Effect of Hydrophobic–Lipophilic Interactions on Chemical Reactivity. 6. The First Example of Correlation of Hydrophobic–Lipophilic Substituent Constants with Chemical Reactivity for a Simple Substrate–Solvent System

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Abstract: Hydrolytic rate constants (k_{obsd}) were determined for 13 para- and meta-substituted phenyl esters of n-hexadecanoates (**16-Y**) in 55:45 (v/v) ($\Phi = 0.55$), 60:40 ($\Phi = 0.60$), 65:35 ($\Phi = 0.65$), and 70:30 ($\Phi = 0.70$) Me_2SO-H_2O solvent mixtures. Rates of 7 n-octanoates (**8-Y**) were also measured in solvents with $\Phi = 0.55$, 0.60, and 0.65. Results reveal that the nature of the substituent effect depends on solvent composition. At $\Phi = 0.70$, the log k_{ob}'s correlate with Hammett's σ constants, whereas at $\Phi = 0.55$, they correlate with Rekker's hydrophobic f constants. In between at $\Phi = 0.65$ and 0.60, the rate constants can be expressed in terms of a two-parameter equation: $\log k = \sigma\rho + hf + C$. The n-octanoates, as expected, follow only the Hammett correlation in disregard of the solvent composition. The present work is the first example of a successful correlation of hydrophobic–lipophilic substituent constants for substrates with different types of substituents and without the involvement of "third-parties", e.g., added host or surfactant molecules. It provides further insights into the effect of hydrophobic–lipophilic interactions on chemical reactivity and yet another new line of evidence for the phenomena of aggregation and self-coiling.

Substituent constants directly reflecting hydrophobic–lipophilic interactions, such as Hansch's π constants and Rekker's f constants (hydrophobic fragmental constants), have already established themselves in an impressively wide range of applications,[1–5] e.g., in structure–property correlations between such constants and pharmaceutical properties or physiological activities of a variety of chemical species,[6–8] as well as in studies on oil–water distribution coefficients and liquid chromatography.[2,3,9,10] In fact, on the basis of Murakami's data, Hansch has already correlated his hydrophobic constants with the rates of hydrolysis catalyzed by an added host (a [20]paracyclophane) for p-nitrophenyl esters of alkanoic acids with 12 different alkyl groups. He has similarly treated Gitler's data for such esters with different alkyl groups hydrolyzed in the presence of mixed micelles of N-myristoyl-L-histidine and cetyltrimethylammonium bromide.[11] Very recently, Fujita and Leo have further treated the effects of electronic and other interactions in multiply substituted aromatic solutes on the magnitude of these hydrophobic constants.[9,12,13] However, to our knowledge as yet no one has established a case which demonstrates that these hydrophobic substituent constants can be directly and quantitatively correlated with chemical reactivity in terms of rate constants for substrates bearing a wide variety of substituents and in the absence of enzymes or their models, e.g., surfactant and host molecules. Thus we would like to present our first successful attempt in addressing such a challenge. Another object of this work is to compare the applicability of the two types of substituent constants, namely, hydrophobic and electronic (Hammett-type σ's), in aqueous–organic solvents of various compositions, and thus to gain further insights into how hydrophobic–lipophilic interactions affect chemical reactivity,[14,15] e.g., to test the validity of Murakami's comment that only monomeric forms of the ester substrates are sufficiently reactive in hydrolysis over the whole concentration range he studied.[16]

Our present approach was worked out from considerations based on the following previous observations:[17,18] (1) Para-substituted phenyl esters of n-hexadecanoates (**16-p-Y**) aggregate and coil-up in Me_2SO-H_2O solvent mixtures with $\Phi = 0.50$ (50:50 (v/v)), where Φ stands for the volume fraction of the organic component of an aqueous–organic binary mixture; they do so also even in the less aggregating aqueous–organic medium with $\Phi = 0.60$ (60:40 (v/v)) if their concentrations are higher than about 2×10^{-5} M. Under these conditions there is no good Hammett correlation for the observed hydrolytic rate constants of these esters. (2) In the same solvent mixtures of $\Phi = 0.40$, 0.50, and 0.60, the n-octanoates **8-p-Y** do not aggregate; in solvents with $\Phi = 0.50$, 0.60, the n-dodecanoates **12-p-Y** also prefer to remain single. At $\Phi = 0.50$, they are both well-behaved in following the Hammett correlation. (3) The ratios of the observed rate constants of the octanoates **8-NO₂** and hexadecanoates **16-NO₂**, k_8/k_{16}, can be used as indicators of their relative degrees of aggregation, and these ratios can even be correlated with Rekker's hydrophobic fragmental constants (f) of the ten organic components of the aqueous–organic solvents. Whereupon we hypothesized the following: (1) the observed rate constants in aggregating solvents are extremely complicated functions of the degrees of aggregation and coiling, besides their dependence on k_m (cf. Scheme I), and (2) the degrees of aggregation and coiling might be correlated with Rekker's f constants of the substituents Y, even though they represent only relatively small portions of the substrates **16-Y** and their effects cannot be relayed to the carbonyl-carbon like the electronic effects.[19]

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Table I. The Hydrolytic Rate Constants k (10^{-3} s^{-1}) of the Substrate **16-Y** at 45 °C in Me$_2$SO–H$_2$O Solvents of Different Compositions and Their σ, f Constants

Y[a]	Φ 0.55	0.60	0.65	0.70	σ[b]	f[c]
p-NO$_2$	2.05	9.10	26.9	27.0	0.95[d]	−0.078
m-NO$_2$	1.95	8.10	21.5	19.2	0.71	−0.078
p-CHO	4.00	10.4	19.2	16.9	0.69[d]	−0.667
m-Cl	0.20	0.77	2.92	4.41	0.37	0.943
p-Cl	0.18	0.70	2.16	3.74	0.23	0.943
p-Br	0.10	0.68	1.95	3.60	0.23	1.168
p-CO$_2$	1.10	1.23	1.61	1.24	0.13	
H	0.37	0.48	1.19	1.80	0	0.175
p-CH$_3$	0.24	0.28	0.89	1.12	−0.17	0.702
m-CH$_3$	0.25	0.29	1.03	1.47	−0.07	0.702
p-OCH$_3$	0.48	0.44	1.10	1.18	−0.27	0.248
p-Ph	0.025	0.071	0.23	0.39	0.01	1.88
p-t-Bu	e	e	0.16	0.19	−0.20	2.26

[a] The rate constant is dependent on the initial substrate concentration, and the k values were obtained at the concentration 3.2×10^{-5} M. [b] The σ constants are cited from: March, J. "Advanced Organic Chemistry", 2nd ed.; McGraw-Hill: New York, 1977; p 253. [c] The f constants are cited from the ref 3. [d] The σ values were calculated according to log $k/k_o = \rho[\sigma + r(\sigma - \sigma^-)]$,[17,22] $r = 0.35$. [e] The rate is too low to be determined.

To test our working hypothesis, 13 n-hexadecanoates **16-Y** and 7 octanoates **8-Y** were synthesized and their hydrolytic behavior investigated in Me$_2$SO–H$_2$O solvent mixtures with Φ = 0.55, 0.60, 0.65, and 0.70. Results of this study yielded new evidence for the phenomena of aggregation and self-coiling. Thus by now we have at least fourteen lines of evidence documented for these phenomena.[20]
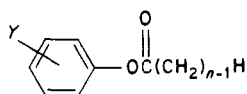
8-Y; n=8; **16-Y**, n=16, Y = p-NO$_2$. m-NO$_2$. p-CHO. p-CO$_2$, p-Cl. m-Cl, p-Br, H, p-CH$_3$, m-CH$_3$, p-OCH$_3$. p-Ph, p-t-Bu

Experimental Section

Substrates. Substituted phenyl carboxylates (**8-Y** and **16-Y**) were prepared by methods reported elsewhere[17] and identified by ^{1}H NMR and elemental analysis. All meta-substituted phenyl hexadecanoates **16-m-Y**, together with **16-p-Ph** and **16-p-t-Bu**, are new compounds. The melting points of these compounds are as follows: **16-p-Ph**, 65–66 °C; **16-p-t-Bu**, 35–36 °C; **16-m-NO$_2$**, 55–56 °C; **16-m-Cl**, 42–43 °C; **16-m-CH$_3$**, 32–33 °C.

Solvent. Water was deionized and dried Me$_2$SO was fractionated as previously described.[21] Aqueous 0.02 M NaHCO$_3$–Na$_2$CO$_3$ solution with pH 9.65 was used as the buffer solution which was mixed with Me$_2$SO in various proportions.[17] The pH values of the mixtures were Φ = 0.70, >14; Φ = 0.65, >14; Φ = 0.60, 13.98; Φ = 0.55, 13.50.

Kinetics. Kinetic measurements were performed on a Perkin-Elmer 559 spectrophotometer equipped with a thermostated cell holder. The wavelengths in nm used for monitoring the formation of the substituted phenol products were the following: p-NO$_2$, 410; m-NO$_2$, 400; p-CHO, 345; p-CH$_3$, 307; p-CO$_2^-$, 295; p-Cl, 310; m-Cl, 305; p-Br, 310; H, 290; m-CH$_3$, 300; p-OCH$_3$, 310; p-Ph, 320; p-t-Bu, 300. Data were treated as previously described.[17] All of the rate constants are accurate to within ±5% for **8-Y** and ±10% for **16-Y**.

Results and Discussion

Table I is a compilation of the pseudo-first-order hydrolytic rate constants (k_{obsd}) for 13 para- and meta-substituted phenyl esters of n-hexadecanoates **16-Y** in Φ = 0.55, 0.60, 0.65, and 0.70 Me$_2$SO–H$_2$O solvent mixtures together with the corresponding

(19) Unlike the Rekker's f constants, values of Hansch's π constants depend on the type of compounds under consideration. Not all of these values for the Y-C$_6$H$_4$OOC(CH$_2$)$_n$H substituents are available, and an attempted correlation with limited values of available π values for the Y substituents with our rate data did not yield a satisfactory result.

(20) In addition to the ten lines of evidence described in ref 17, the effects of added ureas[14] can be counted as the 11th line of evidence. Results reported in ref 18 may be regarded as the 12th and 13th lines of evidence.

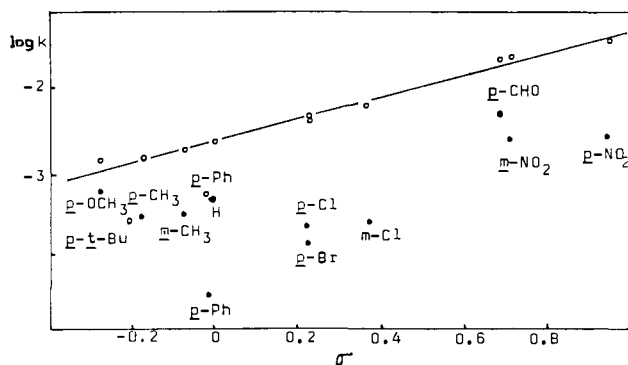(21) Hui Yongzheng; Cheng Xianen; Gu Jianhua; Jiang Xikui *Sci. Sin B* **1982**, *25*, 698.

Figure 1. Solvent composition effect on Hammett correlations of rate constants for the hydrolysis of **16-Y**. (O) Φ = 0.70 Me$_2$SO, ρ = 1.25, r = 0.991 (p-Ph and p-t-Bu deviate from the line); (●) Φ = 0.55 Me$_2$SO, no correlation.
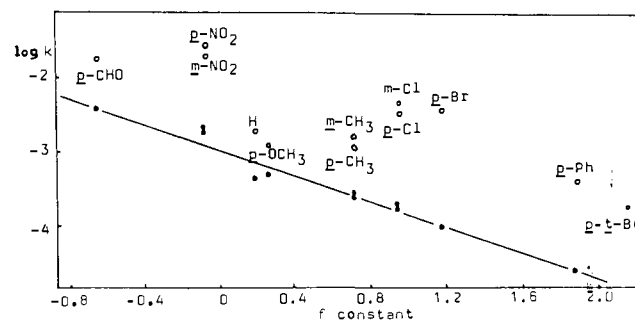

Figure 2. Correlation of rate constants with Rekker's hydrophobic fragmental constants of substituents, f, for the hydrolysis of **16-Y**. (●) Φ = 0.55 Me$_2$SO, h = −0.88, r = 0.974; (O) Φ = 0.70 Me$_2$SO, no correlation.
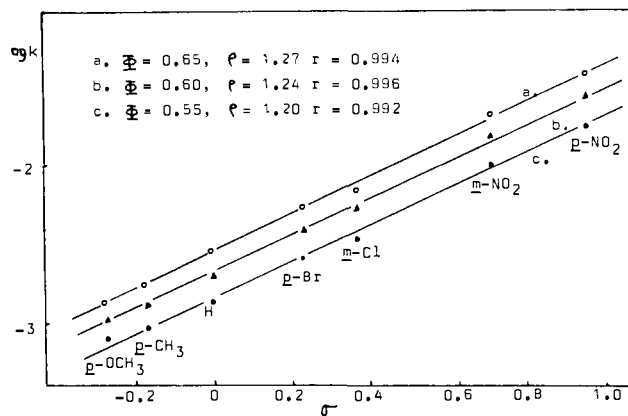

Figure 3. Hammett correlations for the hydrolysis of **8-Y** in Me$_2$SO–H$_2$O solvent mixtures of various compositions.

Table II. The Hydrolytic Rate Constants k (10^{-3} s^{-1}) of the substrates **8-Y** at 45 °C in Me$_2$SO–H$_2$O Solvents of Different Compositions

Y[a]	Φ 0.55	0.60	0.65
p-NO$_2$	20.0	29.5	38.5
m-NO$_2$	12.0	18.2	28.5
m-Cl	3.24	4.80	6.56
p-Br	2.48	3.36	5.25
H	1.25	1.84	2.88
p-CH$_3$	0.914	1.24	1.56
p-OCH$_3$	0.814	1.04	1.28

[a] The rate constant is independent of the initial substrate concentration.

Hammett's electronic and Rekker's hydrophobic substituent constants, i.e., σ and f constants. Figure 1 has the rate data (log k_{obsd}) plotted against σ, and Figure 2 shows log k_{obsd} vs. f. Pertinent data for 7 n-octanoates **8-Y** in similar solvent mixtures with Φ

Scheme I

$$\quad (1,2)$$
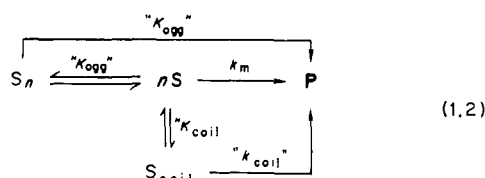
Φ = 0.55, 0.60, and 0.65 are presented in Table II and Figure 3.

We prefer to visualize the hydrolysis of long-chain substrates in aggregating media as an exceedingly complicated process, as might be depicted by Scheme I.

In Scheme I, S and P stand for the monomeric substrate and the hydrolysis product and k_m is the rate constant of the monomeric substrate. Aggregates with a whole spectrum of aggregation numbers, n, are represented by a single symbol S_n; similarly, all coiling conformers which hydrolyze slower than S are represented as S_{coil}. Obviously, the quotation-marked K's and k's are not true thermodynamic and kinetic constants in the usual sense. The points we want to make are the following: (1) Murakami's proposition, i.e., $S_n \to S \to P$, with $k_m \gg$ "k_{agg}", may represent only one of the possible paths operating under certain circumstances. (2) We intuitively believe that aggregates with very low n values, e.g., dimeric and trimeric species, may abound under other circumstances and that the "k_{agg}" values for these low-n species may not be negligible in comparison with k_m. On the other hand, we believe that these aggregates are not necessarily parallelly aligned chains but are mutually entangled molecules coiled up in different degrees, and they may be even more disordered and fluctuant than the Menger micelles since their component species possess no electrical charge.[23] Possibly, their reactivity may drop suddenly and drastically when n reaches certain values. (3) The rates of formation of P from the different paths, $k_m[S]$, "k_{agg}"[S_n], and "k_{coil}"[S_{coil}], may vary widely, thus their relative importance, which ultimately depends on the magnitude of the hydrophobic forces in operation, may change greatly under different conditions. (4) In good (nonaggregating) solvents, **16-Y** should behave "normally", i.e., follow the Hammett $\rho\sigma$ correlation, just like their short-chain analogues, **8-Y**. (5) In strongly aggregating media ("poor solvents"), aggregation and coiling-up might become the overriding factors which control the overall rate and log k might be correlated with some hydrophobic substituent parameters, e.g., Rekker's f constants. (6) Yet another more fascinating possibility might exist, viz., inbetween the two limiting conditions mentioned in points 4 and 5, there might be conditions under which all paths (k_m "k_{agg}", "k_{coil}") depicted in Scheme I were freely traversable thoroughfares, and thus the observed k's might be correlatable with combinations of σ and f.

Gratifyingly, the self-evident messages brought out by Figures 1, 2, and 3 fulfill expectations discussed in points 4 and 5.

In the basically nonaggregating Φ = 0.70 medium, the rate constants of **16-Y** do not correlate with Rekker's f constants but do so with Hammett's σ's with ρ = 1.25 and r = 0.991. Notably, the two most hydrophobic substituents phenyl (f = 1.88) and *tert*-butyl (f = 2.26) have to be excluded from this correlation. This exclusion is justified because it has been established that both **16-p-Ph** and **16-p-Bu** still retain some tendency to aggregate in this medium at concentrations as low as 1×10^{-5} M.[24] Thus they are expected to deviate from the general behavior pattern of the other ten **16-Y** esters.

As expected, the octanoates **8-Y** behave normally in all 3 media of different compositions (cf. Figure 3), to wit, at, at Φ = 0.55, ρ = 1.20, r = 0.992; at Φ = 0.60, ρ = 1.24, r = 0.996; and at Φ = 0.65, ρ = 1.27, r = 0.994. The fact that these ρ values of **8-Y** are almost the same as that of **16-Y** in the Φ = 0.70 medium strongly supports the notion that long-chain and short-chain

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Table III. Values of ρ, h, and C of Equation 3 and Their Standard Deviations,[a] h/ρ Ratios, F-Tests, and Explained SS's for Correlations with 11 or 12 Substrates in Media of Various Φ Values

Φ	ρ	h	C	$-h/\rho$	n[b]	F[c]	explained SS,[d] %
0.55	0.34	-0.78	-3.10	2.29	11	222	98.2
	(0.088)	(0.049)	(0.051)				
0.60	1.12	-0.50	-3.04	0.45	11	119	96.7
	(0.131)	(0.074)	(0.076)				
0.65	1.14	-0.41	-2.64	0.36	12	132	96.7
	(0.134)	(0.064)	(0.076)				
0.70	1.11	-0.33	-2.57	0.30	12	77.8	94.5
	(0.158)	(0.075)	(0.090)				

[a] Values listed in parentheses directly below the ρ, h, or C values. [b] The number of substituents. [c] F-test. [d] explained SS (sum of squares) as percent total, see: Mather, P. M. "Computational Methods of Multivariate Analysis in Physical Geograph"; John Wiley & Sons: London, 1976.

substrates follow the same hydrolytic path in a nonaggregating solvent.

In vivid contrast to the "normal" hydrolytic behavior of the hexadecanoates **16-Y** in the Φ = 0.70 medium described above, the same substrates follow different reaction paths in the Φ = 0.55 medium, in other words, their rate constants show little respect for the electronic effects but correlate fairly well with Rekker's f constants, i.e., log k/k_0 = fh, with h = -0.88 and r = 0.974. Clearly, here hydrophobic–lipophilic forces upon which the degree of aggregation depends become the dominant factor which controls the overall hydrolytic rates. This conclusion is supported by two additional observations: (1) The relative rate orders of some substrates are the reverse of those expected from considerations based on electronic effects, e.g., **16-p-Cl** and **16-p-Br** hydrolyze slower than **16-p-CH₃** and **16-p-OCH₃** and **16-p-NO₂** slower than **16-p-CHO**. (2) The location of the substituent, whether para or meta, has no effect on the rate. Certainly, this agrees with the noteworthy fact that para and meta substituents possess the same hydrophobic constants (f values) but different electronic constants. Summing up, we may conclude that both points 4 and 5 are vindicated and a first example of correlation of hydrophobic substituent constants with chemical reactivity for substrates bearing electronically different types of substituents and without the involvement of added "third-parties" (enzymes, host or surfactant molecules) has been established.

Now we can take on point 6. Inbetween Φ = 0.55 and Φ = 0.70, Φ = 0.60 and Φ = 0.65 were picked for the media of intermediate compositions. Rate constants measured in these media are also listed in Table I. To our great satisfaction, whereas these data are not correlatable with either σ or f alone, they can be correlated by a combination of both through a two-parameter eq 3, in which C is a constant and h reflects the sensitivity of the reaction to hydrophobic–lipophilic forces. The ρ, h, and C values were calculated by multiple linear regression analysis, standard deviations (s) for these values, as well as F-test values, explained SS% values, and h/ρ ratios for these equations are listed in Table III. For Φ = 0.60, we obtained eq 4, and for Φ = 0.65 eq 5. Calculated log k values based on eq 4, 5, 6, and 7 checked well with experimental values (log k_{obsd}) as shown in Table IV.

$$\log k = \rho\sigma + hf + C \qquad (3)$$

$$\Phi = 0.60: \quad \log k = 1.12\sigma - 0.50f - 3.04 \qquad (4)$$

$$\Phi = 0.65: \quad \log k = 1.14\sigma - 0.41f - 2.64 \qquad (5)$$

Since curiosity is always good justification, we tried out the same treatment on data obtained from systems of Φ = 0.55 and 0.70. Again two well-behaved equations were obtained, i.e., eq 6 and 7. Obviously, the numerical value of the h/ρ ratio (cf. Table III) has no meaning, but the ratio or a comparison of these values

$$\Phi = 0.55: \quad \log k = 0.34\sigma - 0.78f - 3.10 \qquad (6)$$

$$\Phi = 0.70: \quad \log k = 1.11\sigma - 0.33f - 2.57 \qquad (7)$$

(cf. Table III) do have significance. Pleasingly, these value (2.29

Table IV. Comparison of Experimental Rate Constants ($-\log k_{obsd}$) and Calculated Values ($-\log k_{calcd}$) Based on Equations 4–7

Y	$\Phi = 0.55$		$\Phi = 0.60$		$\Phi = 0.65$		$\Phi = 0.70$	
	$-\log k_{obsd}$	$-\log k_{calcd}$	$-\log k_{obsd}$	$-\log k_{calcd}$	$-\log k_{obsd}$	$-\log k_{calcd}$	$-\log k_{obsd}$	$-\log k_{calcd}$
p-NO$_2$	2.69	2.72	2.04	1.94	1.57	1.52	1.57	1.49
m-NO$_2$	2.71	2.80	2.09	2.21	1.67	1.80	1.72	1.76
p-CHO	2.40	2.35	1.98	1.93	1.72	1.58	1.77	1.58
p-Cl	3.74	3.76	3.15	3.25	2.67	2.76	2.43	2.63
m-Cl	3.70	3.71	3.11	3.10	2.53	2.60	2.36	2.47
p-Br	4.00	3.93	3.17	3.37	2.71	2.86	2.44	2.70
H	3.43	3.24	3.32	3.13	2.92	2.71	2.74	2.63
p-CH$_3$	3.62	3.71	3.55	3.58	3.05	3.12	2.95	2.99
m-CH$_3$	3.60	3.67	3.54	3.47	2.99	3.01	2.83	2.88
p-OCH$_3$	3.32	3.39	3.36	3.47	2.96	3.05	2.93	2.95
p-ph	4.60	4.56	4.15	3.97	3.64	3.40	3.41	3.18
p-t-Bu					3.80	3.79	3.72	3.54

Table V. Values of ρ, h, and C of Equation 3 and Their Standard Deviations (in parentheses), h/ρ Ratios, F-Test, and Explained SS's for Correlations with 10 Substrates in Media of Various Φ Values
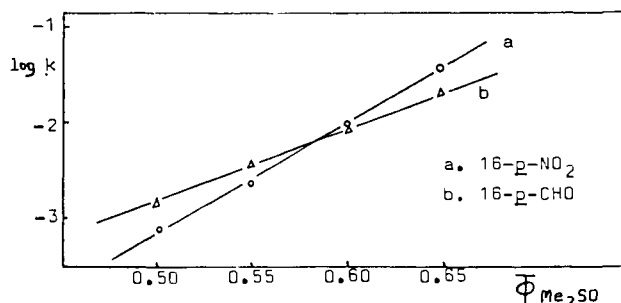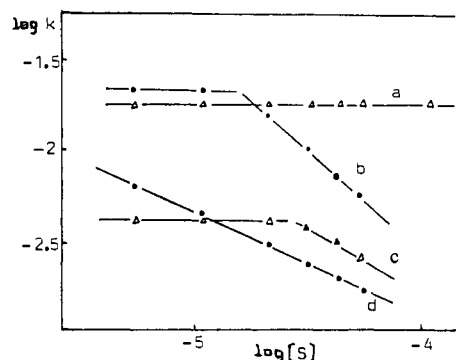
Φ	ρ	h	C	$-h/\rho$	F	explained SS, %
0.55	0.35 (0.094)	−0.76 (0.066)	−3.10 (0.055)	2.17	126	97.3
0.60	1.16 (0.11)	−0.40 (0.081)	−3.08 (0.067)	0.34	117	97.1
0.65	1.20 (0.099)	−0.27 (0.07)	−2.69 (0.058)	0.23	139	97.5
0.70	1.19 (0.06)	−0.093 (0.042)	−2.65 (0.035)	0.08	291	98.8

Table VI. Rate Constant Dependence on Initial Substrate Concentration of 16-p-NO$_2$ and 16-p-CHO and $\Phi = 0.55$ and $\Phi = 0.60$ Me$_2$SO–H$_2$O Media, 45 °C

Φ	substrate	concentration of substrate (10^{-5} M)					
		0.535	1.07	2.14	3.21	4.28	5.35
0.55	16-p-NO$_2$	5.90	4.55	3.07	2.05	1.92	1.66
	16-p-CHO	4.22	4.12	4.18	4.00	2.90	2.50
0.60	16-p-NO$_2$	20.9	21.1	15.6	9.10	7.00	6.07
	16-p-CHO	9.62	9.72	9.54	10.4	9.22	

Figure 4. Rate constant dependence on the solvent composition for the hydrolysis of 16-p-NO$_2$ and 16-p-CHO.

Figure 5. Rate constant dependence on initial substrate concentration of 16-p-NO$_2$ and 16-p-CHO in $\Phi = 0.55$ and $\Phi = 0.60$ Me$_2$SO–H$_2$O media. (a) $\Phi = 0.60$ 16-p-CHO; (b) $\Phi = 0.60$, 16-p-NO$_2$; (c) $\Phi = 0.55$, 16-p-CHO; (d) $\Phi = 0.55$, 16-p-NO$_2$.

> 0.45 > 0.36 > 0.30) decrease with increasing Φ.[25] This fact, therefore, together with eq 4, argues convincingly for the proposition that under certain circumstances the nature of the substituent effect may change with the nature of the medium, and for one particular mixed-solvent system the relative importance of electronic and hydrophobic contributions is a function of the solvent composition. All in all, we do not claim that the whole of our results so far discussed has proven the ideas speculated in points 1, 2, 3, and 6, but we do believe that together they form a consistent picture which might inspire or even provoke others toward further investigations.

Notably, for the $\Phi = 0.70$ system the treatment leading to eq 7 and a ρ value (1.18) similar to those of the octanoates includes 16-p-Ph and 16-p-t-Bu, which have to be excluded from the Hammett $\rho\sigma$ plot previously discussed. This fact lends support to our earlier proposition that it is justifiable to exclude 16-p-Ph and 16-p-t-Bu from the Hammett treatment (vide supra). As a matter of fact, we can make a separate treatment without the data on 16-p-Ph and 16-p-t-Bu. The results are tabulated in Table V. This treatment has the advantage that there is definitely no meaningful cross-correlation between σ and f ($r = 0.237$, $F(1,8) = 0.47$), whereas the previous treatment has an apparent cross-correlation ($r = 0.575$, $F(1,10) = 4.94$, CI $\sim 95\%$). In like manner, results similar to those summarized in Tables III and V can also be obtained without the data for the halogen substituted substrates. In this treatment there is also no cross-correlation between σ and f ($r = 0.159$, $F(1,7) = 0.18$). Clearly, the "cross-correlation" between σ and f of the first treatment (Table III) is a reflection of chance, or a consequence of the use of all

the substrates which happened to be obtainable.[26,27] In order to further verify the above judgment, we have made the following supplementary investigation.

As substrates, 16-p-CHO was pitted against 16-p-NO$_2$ because their "pecking-order" is reversed when they are transposed between the σ value and f value totem poles. The –CHO group is much

(25) The h/ρ ratios for 8-Y are smaller than 0.08.

(26) This rather poor cross-correlation (significance level, $\sim 5\%$) is not necessairly meaningful, e.g., as stated in a recent text (Shorter, J. "Correlation Analysis of Organic Reactivity"; John Wiley: New York, 1982; p 16): "... any apparent correlation at a significant level of 5% or worse should be regarded with suspicion. We should usually expect 1% or better".

(27) In addition to all the pertinent arguments already presented, there are other good reasons to believe that our results (eq 4–7) are not an outcome of fortuity. For instance: (1) Hydrophobic constants such as π and f are completely derived from partition data, and they are intrinsically different from Hammett cosntants. In fact, both strongly electron-attracting and electron-donating polar groups are highly hydrophilic. (2) Two "classes" of substrates differing in their chain lengths were compared, and each class was equipped with many substituent groups of different electronic properties. They behaved differently; the hydrolytic behavior of the shorter substrates was not dependent on the nature of the reaction medium, and that of the longer ones was. (3) Four solvent mixtures of different composition (Φ) were tested, and each system conformed to the same equation (eq 3). (4) The trend of the h/ρ ratios (2.29 > 0.45 > 0.36 > 0.30, Table III; 2.17 < 0.34 < 0.23 < 0.08, Table V) for the $\Phi = 0.55$, 0.60, 0.65, 0.70 media could hardly be fortuitous.

more hydrophilic ($f = -0.667$) than the ~NO_2 group ($f = -0.078$), whereas electronically the latter is more electron attracting. Thus if we obtain straight lines by plotting their log k's against the solvent composition Φ, we may expect a crossing of these lines somewhere in between the two limiting Φ values. This expectation is nicely borne out by Figure 4.

Finally, the rate dependence on initial substrate concentration of **16-p-NO₂** and **16-p-CHO** was studied (Table VI). The log k–log [S] plots are shown in Figure 5. The information provided

by these curves has been discussed previously.[17] Suffice it here to conclude on the basis of Figure 5 that the more hydrophobic **16-p-NO₂** has a greater tendency to aggregate. It seems amazing that differences in hydrophobicity of a small part of a molecule can manifest themselves so clearly sometimes.

Induction of Peptide Conformation at Apolar/Water Interfaces. 1. A Study with Model Peptides of Defined Hydrophobic Periodicity

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Abstract: To investigate the role of hydrophobic periodicity in the amino acid sequence of peptides and proteins in determining secondary structure at apolar/water interfaces, isocompositional peptides of leucine (L) and lysine (K) were synthesized. Peptides LKKLLKL (I), (LKKLLKL)₂ (II), and LKLKLKL (III) were prepared by solid-phase synthesis with a p-nitrobenzophenone oxime ester support. Peptides I and II have hydrophobic repeat periods matching that of an α-helix whereas peptide III has a repeat matching that of a β-sheet. Their conformations in aqueous solution were studied by circular dichroism spectroscopy. Peptide I appears too short to form α-helices as it gave spectra typical of an unordered conformation, while peptides II and III aggregated to form α-helical tetramers and β-sheets, respectively. To further evaluate the effect of apolar/water interfaces on conformation, properties of the peptides at the air/water interface were investigated. Peptides II and III formed much more stable monolayers than I due to their ability to form amphiphilic secondary structures. Transfer of monolayers to solid supports was accomplished by the Langmuir–Blodgett technique. Fourier transform attenuated total reflectance infrared spectroscopy was found to be a powerful technique for examining their conformations. The positions of the amide I and amide II bands observed for peptide II and peptide III were in close agreement with the established values for α-helix and β-sheet structures, respectively. Ultraviolet circular dichroism spectroscopy of the monolayers further confirmed their conformations. These results show that hydrophobic periodicity can determine the structure of peptides and perhaps other polymeric molecules at apolar/water interfaces, offering intriguing possibilities for molecular scale design of surfaces.

The hydrophobic effect[1] is a prime contributor to the folding and stabilization of protein structures.[2] Hydrophobic residues tend to cluster into the solvent-inaccessible interiors of globular proteins while hydrophilic residues tend to project outward and are more solvated.[3] α-Helices and antiparallel β-sheets often lie along the surface of proteins[4] and are amphiphilic; their solvent-exposed faces are more hydrophilic than their opposite faces which are in contact with the apolar interior of the protein.[5] Amphiphilicity is also important for the stabilization of the secondary structures of peptides and proteins which bind in aqueous solution to extrinsic apolar surfaces including phospholipid membranes,[6,7] air,[6,7] and the hydrophobic binding sites of the regulatory protein, calmodulin.[8] The amino acid sequences of such secondary structures show periodic distributions of hydrophobic and hydrophilic amino acids along the chain[9] with repeat periods corresponding to those of the appropriate structures (e.g., 3.6 for α-helices, 2.0–2.3 for β-sheets). Figure 1 illustrates this concept.

An interesting and important issue is the extent to which hydrophobic periodicity determines secondary structure. Short-range interactions are also known to be important factors in the formation of secondary structures; it has long been known that each amino acid has unique conformational preferences. This conclusion stems from Chou and Fasman's analysis of the different frequencies of occurrence of a given amino acid in specific conformations documented by X-ray crystallography of proteins,[10] studies with amino acid homo- or copolymers,[11] and model building.[12] To investigate the influence of hydrophobic periodicity independent from short-range interactions we synthesized a series of peptides (**1–3**) which contain leucyl and lysyl residues in identical ratios, but with different hydrophobic periodicities.

peptide **1**, FMOC (Leu-Lys-Lys-Leu-Leu-Lys-Leu)₁

peptide **2**, FMOC (Leu-Lys-Lys-Leu-Leu-Lys-Leu)₂

peptide **3**, FMOC (Leu-Lys-Leu-Lys-Leu-Lys-Leu)₁
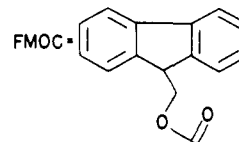
Chou–Fasman parameters[10] ($\langle P_\alpha \rangle = 1.19$, $\langle P_\beta \rangle = 1.06$) were, of course, identical for all three peptides, as were the Zimm–Bragg s values[11] for helix formation. These peptides differ only in their hydrophobic periodicities and chain lengths allowing the effect

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