franks[8] of −3.45 kJ/mol for the complete removal of water and substitution by hydrocarbon solvent and that reported by Seeman[9] of −2.91 kJ/mol for the absorption of alkanols into erythrocyte membranes. The equilibrium distribution of fatty acids between *n*-heptane and aqueous buffer solutions was studied by Smith and Tanford.[10] A linear relationship between free energy and chain length was observed for the series of compounds up to 22 carbons long. The average free energy change per methylene group was −3.43 kJ/mol.

Amphiphilic compounds such as those described here have two sites, one hydrophilic and one hydrophobic. The polar head tends to orient the water molecules around it. However, since this effect is short range (ca. four carbons), the hydrophobic effect of the addition of a methylene group to compounds four carbons and longer will be completely independent of influence by the hydrophilic part.[11] For compounds less than four carbons, the polar head predominates. The $\Delta(\Delta G)$ per methylene group is then less than it is for the larger compounds. The values of $\Delta(\Delta G)$ for the addition of a methylene group to formate, acetate, and propanoate are −1.3, −2.8, and −2.2 kJ/mol, respectively. The variation in these values is probably due to the substantial changes in charge and environment of the carboxylate group and, hence, its interaction with the surrounding solvent as the first few methylene groups are added.

Alicyclic carboxylic acids gave an average $\Delta(\Delta G)$ of −2.7 kJ/mol (Table I, part B), if the exceptionally high values for the addition of a methylene group to the acetates are excluded. With further additions of methylene groups, the added free energy returns to normal. Comparison of compounds with the ring equidistant from the carboxylate group but differing in ring size by one methylene group gives an average $\Delta(\Delta G)$ of −2.6 kJ/mol. Apparently, branching one, two, or three carbons removed from the carboxylate group affects the electrode response in a different but characteristic manner. Studies now underway[12] of aliphatic compounds with high degrees of branching near the carboxylate group ("crowded acids") should help establish the reason for this anomaly.

Aromatic Compounds. Addition of carbon groups para to benzoic acid or between the phenyl group and the carboxylate, as in the phenylacetate series, gives values of $\Delta(\Delta G)$ less than those just described. For example, the free energy increment for the addition of a *p*-methyl group

(4) Lelerc, G.; Bizec, J. C. *J. Med. Chem.* **1980**, *23*, 738–744.
(5) Vinek, W. C.; Aldrich, C. S.; Borchardt, R. T.; Grunewald, G. L. *J. Med. Chem.* **1981**, *23*, 1350–1357.
(6) Davis, D. P.; Borchardt, R. T.; Grunewald, G. L. *J. Med. Chem.* **1981**, *24*, 12–16.
(7) Hirsch, R. F.; Olderman, G. M. *Anal. Chem.* **1976**, *48*, 771–776.
(8) Franks, F. *Nature (London)* **1977**, *270*, 386–387.
(9) Seeman, P.; Roth, S.; Schneider, H. *Biochim. Biophys. Acta* **1971**, *225*, 171–184.
(10) Smith, R.; Tanford, C. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, 289–293.
(11) Tanford, C. "The Hydrophobic Effect"; Wiley: New York, 1973, Chapter III.
(12) Hirsch, R. F.; Rachlin, E. M.; Zhou, X.; Chretien, J. R.; Dubois, J. E. unpublished work, Seton Hall University and University of Paris, 1982.

Table II. $-\Delta(\Delta G)^a$ of CH_2 Groups Resulting in Loss of Resonance

compd 1	compd 2	$-\Delta(\Delta G)$, kJ/mol
m-chlorophenylacetate	*m*-chlorobenzoate	−4.2
m-bromophenylacetate	*m*-bromobenzoate	−4.2
phenylacetate	benzoate	−3.3
m-tolylacetate	*m*-toluate	−2.2
o-tolylacetate	o-toluate	5.6

[a] In kilojoules per mole.

to benzoate is −2.4 kJ/mol. For benzoate the charge is delocalized throughout the aromatic system, decreasing the charge density and extent of hydration of the anion. Placement of a methyl group in the para position increases the intensity of the charge by electron donation through resonance and inductive effects. This increase in charge density facilitates the water structuring partially offsetting the size effect of the methyl group. These electronic effects decrease with increasing distance from the ring. The hydrophobic interaction of an alkyl group with the solvent then becomes the dominant factor in the determination of $\Delta(\Delta G)$ per methylene group for long chain lengths, approaching the value obtained for the linear compounds (Table I, part C). Incorporation of the alkyl chain between the phenyl group and the anionic center gives constantly low values of $\Delta(\Delta G)$ as seen for the series from phenyl acetate through 5-phenylpentanoate (Table I, part D). This is in agreement with the observations of Chawla et al.[13] for the study of the enthalpies and heat capacities of dissolution for some carboxylates. They explain this phenomenon by the opposing effects of the phenyl ring and the methylene groups on the charge of the carboxylate group. Electron withdrawal lowers the contribution of the methylene groups to the overall solvation of the molecule.

The dramatic change in the electrode response in the conversion of benzoate to phenylacetate is worthy of discussion. Introduction of the methylene group between the phenyl and carboxylate groups results in a localization of charge on the latter and an increase in the hydration of that group. Added energy is required for desolvation upon partitioning into the organic ion exchanger. This corresponds to a free energy increment of 3.3 kJ/mol (note sign) for the added methylene group. This means that resonance delocalization enhances electrode response by 5.7 kJ/mol (3.3 + 2.4 kJ/mol). The effect of localization of charge is demonstrated in Table II. Electron-withdrawing and electron-donating groups have been substituted in the meta and ortho positions to study the consequence of inductive and steric effects on the relative electrode responses to these two compounds.

Placement of electron-withdrawing groups in the meta position of phenylacetate and benzoate results in a $\Delta(\Delta G)$ 1, than that calculated for the unsubstituted compounds. This is shown for both the *m*-chloro and *m*-bromo compounds. Two effects determine electrode sensitivity: size of the substituted group and extent of hydration of the compound. Since the groups are the same within each comparison, only the hydration of the carboxylate will be considered. Electron withdrawal from the ring results in a decrease in charge density and hydration. The effect is smaller for the phenylacetates than for the benzoates. Thus, an average $\Delta(\Delta G)$ of 4.2 kJ/mol for the *m*-halophenylacetates relative to the corresponding *m*-halobenzoates is not surprising.

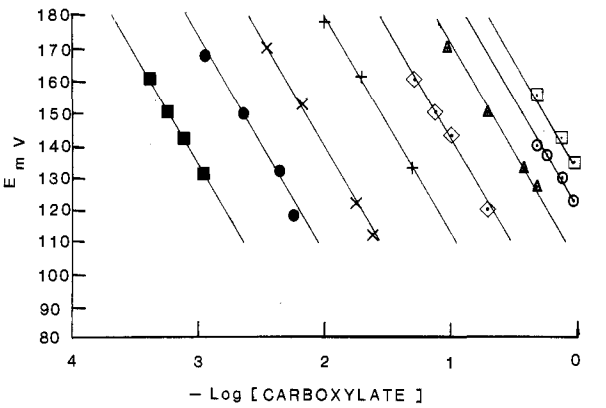

Figure 1. Electrode potential as a function of log concentration for the linear carboxylates: (○) formate, (□) acetate, (△) propanoate, (◇) butanoate, (+) pentanoate, (x) hexanoate, (●) heptanoate, (■) octanoate.

Similar arguments can be used to account for the effects of electron-donating groups. Electron donation by the *m*-methyl group of *m*-toluate increases the electron density on the anion and the extent of hydration. Conversely the increased electron density of the phenylacetate ring is more effective in offsetting the electron-donating effect of the methyl group in the *m*-tolylacetate than in phenylacetate itself. Therefore, a $\Delta(\Delta G)$ of 2.2 kJ/mol is reasonable.

Sign inversion is not observed for the *o*-methyl-substituted compounds. The ortho substituent skews the carboxylate of the toluate out of the plane of the ring, the consequence of a steric interaction of the *o*-methyl with that group.

Application to Substituent Constants and Partition Data. We have thus far demonstrated that thermodynamic constants can be calculated from potentiometric data. The value assigned to the group depends upon its position in the molecule as well as the effect of neighboring groups on it. Through summation of these effects it should then be possible to arrive at a free energy representative of the hydrophobicity of the entire molecule.

The partitioning of many compounds between octanol and water has been studied by Hansch.[14,15] Partition coefficients (P) were then used to calculate fragment constants, which represent the changes in the partitioning properties caused by structure modification. Tables of these fragment constants are available.[16] Summation of these along with the appropriate bonding constants (to account for unsaturation, aliphatic ring systems, etc.) can be used to predict log P values for new compounds. Where partition data were unavailable for our compounds, log P values were calculated in this manner as a substitute. Then constants for selected comparisons were calculated from the differences of the log P values.

The relationship between π and $\Delta(\Delta G°)$ of transfer in different solvent systems is shown in eq 1, where K_x and

$$\pi = 2.303RT \log (K_x/K_H) = \Delta G_x° - \Delta G_H° \quad (1)$$

K_H are the distribution coefficients of compounds R_x and R_H, respectively, and $\Delta G_i°$ is the free energy of transfer for compound i. Two biphasic systems, one having the solvents more similar in polarity than the other, give different π values for the compounds being compared, since the partition coefficients will be more dissimilar in

(13) Chawla, B.; Ahluwalia, J. C. *J. Solution Chem.* **1975**, *4*, 383–389.

(14) Iwasa, J.; Fujita, F.; Hansch, C. *J. Med. Chem.* **1965**, *8*, 150–153.

(15) Leo, A.; Hansch, C.; Elkins, D. *Chem. Rev.* **1971**, *71*, 525–616.

(16) Hansch, C.; Leo, A. "Substituent Constants for Analysis in Chemistry and Biology"; Wiley: New York, 1979; 171–130.

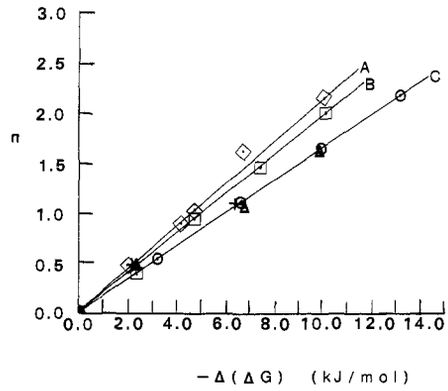

Figure 2. Calculated π values as a function of $-\Delta(\Delta G)$: (○) linear carboxylates, (+) cyclopentane alkanoates, (△) cyclohexanealkanoates, (□) benzoates, (◇) phenylacetates.

the one system than in the other. The slope of a plot of π vs. our potentiometrically determined $-\Delta(\Delta G)$ values should likewise be linear. Examples are shown for the aliphatic carboxylates, benzoates, and phenylacetates (Figure 2), where for the aliphatic carboxylates

$$\pi = 0.17[-\Delta(\Delta G)] \quad (2a)$$

for the benzoates

$$\pi = 0.20[-\Delta(\Delta G)] \quad (2b)$$

and for the phenylacetates

$$\pi = 0.21[-\Delta(\Delta G)] \quad (2c)$$

Using the relationships just described, one can predict π from $-\Delta(\Delta G)$. An interesting use of this technique is seen in the determination of π for cyclopentanepropanoate. Since it is an aliphatic compound it is reasonable to assume that it will fall in the series with the linear aliphatic carboxylates. A value of $\Delta(\Delta G)$ of −6.32 kJ/mol was determined potentiometrically (using cyclopentane formate as the parent compound). A value of π of 1.1 was calculated by equation 2a, while π from octanol–water partition data is 1.08. With careful choice of the parent compound it should be possible to define the hydrophobic interactions for a vast number of compounds. Furthermore, where partition constants have in the past been used to study these interactions, potentiometric data should be useable as well.

Application to Structure–Activity Studies. Quantitative structure–activity relationships have been used to correlate experimentally determined hydrophobicity parameters with drug activity for several kinds of drugs and biological systems.[17]

Octanol–water partition coefficients are now commonly used as a measure of biological effects.[18,19] Toxicity, for example, has been found to be related to the lipophilicity of the toxic compound.[20] Hydrophobic parameters such as the π constants have been used to measure this effect. In one such study[21] it was shown that the log of the reciprocal of the isotoxic concentrations of substituted benzoic acids for the 50% mortality of mosquito larva were directly related to π (eq 3), where C is the LD_{50} value of

$$\log (1/C) = k\pi + b \quad (3)$$

Table III. Comparison of Toxicity of Substituted Benzoic Acids with Electrode Response

compd	log $(1/LD_{50})^a$	$-\Delta(\Delta G)^b$
p-hydroxybenzoic acid	1.29	−3.06
p-methoxybenzoic acid	1.60	1.10
p-toluic acid	1.66	1.10
p-chlorobenzoic acid	2.06	6.65
p-bromobenzoic acid	2.03	7.95
m-chlorobenzoic acid	2.00	8.11
3,4-dichlorobenzoic acid	2.27	11.8

[a] Calculated from the isotoxic concentrations of Casida reported by Hansch and Fujita.[21] [b] Calculated relative to benzoate.

Table IV. Comparison of Inhibition of Spore Generation by Fatty Acids with Electrode Response

compd	log $(1/I_{50})^a$	$-\Delta G$
heptanoate	0.82	15.2
octanoate	1.27	18.5
nonanoate	2.05	21.8
decanoate	2.22	25.1
undecanoate	2.35	28.4
dodecanoate	2.70	31.7
tridecanoate	3.12	35.0

[a] Reference 22.

the substituted acid. It is reasonable that a relationship between toxicity and $\Delta(\Delta G)$ should likewise exist. The logs of the reciprocal of Casida's LD_{50} values[21] were regressed (using Bartlett's method[22]) against $\Delta(\Delta G)$ for meta- and para-substituted benzoic acids (using benzoate as the parent compound) demonstrating that (Table III)

$$\log (1/C) = -0.060\Delta(\Delta G) + 1.54 \quad (4)$$

where the 95% confidence limits are ±0.012 for the slope and ±0.06 for the intercept, and R^2 was 97.7%.

In the second example, the concentrations of fatty acids necessary to cause a 50% inhibition of *Bacillus subtilis* spore germination were reported.[23] This included the compounds from heptanoic through tridecanoic acid. A linear relationship was found to exist between log $(1/I_{50})$ and the octanol–water partition coefficients (log P). A linear relationship should thus exist between log $(1/I_{50})$ and $-\Delta G$. A plot of log $(1/I_{50})$ vs. ΔG (Table IV) follows eq 5, where the 95% confidence limits are ±0.02 for the

$$\log (1/I_{50}) = -0.12\Delta G - 0.9 \quad (5)$$

slope and ±0.5 for the intercept, and R^2 was 95.0%.

The results discussed here demonstrate the utility of ion-selective potentiometry for the study of hydrophobic interactions in biological systems. The technique is a nondestructive bench-top method that allows the chemist to measure directly the activity of many organic anions in aqueous solution. Furthermore, this could be extended to the study of cations through the use of other liquid membrane electrodes.

The free energy relationships may not be as simple as those described here. In some cases they will be nonlinear. It may be necessary to include electrostatic parameters such as the Hammett σ or Taft σ^* substituent constants

(17) Ariens, E. J. *Drug Des.* **1980**, *9*, 47–119.
(18) Ariens, E. J. *Drug Des.* **1971**, *1*, 271–342.
(19) Levin, V. A. *J. Med. Chem.* **1980**, *23*, 682–684.
(20) Cheng, C. Y.; Hanssen, E. B.; Waters, J. A. *J. Med. Chem.* **1982**, *25*, 145–152.
(21) Hansch, C.; Fujita, T. *J. Am. Chem. Soc.* **1964**, *86*, 1616–1626.
(22) Kendall, M. G.; Stuart, H. "The Advanced Theory of Statistics"; Hafner Publishing Co.: New York, 1967; Vol. 2, pp 400–405.
(23) Yasida, Y.; Tochikubo, K.; Hachisuka, Y. *J. Med. Chem.* **1982**, *25*, 314–320.

in the structure–activity expression. In any event, through extension of this work it will be possible to develop biologically significant QSAR's by using potentiometric data.

Experimental Section

Instrumentation. Potentiometric measurements were made with an Orion Research (Cambridge, MA) Model 93-07-01 nitrate electrode. The reference potential was supplied by a double junction reference electrode, Orion Research Model 93-02-00. Readings were taken with a Corning (Corning, NY) Model 12 millivoltmeter and monitored with a Leeds and Northrup (Philadelphia, PA) Model S6000 Speedomax Type G strip chart recorder. Recording was found to be helpful in determining the point at which equilibration of the electrode was reached. It also aided in detecting electrical or experimental instabilities.

The electrode response is dependent on the prior use of the electrode, particularly when it has been used with a sample to which it responds strongly. The nitrate electrode was selected for this study because it senses all of the ions of interest adequately, and only a very few ions so strongly that the electrode response became distorted. The commercially available chloride electrode would show this hysteresis effect with a much larger number of the samples, since it responds more strongly to all of them, while a perchlorate electrode would not sense some of the ions of interest at all, since it shows a much weaker response to a given ion than does the nitrate electrode. Since the order of response within a group of ions is the same for the nitrate and the perchlorate electrode,[7] the choice of the nitrate electrode is mainly a matter of experimental convenience. A set of ions showing a strong response with the nitrate electrode might well be studied more conveniently with the perchlorate electrode, for example.

The samples were tested in 30-mL beakers placed in a water-jacketed system for maintaining a constant temperature. Water regulated at 25.0 ± 0.1 °C was circulated throughout this system. A layer of water between the beaker and the water jacket assured thermal contact between the sample and the thermostatted liquid. All solutions were stirred with a magnetic stirrer.

Materials. The cyclopentanecarboxylic acids, straight-chain aliphatic carboxylic acids, 4-phenylbutanoic, 5-phenylpentanoic, *m*-bromophenylacetic, *o*- and *m*-tolylacetic, meta- and para-substituted benzoic, and 3,4-dichlorobenzoic acids were obtained from the Aldrich Chemical Co., Inc. (Milwaukee, WI). The *p*-*n*-butylbenzoyl chloride and *p*-toluic, *p*-ethylbenzoic, *p*-pentylbenzoic, and all sulfonic acids were obtained from Eastman Organic Chemicals (Rochester, NY). The *p*-*n*-butylbenzoic acid was prepared by heating *p*-butylbenzoyl chloride in basic solution with constant stirring for 2 h. The solution was allowed to cool slowly and then acidified with 6 M HCl until acid to litmus (the pH was also checked with pH paper). The precipitate was collected on a Buchner funnel and dried in a vacuum oven overnight at 66 °C: mp 95–96 °C (lit.[24] mp 100 °C); ^{1}H NMR δ 0.9 (d, 3, CH_3), 1.1–1.9 (m, 4, $CH_3CH_2CH_2$), 2.7 (t, 2, CH_2Ar), 7.2 (dd, 2, ArH), 8.0 (dd, 2, ArH), 12.3 (s, 1, CO_2H).

Sample Preparation and Measurement. Buffer was prepared by dissolving 0.050 mol of dibasic sodium phosphate in boiled deionized water and diluting to 1 L after adjusting the pH to 7.0 with sulfuric acid. Sulfuric acid was chosen to adjust the pH so as not to alter the total phosphate concentration. In addition, sulfate ion is sensed very weakly by the nitrate electrode.

Each sample was dissolved in the buffer to prepare a stock solution. The pH was readjusted to 7.0 with dilute sodium hydroxide. The stock solutions were each then diluted with buffer to give a series of test solutions that would give electrode response within the range of 100 to 200 mV.

Sodium sulfate was used to adjust the outer filling solution (buffer) of the reference electrode as suggested by Orion Research.[25] The ionic strength adjuster was found not to be necessary for the samples themselves, since the phosphate buffer provided a high and constant background ionic strength.

After the solutions of a given acid were allowed to equilibrate to 25 °C, they were tested in a random sequence with the electrode system. Between each of the sample measurements the electrode was allowed to equilibrate with a 0.0100 M potassium nitrate solution. The reading with this reference solution was corrected to 40 mV by subtracting the appropriate quantity. The succeeding sample readings were corrected accordingly.

Principle of Calculation. The observed electrode potentials may be expressed by the Nernst equations for a substituted compound (i) and its parent (j)

$$E_i = E_i^\circ - RT/nF \ln a_i$$

$$E_j = E_j^\circ - RT/nF \ln a_j$$

The difference ΔE represents the effect of the substituent on the ion–solvent interaction:

$$\Delta E = E_i - E_j = E_i^\circ - E_j^\circ - RT/nF \ln a_i/a_j$$

At constant potential

$$\Delta E = 0 = \Delta E^\circ - RT/nF \ln a_i/a_j$$

so

$$\Delta E^\circ = RT/nF \ln a_i/a_j$$

The free energy increment then is

$$\Delta(\Delta G^\circ) = -RT \ln a_i/a_j$$

At a high and constant ionic strength, activity coefficients are constant, so concentrations are used in place of activities.

$$\Delta(\Delta G^\circ) = -RT \ln C_i/C_j$$

A plot of membrane electrode potential as a function of log sample concentration is drawn for each compound studied. An example is shown for the linear carboxylates (Figure 1). These plots are linear over at least a tenfold concentration range. Concentrations read at a chosen value of E from the graph are used in the calculation of ΔG and $\Delta(\Delta G)$, the latter being the difference of log concentrations of the compounds compared, and may be estimated from the distance between the lines for these compounds. To ensure constancy of $\Delta(\Delta G)$ for any chosen potential, the slopes for all compounds being compared must be the same. In our study it was found that this requirement was met best at 140 mV. The precision of the calculated $\Delta(\Delta G)$ values that result is estimated to be ±0.15 kJ/mol, based on a ±2 mV reproducibility.

Registry No. Formate, 71-47-6; acetate, 71-50-1; propanoate, 72-03-7; butanoate, 461-55-2; pentanoate, 10023-74-2; hexanoate, 151-33-7; heptanoate, 7563-37-3; cyclohexaneformate, 3198-23-0; cyclohexaneacetate, 70554-37-9; cyclohexanepropanoate, 86260-90-4; cyclopentaneformate, 45520-91-0; cyclopentaneacetate, 70554-35-7; cyclopentanepropanoate, 86260-91-5; benzoate, 766-76-7; *p*-toluate, 5118-31-0; *p*-ethylbenzoate, 79599-76-1; *p*-*n*-propylbenzoate, 63684-64-0; phenylacetate, 7631-42-7; 3-phenylpropanoate, 826-17-5; 4-phenylbutanoate, 4358-94-5; butanesulfonate, 24613-77-2; pentanesulfonate, 24708-25-6; hexanesulfonate, 24613-78-3; heptanesulfonate, 24708-26-7; *m*-chlorobenzoate, 16887-60-8; *m*-bromobenzoate, 16887-61-9; *m*-toluate, 16887-59-5; *o*-toluate, 16887-74-4; *p*-hydroxybenzoic acid, 99-96-7; *p*-methoxybenzoic acid, 100-09-4; *p*-toluic acid, 99-94-5; *p*-chlorobenzoic acid, 74-11-3; *p*-bromobenzoic acid, 586-76-5; *m*-chlorobenzoic acid, 535-80-8; 3,4-dichlorobenzoic acid, 51-44-5; octanoate, 74-81-7; nonanoate, 3342-79-8; decanoate, 3398-75-2; undecanoate, 23815-71-6; dodecanoate, 115-05-9; tridecanoate, 86260-92-6; *p*-*n*-butylbenzoic acid, 20651-71-2; *p*-butylbenzoyl chloride, 28788-62-7.

(24) "Sadtler Standard N.M.R. Spectra"; Sadtler Research Laboratories: Philadelphia, PA, 1972.

(25) Orion Research, "Instruction Manual—Nitrate Electrode Model 93-07"; Orion Research Inc.: Cambridge, MA, 1973; p 4.