Structure–Lipophilicity Relationships of Zwitterionic Amino Acids

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The lipophilicity of zwitterionic amino acids was determined by measuring their distribution coefficients in an octan-1-ol–buffer system at pH values near their isoelectric point (log D^{I}) using centrifugal partition chromatography (CPC). The observed differences in the lipophilic expression of methylene groups in the side-chain of α-amino acids are interpreted as a consequence of their different electronic structure and/or hydration features due to proximity effects exerted by the dipolar $^{+}H_3N-CH-CO_2^{-}$ moiety. A comparison of non-additive log D^{I} increments of methylene groups in a flexible alkyl and relatively rigid cycloalkyl side-chain of α-amino acids leads to the conclusion that the flexible alkyl side-chain is not fully accessible to solvents. The influence of intercharge distance on lipophilicity was examined with homologous piperidinyl carboxylic acids, indicating a decrease 0.4 log D^{I} units per increase of 1 Å between opposite charges. Among flexible non-α-amino acids, an increase in lipophilicity increment is observed only when the seventh carbon is added between the two opposite charges. As for the lipophilicity of *N*-alkylated zwitterions, *N*,*N*-dimethylglycine is shown to be less lipophilic than *N*-methylglycine, a finding of potential interest in the context of metabolic reactions of *N*-dealkylation. It is postulated that amino acids are transferred into lipidic phases in the form of hydrates, implying that the latter may have biological significance.

The relationships between molecular structure and its expressed physicochemical properties have been of long-standing interest to chemists and pharmacochemists.[1] Based on a large body of existing experimental data, the constitutive–additive (group contribution) approach has proven successful in a number of cases for deriving or deducing unknown properties from a known ensemble of atoms and their connectivity. More generally, this approach can sustain chemical intuition in terms of structure–property relationships. Examples such as hydration energy and lipophilicity are well documented in the literature.[2–5] However, significant deviations between predicted and experimental results do occur inasmuch as the number of atoms exceeds a limit, or the pattern of connectivity and non-bonded intramolecular interactions is unfamiliar to the database. Remedies have therefore been sought to narrow the gap between predicted and experimental values; in this context, intramolecular interactions tend to play critical and subtle roles.

Molecular lipophilicity, conventionally expressed as the partition coefficient of a solute in an octan-1-ol–water biphasic system (log *P*), has proven to be one of the most important properties of drugs as far as their pharmacokinetic and pharmacodynamic behaviours are concerned.[6–9] Efforts to rationalize and further to predict lipophilicity from chemical structure have been made in the last two decades. In these endeavours, Rekker's fragmental approach used multiples of a constant term generated by statistical analysis to characterize intramolecular interactions of polar groups, and to correct the calculated log *P* values.[2] The physical meaning of this constant term has, however, been left uncharacterized. The influence of proximity effects on lipophilicity due to the 'field' generated by polar groups has been difficult to explain and quantify. Also, '*ortho* effects' in aromatic rings were shown to express complex electronic and steric influences.[10] While recognizing that the partitioning process in an octan-1-ol–water system results from hydration/solvation processes, van de Waterbeemd and Testa proposed a hydration factor ω to quantify proximity effects and to relate the hydration/dehydration phenomena of solutes to the deviations resulting from the additive values.[11] One of the most anomalous proximity effects may lie in the lipophilicity of positively charged ammonium ions, in which the highly hydrophilic ionic nature of a N^{+} centre influences the lipophilicity of neighbouring methylene groups even as far as the eighth carbon atom.[2] Models explaining this anomaly remain unsatisfactory.

In the present study, we have attempted to explore the structure–lipophilicity relationships of dipolar amino acid zwitterions and their hydrational features. Indeed, unusual intramolecular interactions in zwitterions are expected to affect hydration/solvation features and hence lipophilicity. Recent studies by Manners *et al.*,[12,13] which indicated that zwitterionic metabolites of β-adrenergic antagonists produced by sulphate conjugation exhibited remarkably different lipophilicity depending upon the distance between the negatively charged sulphate moiety and the positively charged ammonium group, further suggested that accurately assessing the lipophilicity of zwitterionic species might uncover intramolecular interactions. To achieve this goal, we have used the recently developed centrifugal partition chromatography (CPC) technique to precisely and accurately determine partition coefficients in an octan-1-ol–water system. The many advantages of the CPC technique over other experimental methods for measuring partition coefficients have been described elsewhere.[14,15]

Previous measurements of distribution coefficients of several amino acids and derivatives performed at a fixed pH by Klein *et al.*[16] and Younger and Cramer[17] could not assure a maximum population of zwitterionic species due to the variation in their isoelectric points (pI). In the present study, the partition coefficient of zwitterionic species was derived from distribution coefficients (log *D* values) measured at three pH values close to the pI values of the amino acids.

Experimental

Materials.—The L-amino acids were purchased from Fluka (Buchs, Switzerland) or Sigma (St. Louis, Missouri, USA) and were of >99% purity. 2-Morpholinoethanesulphonic acid (MES, >99% purity) and 3-morpholinopropanesulphonic acid (MPS, >99% purity) were from Merck (Darmstadt, Germany), and octan-1-ol (purum, *ca.* 98%) from Fluka. All compounds were used without further treatment.

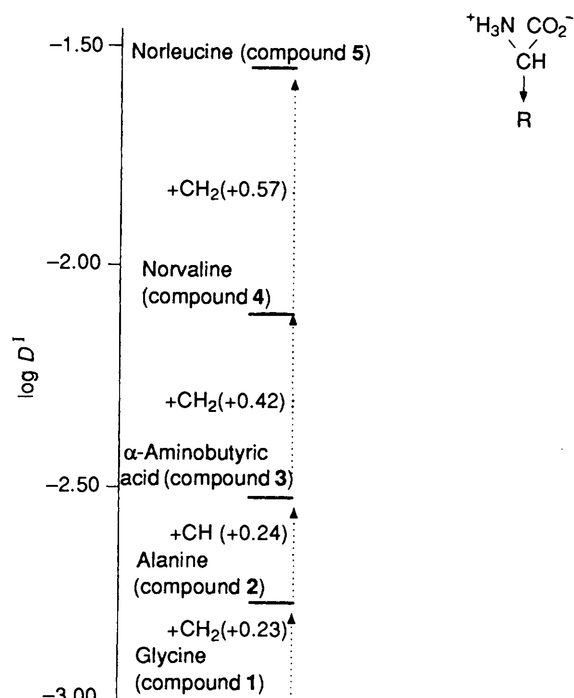

Fig. 1 Nonadditive log D^{I} increments of methylene groups in the flexible side-chain of amino acids

Measurement of Distribution Coefficient (*log* D).—Measurements of distribution coefficients in octan-1-ol–aqueous buffer systems were performed by horizontal flow-through centrifugal partition chromatography using a coil planet type centrifuge. The design principle of the instrument has been described.[18,19] The apparatus from Pharma-Tech Research Corp. (Baltimore, Maryland, USA) used three columns, each of which was helically wound with five layers of PTFE tubing (3.00 mm ID, 3.94 mm OD). The three columns made a total capacity of 350 cm^3. A Kontron model 420 HPLC pump was used to propel the mobile phase, and a Kontron model 432 UV–VIS detector coupled with a Hewlett-Packward 3392A integrator to detect the solutes. A Phase Separations flowmeter was used to measure flow rates precisely.

Measurements began by filling up the columns with octan-1-ol pre-saturated with 0.02 mol dm^{-3} MES or MPS buffer depending upon the pH used. While the columns were then revolving at a speed of 1000 rpm along the central axis, they were also rotating along their own axis in a mode of planetary motion. The mobile phase (aqueous phase) was then pumped into the columns in a 'head-to-tail' mode during the rotation. Depending upon expected distribution coefficients, the flow rate of the mobile phase was adjusted from 0.5 to 6.0 cm^3 min^{-1} (flow rates of 0.5 cm^3/min^{-1} for compounds with log $D < -2.3$; 1 cm^3/min^{-1} for those with log D between -2.3 and -1.3; and 6 cm^3/min^{-1} for those with log D between -1.3 and 0). A Merck injector was used to inject samples of 200 mm^3 (aqueous buffer, 1–50 mmol dm^{-3}). All amino acids were detected at the same wavelength (220 nm), and all measurements performed at 25 ± 0.1 °C and in triplicate. Concentration effects were negligible, the difference in calculated log D values being smaller than 0.05 units.

Under flow rates of 0.5, 1 and 6 cm^3/min^{-1}, *ca.* 310, 305 and 270 cm^3 of the stationary phase were retained, respectively. The retention time of the solvent front (t_0) was measured by non-retained solutes (potassium dichromate or lysine). It follows

$$\log D = \log \frac{(t_R - t_0)\cdot U}{V_t - U\cdot t_0} \qquad (1)$$

that the distribution coefficients can be calculated by eqn. (1) where t_R is the retention time of the solute, U is the flow rate of the mobile phase, and V_t is the total capacity of the three columns.

pK_a *Measurements.*—The pK_a values of some amino acids were determined by potentiometry at 25.0 ± 0.1 °C and an ionic strength of 0.1. The initial concentration of the compound investigated was 2 mmol dm^{-3}. The method and equipment used have been previously described.[20]

Results

For zwitterionic molecules, distribution coefficients form a broad plateau when measured at pH values near the isoelectric point. It was also observed by Akamatsu *et al.*[21] that the distribution coefficient of zwitterionic peptides tends to plateau over a few pH units and drop beyond this pH range due to the increased population of positively or negatively charged species. In this study, the distribution coefficients measured at two or three pH values showed little or no variation with pH (Table 1); the highest log D value, measured near the pI, was retained as log D^{I}, the partition coefficient of the zwitterion. There may be some uncertainty in this selection, but differences are negligible and cannot affect the conclusions. For example, in the case of compound **14** (β-alanine), the log D value measured at pH 7.4 is determined as log D^{I} value, the pH being 0.5 units away from the pI value (6.9). In view of the negligible averaged difference (0.02) between the log D values measured at pH 6.0 and 7.4, the 'true' log D^{I} value for β-alanine cannot be detectably different from that measured at pH 7.4.

Discussion

Abnormal log D$^{\text{I}}$ *Increments of Methylene Groups in Side Chains of Flexible Amino Acids.*—Methylene groups unperturbed by detectable electronic or conformational effects display lipophilic increments close to 0.6 in the log P (octanol) scale, *i.e.* 0.52 according to Rekker[2] and 0.66 according to Leo and Hansch.[3] Abnormal lipophilic increments of methylene groups are usually observed when they are in close proximity to polar moieties. This phenomenon is due, at least in part, to the electronic structure of methylene groups near polar entities differing from that of distal ones. The influence of proximity effects on lipophilicity, is, however, limited to a distance of three carbon atoms for neutral molecules.[2] The non-constant lipophilicity increments of methylene groups in the zwitterionic α-amino acids are shown in Fig. 1. Clearly, methylene groups proximal to the dipolar moieties are much less lipophilic than distal ones; only the ε-CH_2- has recovered a 'normal' lipophilicity (0.57). This phenomenon may well be due to differences in electronic structure and hence charge density.[2] However, hydration features must also be considered here since proximity effects have been postulated to be a consequence of hydration effects.[11]

Theoretical studies have confirmed the structuring of water molecules around solutes, leading to the hydration shell model.[24–29] Upon dissolution in water, polar groups capable of forming H-bonds display large favourable free energy changes as compared to alkanes (ΔG° for alkanols is *ca.* −3 to −7 kcal mol^{-1}, and 0–3 kcal mol^{-1} for alkanes),* charged groups such as -NH_3^+ and -CO_2^- being more 'tightly' or 'extensively' hydrated than neutral ones (ΔG° for protonated amines is *ca.* −50 to −70 kcal mol^{-1}, and −80 kcal mol^{-1} for carboxylate

* 1 cal = 4.184 J.

Table 1 Distribution coefficients in an octan-1-ol–water system, isoelectric points and dissociation constants of amino acids

Compound	Chemical structure	log D 5.0[a] (s.d.)	log D 6.0[b] (s.d)	log D 7.4[c] (s.d.)	log D^{I}[d]	pK_a 1[e] (s.d.)	pK_a 2[f] (s.d.)	pI[g]
1	H_3N^+ CO_2^-	−3.02 (0.01)	−3.00* (0.01)	−3.01 (0.01)	−3.00	2.34[h]	9.60[h]	5.97
2	H_3N^+ CO_2^-	−2.78 (0.00)	−2.77* (0.01)	−2.79 (0.01)	−2.77	2.34[h]	9.69[h]	6.02
3	H_3N^+ CO_2^-	−2.53 (0.01)	−2.53* (0.01)	−2.54 (0.02)	−2.53	2.55[h]	9.60	6.08
4	H_3N^+ CO_2^-	−2.20 (0.01)	−2.11* (0.00)	−2.11 (0.00)	−2.11	2.30[h]	9.79[h]	6.04
5	H_3N^+ CO_2^-	−1.57 (0.00)	−1.54* (0.01)	−1.55 (0.00)	−1.54	2.39[h]	9.76[h]	6.08
6	H_3N^+ CO_2^-	—	−2.78* (0.01)	−2.78 (0.01)	−2.78	2.73 (0.01)	8.45 (0.01)	5.59
7	H_3N^+ CO_2^-	—	−2.28* (0.01)	−2.28 (0.01)	−2.28	2.62 (0.01)	10.01 0.01)	6.32
8	H_3N^+ CO_2^-	—	−1.87* (0.01)	−1.87 (0.01)	−1.87	2.62[h]	10.03[h]	6.33
9	$H_2\overset{+}{N}$ CO_2^-	—	−2.84* (0.01)	−2.84 (0.01)	−2.84	2.01 (0.01)	10.21 (0.01)	6.11
10	$H_2\overset{+}{N}$ CO_2^-	−2.64 (0.00)	−2.62* (0.01)	−2.65 (0.00)	−2.62	1.99[h]	10.60[h]	6.30
11	H_2 ^+N CO_2^-	—	−2.31* (0.01)	−2.38 (0.01)	−2.31	2.36 (0.01)	10.33 (0.01)	6.35
12	H_2 ^+N CO_2^-	−2.96 (0.01)	−2.89* (0.01)	−2.91 (0.01)	−2.89	3.45 (0.01)	10.20 (0.01)	6.83
13	H_2 ^+N CO_2^-	—	−3.10 (0.01)	−3.05* (0.01)	−3.05	3.86 (0.01)	10.33 (0.01)	7.10
14	H_3N^+ CO_2^-	−3.12 (0.01)	−3.11 (0.01)	−3.05* (0.02)	−3.05	3.60[h]	10.19[h]	6.90
15	H_3N^+ CO_2^-	−3.34 (0.02)	−3.10 (0.01)	−3.09* (0.01)	−3.09	4.23[h]	10.43[h]	7.33
16	H_3N^+ CO_2^-	−3.36 (0.02)	−3.10 (0.01)	−3.10* (0.01)	−3.10	4.37[h]	10.85[h]	7.61

Table 1 *continued*

Compound	Chemical structure	log D 5.0[a] (s.d.)	log D 6.0[b] (s.d)	log D 7.4[c] (s.d.)	log D^{1}[d]	pK_a 1[e] (s.d.)	pK_a 2[f] (s.d.)	pI[g]
17	H_3N^+(CH₂)₇CO_2^-	—	−3.02 (0.01)	−3.03* (0.02)	−3.03	4.52 (0.01)	10.50 (0.01)	7.51
18	H_3N^+(CH₂)₈CO_2^-	—	−2.54 (0.02)	−2.55* (0.01)	−2.55	4.59 (0.01)	10.46 (0.01)	7.53
19	H_2N^+ CO_2^-	—	−2.78* (0.01)	−2.79 (0.01)	−2.78	2.23[g]	10.01[g]	6.12
20	HN^+ CO_2^-	—	−2.91* (0.01)	−2.94 (0.01)	−2.91	2.04[g]	10.47[g]	6.26
21	NH^+ CO_2^-	—	−2.45* (0.01)	−2.50 (0.02)	−2.45	3.58 (0.01)	9.92 (0.01)	6.77

[a] Distribution coefficients measured at pH 5.0. [b] Distribution coefficients measured at pH 6.0. [c] Distribution coefficients measured at pH 7.4. [d] The log D^1 value is determined as the highest log D value, marked with *. [e] Dissociation constants of the carboxylic group. [f] Dissociation constants of the amino group. [g] The isoelectric points are taken as the arithmetic mean of pK_a 1 and pK_a 2 (ref. 22). [h] Data are taken from ref. 23.

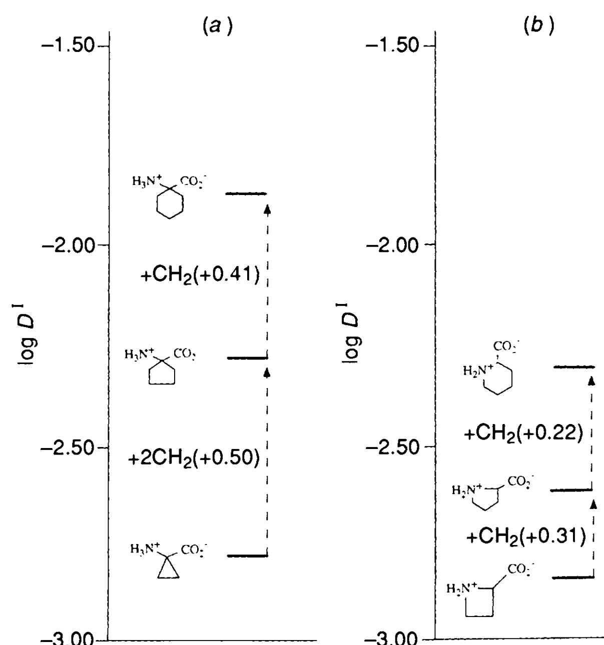

Fig. 2 Nonadditive log D^1 increments of methylene groups in the side-chains of relatively rigid amino acids

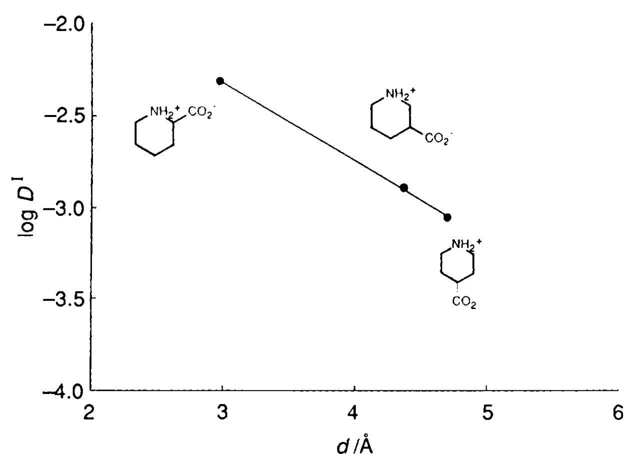

Fig. 3 Intercharge-distance-dependent lipophilicity of relatively rigid amino acids

ions).[30] It is then plausible that the structuring of water molecules around a dipolar moiety is so strong that it extends as far as the ε-CH_2 group. The effects exerted by neutral polar groups are usually within the domain of three carbon atoms,[2] while protonated amino moieties tend to have an influence up to eight carbon atoms. The extent of proximity effects exerted by the dipolar moieties of zwitterionic α-amino acids appears to lie between those of neutral and charged groups. The consequence of different hydration features of alkyl side chains is also manifested in the internal rotational motion of each methylene group. As evidenced from ^{13}C spin–lattice relaxation time (T_1) of norleucine measured by NMR spectroscopy, the rate of internal rotational motion of methylene groups increases with their distance from the dipolar moieties.[31]

log D[1] *Increments of Methylene Groups Relatively Rigid in Cycloalkyl Residues of α-Amino Acids.*—Due to the flexibility of aliphatic side-chains, the non-bonded distance between each methylene or methyl group and the dipolar moiety can only be an average one. Relatively rigid aliphatic side-chains should further corroborate or offer additional information on distance-dependent through-space and through-bond proximity effects.

A comparison of log D^1 values of alanine and 1-aminocyclopropanecarboxylic acid (compound **6**) reveals that the latter (log D^1 = −2.78) with one additional methylene group is not more lipophilic than the former (log D^1 = −2.77). It is likely that the rigidity of the cycloalkyl side-chain reduces its lipophilicity since it is known that conformational entropy should be gained when transferring a flexible molecule from an ordered liquid, water, to an organic medium.[32] Another interpretation for this phenomenon is that the unusual delocalization of σ electrons in the cyclopropane ring[33,34] increases the polarizability of the molecule. The σ aromaticity in the cyclopropane ring is also reflected in the reduced basicity of the amino group (pK_a = 8.45, shown in Table 1) as compared with the same group attached to five- or six-membered rings (pK_a *ca.* 10).

As shown in Fig. 2(*a*), the log D^1 increment of each additional γ-CH_2- group in 1-aminocyclopentanecarboxylic acid (compound **7**) when compared to 1-aminocyclopropanecarboxylic acid is 0.25, which is close to that of a γ-CH_2- group in α-aminobutyric acid (0.24). The difference in log D^1 values between compounds **7** and **8** assigns an increment of 0.41 to a δ-CH_2-group, which again is close to that of a δ-CH_2-group in a flexible alkyl side-chain (0.42, shown in Fig. 1).

Fig. 2(*b*) illustrates the log D^1 increment of methylene groups in relatively rigid heterocyclic structures. It is shown that the log D^1 increment of a γ-CH_2- group in compound **10** (proline, 0.22) is close to that in α-aminobutyric acid (0.24). Further addition of a methylene group into the five-membered ring (compound **11**), however, yields an increment of 0.31.

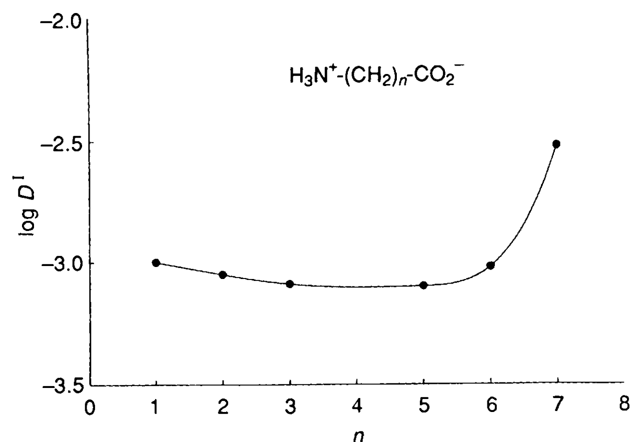

Fig. 4 Intercharge-distance-dependent lipophilicity of flexible amino acids

The results from the two series of relatively rigid analogues suggest that the flexible side-chains are not in a fully self-coiled conformation when partitioning into the octan-1-ol phase, but are partially exposed to the solvent.

Intercharge Distance-dependent Lipophilicity of Relatively Rigid Amino Acids with Distal NH_3^+ *and* CO_2^- *Moieties.*—Before examining the effects of intercharge distance on the lipophilicity of homologous amino acids, it is appropriate to study conformationally defined positional isomers of piperidinyl carboxylic acids, *i.e.* compound **11**, nipecotic acid (compound **12**, a potent neuronal GABA uptake inhibitor[35]) and isonipecotic acid (compound **13**, an agonist of the GABA receptor[36]). In Fig. 3 a plot of log D^1 against the intercharge distance as obtained from crystallography data[37,38] reveals that increasing the intercharge distance decreases the log D^1 values with a slope of *ca.* 0.4 units $Å^{-1}$. The consequence is significant in terms of structure–lipophilicity relationships and prediction of the fate of zwitterionic metabolites.

Intercharge Distance-dependent Lipophilicity of Flexible Amino Acids with Distal NH_3^+ *and* CO_2^- *Moieties.*—In a study of distribution coefficients of propranolol and its metabolites, Manners *et al.*[13] discovered that positional isomers of sulphate conjugates can have distribution coefficients differing by as much as 2.9 log *D* units depending upon the site of conjugation in the molecule. This study clearly demonstrated that lipophilicity decreases with increasing distance between opposite charges in the molecule, the consequence being of marked relevance to the fate of the conjugated metabolites. It is therefore of interest to examine systematically how the distance between opposite charges may influence lipophilicity in homologous amino acids. Compounds under investigation include amino acids with one to seven carbon atoms between the two charged groups. Fig. 4 shows unambiguously that their log D^1 values (*ca.* −3) differ only minimally when the number of methylene groups between the two charged moieties varies from one to six. Only the seventh methylene group shows a 'normal' increment of 0.50.

The results can be interpreted in terms of two opposite influences on lipophilicity, namely its increase with increasing number of methylene groups, and its decrease with increasing distance between the two ionic groups, as demonstrated above (Fig. 3). It appears that the two effects more or less compensate each other for a distance of one to six carbon atoms. Through-bond proximity effects (Fig. 1 and 2) and through-space effects (reflecting self-coiling and other conformational factors[39]) must be operative.

While no quantitative assessment of these effects is possible at present, the relatively modest decrement in log *D* with increasing distance (Fig. 3) cannot alone explain the constant log *D* of compounds **1**, **14**–**17**. Self-coiling must thus be hypothesized as a factor, partly shielding methylene groups from the solvent and preventing them from expressing their hydrophobic increment. By means of a NMR chemical shift method, Chevalier and Le Perchec studied the mean interchange distance of flexible zwitterionic (trimethylammonio)-alkanoates in aqueous and methanolic solutions, and showed that coiling is more pronounced in aqueous solutions as the number of methylene groups increases, probably due to electrostatic interactions between the two charged moieties and hydrophobic coiling of the lipophilic arm.[40]

A distance of as much as seven CH_2 groups (compound **18**) is necessary for one CH_2 group to express its hydrophobic increment, *i.e.* the one separated by three carbons from each of the ionic groups. This is in excellent agreement with the results of compounds **1**–**5**, where only the δ- and ε-CH_2 groups can more or less completely express their hydrophobic increment.

Influence of N-*Methylation on the Lipophilicity of Amino Acids.*—Molecules containing ammonium moieties often exhibit anomalous lipophilicity effects due to *N*-alkylation.[2] For glycine (compound **1**) and its analogues with secondary and tertiary amines (compound **19** and **20**), *N*-monomethylation produces an increment of 0.22 log *D* units, whereas *N*,*N*-dimethylglycine has a decreased log D^1 value as compared with *N*-methylglycine despite its additional methyl group. Instead of being localised on either *N*-atom or amino protons the positive charge is distributed over all atoms of the ammonium moiety.[41] It is thus likely that *N*-methylation may increase the polar surface area due to the diffused charge, and hence decrease lipophilicity as proposed by Rekker.[2] In the case of zwitterionic amino acids, the situation is further complicated by the presence of the carboxylate moiety. It has been pointed out that increasing bulk of the amine moiety may prevent its complete engulfment by folding,[40] and hence allow its hydrophilic increment to be better expressed. In any case, the interchange distance in *N*-methylated amino acids is made uncertain by delocalization rendering very difficult any understanding of the increments associated with compound **21**.

As *N*-dealkylation and *N*-methylation are important reactions in xenobiotic metabolism, their influence on the lipophilicity of metabolites may affect both pharmacokinetic and pharmacodynamic behaviour and should be better understood.

Conclusions

It has been estimated by Harvey[42] that moving an atom carrying a positive or negative charge from water to media of low dielectric constant (ε in the range 2–4) would cost 25–50 kcal mol^{-1} of free energy. By analogy, it would take *ca.* 10 kcal mol^{-1} to move it from water to water-saturated octan-1-ol (dielectric constant = 8.58[43]), namely a decrease of *ca.* 5 log *D* units at ambient temperature. Testa and Murset-Rossetti[44] measured the partition coefficients of protonated (log P_+) and neutral (log *P*) antihistamines, the average difference (log P_+ − log *P*) being *ca.* −3.5. Zwitterionic α-amino acids are shown here to be far less hydrophilic than deduced from the presence of two charged groups. Intramolecular charge compensation and overlap of hydration spheres may be evoked as partial explanations, but it is also conceivable that the solutes are transferred into octanol as particularly stable hydrates. Abraham and Leo have speculated that polar residues may be transferred as hydrates into octanol,[45] and studies from this laboratory have demonstrated a 'water-dragging effect' when polar solutes partition into an organic phase.[46]

As for the zwitterionic non-α-amino acids, conformational behaviour becomes a major factor influencing lipophilicity. Completely folded conformations resulting from an internal ionic bond are seldom the preferred ones, but here again hydrates, and particularly water bridges of the type $-CO_2^- \cdots H_2O \cdots {}^+HN<$, can be hypothesized to exist and decrease the energy of dehydration associated with transfer into octanol. Work is in progress in our laboratory to investigate these hydrational features.

Based on highly refined atomic structures of the complexes of L-arabinose binding protein with L-arabinose, D-fucose, and D-galactose. Quiocho *et al.*[47] found that bound water molecules can govern substrate specificity and affinity. Given the fact that some amino acids such as glycine, β-alanine, and GABA play important physiological roles as neurotransmitters, one is led to speculate whether a hydrate can be the functional form binding to their receptors in a microenvironment of low polarity.

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