# Zwitterions Can Be Predominant in Membrane Penetration of Drugs: Experimental Proof

Károly Mazák* and Béla Noszál

Research Group of Drugs of Abuse and Doping Agents, Department of Pharmaceutical Chemistry, Semmelweis University, Hungarian Academy of Sciences, Högyes E. u. 9, H-1092 Budapest, Hungary

ABSTRACT: The complete set of species-specific partition coefficients was determined, and the predominant contribution of zwitterionic species to the overall lipophilicity was experimentally proven for the first time. The compounds studied were the amphoteric eburnane alkaloid *cis-* and *trans-*apovincaminic acids of therapeutic interest. Partition of the individual microspecies was mimicked by model compounds of the closest possible similarity, and then correction factors were determined and introduced. The noncharged microspecies of the *cis*-epimer is 30 900 times as lipophilic as its zwitterionic protonation isomer, while the analogous ratio for the *trans*-epimer is around 15 800. Due to the overwhelming dominance of the zwitterionic form, however, its contribution to the overall lipophilicity exceeds 8 and 5 times that of the noncharged form for the two epimers, respectively. The lipophilicity profile of these zwitterionic compounds is expressed, calculated, and depicted in terms of species-specific lipophilicities over the entire pH range.
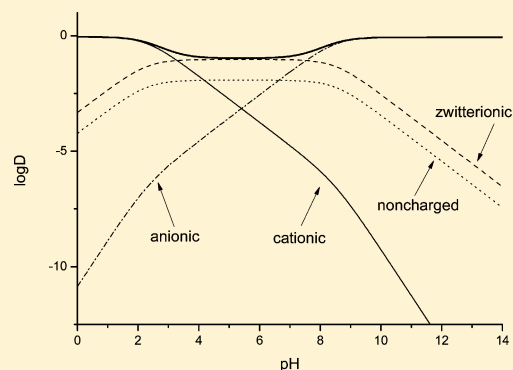
1. INTRODUCTION

Lipophilicity is a molecular property of immense importance in pharmacy, biochemistry, and medicinal chemistry. Its applications include such apparently diverse fields as drug design for targeted delivery and development of chromatographic separations. The ability of drugs to diffuse passively through biological membranes has long been known to be largely influenced by their lipophilicity.[1] The pH−partition hypothesis postulates that absorption of ionizable drugs mainly takes place in compartment(s) where the local pH ensures the maximum concentration of the noncharged form relative to the ionized form(s).[2] In addition, lipophilicity is a tool to unravel biologically relevant intramolecular interactions and intermolecular forces of recognition.[3,4]

To quantitate lipophilicity, the commonly accepted parameter is log *P*, the logarithm of the partition coefficient. It is the concentration ratio of a solute in a single electrical state, being in equilibrium between two immiscible solvents. Octanol is the most often used organic solvent, and the octanol−water partition coefficient is the prime descriptor of lipophilicity in quantitative structure−activity relationship (QSAR) studies.[5] When more than one electrical species are present in solution, the observed ratio of concentrations is the distribution coefficient (*D*), which takes into account the intrinsic lipophilicity of the various electrical species present (p_i) and their mole fractions in the aqueous phase (x_i):

$$D = \sum (x_i p_i)$$

(1)

The variation of log *D* as a function of the aqueous pH is the lipophilicity profile. It is a sine qua non condition to understand the pharmacokinetic, toxicokinetic, and even pharmacodynamic properties.[6]

For a long time the lipophilicity of ionizable drugs and solutes has been underrepresented in the literature, due mainly to the lack of reliable methods to determine the partition coefficients of the ionic forms. This is especially true for ionization/protonation isomers, such as the zwitterionic and noncharged forms of amphoteric compounds.

Earlier attempts at the quantitation of microspecies-specific partition coefficients were usually restricted to some of the partitioning microspecies, due to the apparently unsurmountable difficulties in determining all the microscopic partition coefficients.[7,8] Abraham et al.[9] developed an equation to predict the blood−brain distribution coefficient of a drug based on the microscopic partition coefficient of the noncharged species, its hydrogen-bond acidity, and its hydrogen-bond basicity. These models assumed, however, that only the noncharged species partitions into the organic phase, although the contribution of charged species to the distribution coefficient may not be insignificant in a number of cases.[6,10−12]

To gain insight into the partition microequilibria of amphoteric drugs at the species-specific level, we have recently elaborated a method and studied two systems.[13,14] The partition properties of the compound in question and its microspecies-mimicking synthetic derivatives were investigated and exemplified on niflumic acid, a highly lipophilic nonsteroidal anti-inflammatory drug. We reported, for the first time for any compound, experimental microscopic partition

coefficients for the two protonation isomers.[13] Subsequently, we reported the complete set of experimental microscopic partition coefficients of morphine, the best known opiate alkaloid. The lipophilicity profile of morphine was expressed, calculated, and depicted in terms of species-specific lipophilicities over the entire pH range.[14]

As expected, the noncharged form was much more lipophilic than its zwitterionic protonation isomer for both compounds. In addition, for morphine there are approximately 3 times as many noncharged microspecies as zwitterionic microspecies, irrespective of the pH, ensuring that the contribution of the noncharged form to the overall lipophilicity is indeed the dominant factor. Niflumic acid is a dominantly zwitterionic compound, having 16 times as many zwitterionic as noncharged microspecies in aqueous solution. Nevertheless, because of the orders of magnitude larger lipophilicity of the noncharged form, its contribution to the overall lipophilicity is around 25 times more important than that of the zwitterionic protonation isomer.

Concerning the hypothetic zwitterionic predominance in the lipophilicity profile and the concomitant zwitterionic superiority in membrane penetration of any drugs, Pagliara et al.[6] described this possibility, but to the best of our knowledge, the complete set of such experimental microscopic partition coefficients has not so far appeared.

Here we report the first case of zwitterionic dominance in the lipophilicity profile. Our compounds of choice were *cis-* and *trans-*apovincaminic acid. Vinpocetine (the ethyl ester of *cis-*apovincaminic acid) and related eburnane derivatives are valuable therapeutic agents mainly in cardiovascular and cerebral insufficiencies.[15] The pharmacological activity is associated with the fused, five-membered eburnane ring system (Figure 1). The *cis-* and *trans-*derivatives in Figure 1 are epimers, diastereomers differing only in the configuration of the C(3) carbon.

(3S,16S) ethyl *cis*-apovincaminate (1)
(vinpocetine)

(3R,16S) ethyl *trans*-apovincaminate (2)

(3S,16S) *cis*-apovincaminic acid (3)

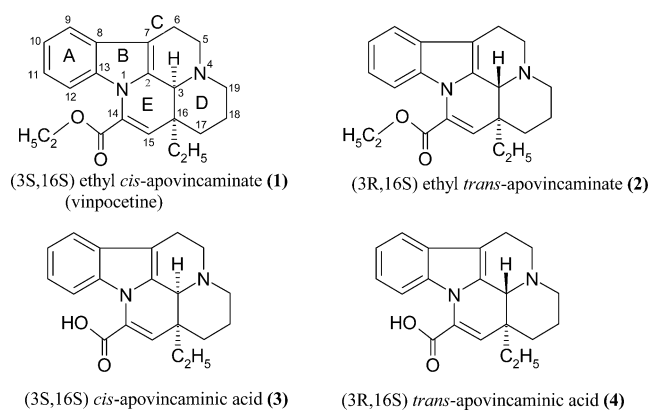(3R,16S) *trans*-apovincaminic acid (4)

Figure 1. Structures of the investigated eburnane derivatives.

Compound **1** has become a reference in the pharmacological research of cognitive deficits in the central nervous system caused by hypoxia and ischemia.[16] The medicinal success of **1** and related compounds has inspired continual synthetic efforts to produce further derivatives of *cis*-D/E ring anellation, i.e., the 3α-H,16α-ethyl (3S,16S) configuration, and those of *trans*-D/E ring anellation.[17,18]

Despite the decades-long therapeutic use of eburnanes, and the well-known pharmacokinetic significance of lipophilicity and basicity, scarcely any physicochemical data appeared on

these compounds. The obvious reason is the typically poor water solubility of eburnanes arising from the rigid ring system and the relatively small number of polar groups. In our proton speciation study of the above compounds, the aqueous acid−base properties have been elucidated indirectly by the extrapolation of protonation constants in methanol−water mixtures of gradually changing solvent composition.[19] The lipophilicity of their noncharged form was quantified by a reversed-phase thin-layer chromatographic (RP-TLC) method, verified by stir-flask studies.[20]

In the present study the partition microequilibrium analysis of compounds **3** and **4** was quantified, the inherent species-specific partition coefficients were determined, and the contribution of all four microspecies to the lipophilicity profile was calculated, interpreted, and transformed into diagrams.

2. RESULTS

2.1. Acid−Base Equilibria. Compounds **3** and **4** contain one acidic and one basic site each; thus, they exist in solutions in four microscopic protonation forms (microspecies), the cationic, zwitterionic, noncharged, and anionic forms.[21,22]

The protonation scheme of compound **3** is depicted in Figure 2. k^N, k^O, k_N^O, and k_O^N are the microconstants. Indices O and N designate oxygen and nitrogen atoms of the basic carboxylate and tertiary amino sites, respectively. Superscripts of the microscopic protonation constants indicate the group protonating in the given microequilibrium protonation process, whereas the subscript (if any) stands for the group holding the proton during the process.

The aqueous protonation constants of the four investigated compounds from our earlier work[19] can be seen in Table 1. Compounds **3** and **4** were soluble in water, and their macroconstants could directly be determined. The two esters had poor water solubility at alkaline pH, so their macroconstants were obtained by the Yasuda−Shedlovsky extrapolation procedure from protonation constants in methanol−water mixtures.

For the determination of microconstants we chose the deductive method, which requires an appropriate model compound to mimic the minor species.[22] The minor protonation pathway is the one containing the noncharged microspecies as opposed to the route containing the zwitterion. Conditions of the accurate calculation of microconstants are better if a microconstant related to the minor protonation pathway is determined.[22] Electronic (withdrawing or donating) effects of the carboxyl and ester groups on the rest of the molecule are highly similar; thus, k_O^N of compound **3** or **4** and K_1 of the respective apovincaminic acid ester are also practically equal. This enabled us to calculate the values of all the other microconstants.[19] The microscopic constants determined for these molecules can be seen in Table 1.

2.2. Lipophilicity. The distribution coefficient for compounds **3** and **4** can be expressed as follows:

$$D_{pH} = x_{Ani}p^{Ani} + x_{Non}p^{Non} + x_{Zwi}p^{Zwi} + x_{Cat}p^{Cat} \qquad (2)$$

where x_i stands for the aqueous mole fractions of the anionic (Ani), noncharged (Non), zwitterionic (Zwi), and cationic (Cat) species. The aqueous mole fractions include protonation microconstants and acidity values, expressing thus the pH dependence of the D distribution coefficient. For example
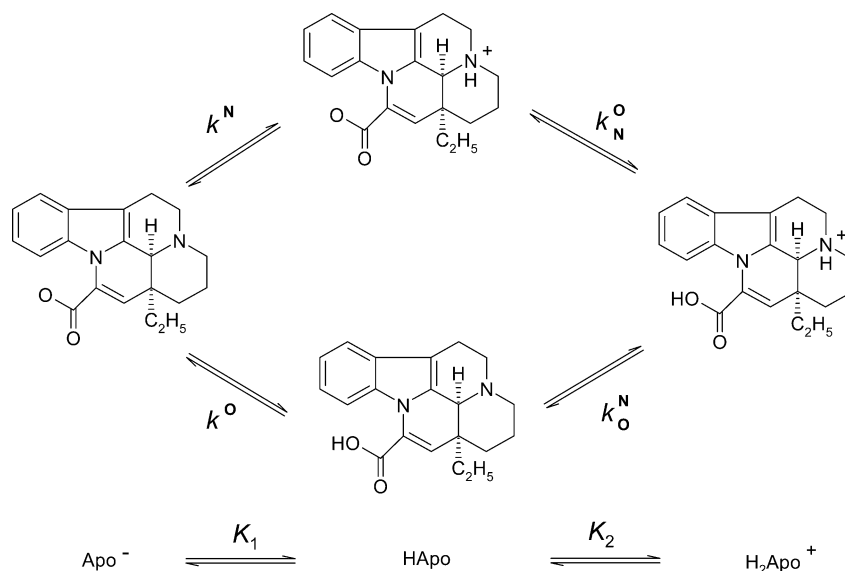
Figure 2. Protonation equilibria of compound **3** in terms of macroscopic (K_1, K_2) and microscopic (k^N, k^O, k_N^O, k_O^N) protonation constants.

Table 1. Protonation Macro- and Microconstants of the Four Investigated Compounds[19]

compd	$\log K_1$	$\log K_2$	$\log k^N$	$\log k^O$	$\log k_O^N$	$\log k_N^O$
3	8.49	2.30	8.49	3.10	7.69	2.30
4	7.72	2.19	7.72	2.84	7.07	2.19
1	7.69					
2	7.07					

$$x_{Ani} = \frac{1}{1 + (k^N + k^O)[H^+] + k^N k_N^O [H^+]^2} \quad (3)$$

$$x_{Zwi} = \frac{k^N[H^+]}{1 + (k^N + k^O)[H^+] + k^N k_N^O [H^+]^2} \quad (4)$$

The knowledge of each microscopic partition coefficient is especially important for a thorough understanding of the pharmacokinetic and pharmacodynamic properties of drugs acting in the central nervous system, a particularly lipophilic medium. Furthermore, the p^{Non}/p^{Zwi} ratio of the noncharged vs zwitterionic partition coefficients is an important structural parameter to gain insight into intramolecular effects.[6,23]

To obtain species-specific $\log p$ values for all four microspecies, we applied our recently developed method to determine the microscopic partition coefficients.[13,14] We used the ethyl ester derivatives of apovincaminic acids to mimic the noncharged form and then determined and introduced correction factors to minimize effects of chemical derivatization. The effect of ethylation in both model compounds was taken into account by comparing their lipophilicity to that of their parent acid in their uniformly cationic ionization state, which exists overwhelmingly in sufficiently acidic solutions.

We chose a standardized HCl solution of 0.15 M (pH 0.82), as the majority of $\log K$ and $\log P$ values are determined at this physiological ionic strength. The results are listed in Table 2, including results obtained with a standardized NaOH solution of 0.15 M (pH 13.18). Table 2 also lists $\log D$ values measured at the isoelectric point (pI) of the apovincaminic acids. For compound **3**, this is pH 5.40, and for compound **4**, it is pH 4.96.

Table 2. log D Values of the Investigated Compounds in the Octanol/Water System[a]

compd	$\log D$ at pH 0.82	$\log D$ at pI	$\log D$ at pH 13.18
3	−0.05 (0.03)	−0.97 (0.03)	−0.07 (0.01)
4	0.13 (0.01)	−0.14 (0.01)	0.21 (0.01)
1	0.94 (0.03)		
2	0.90 (0.02)		

[a]Standard deviations are given in parentheses.

In our previous study[20] we already determined the $\log P$ for the neutral form of the two esters. Due to their low water solubility, the partition coefficients of the neutral forms of these esters were calculated from distribution coefficients in various acidic solutions and resulted in $\log P$ values of 4.45 (0.01) and 4.75 (0.02) for compounds **1** and **2**, respectively.

Due to the predominance of the cation at low pH, the reported $\log D$ values in Table 2 characterize overwhelmingly the lipophilicity of the cationic species at the physiological ionic strength. For the same reason, the $\log D$ values in the highly alkaline solution characterize the lipophilicity of the anionic form of apovincaminic acids. On the basis of our data in Table 2, the lipophilicity of the cationic form of the apovincaminic acids is lower than that of their ethyl esters; the differences are 0.99 and 0.77 in $\log D$ units for the *cis*- and *trans*-epimers, respectively. These differences are valid only for the physiological ionic strength, as the lipophilicity of ionic species depends on the type and concentration of the background electrolyte.[11,24−27]

It is plausible that the ethylation-derived differences in the lipophilicity of the cationic forms will be very close to the differences between the noncharged forms of the acids and the esters. These differences can serve as appropriate correction factors.

Using the experimentally determined partition coefficients of the model compounds, and taking into account the correction factors, we calculated the logarithm of the microscopic partition coefficients ($\log p$) of the noncharged forms of the apovincaminic acids. For compound **3** $\log p^{Non} = 4.45(\log P$ of the noncharged form of its ethyl ester) − 0.99(correction factor) = 3.46, whereas for compound **4** $\log p^{Non} = 4.75(\log P$

of the noncharged form of its ethyl ester) − 0.77(correction factor) = 3.98.

To determine p^{Zwi}, eq 2 has to be rearranged as follows:

$$p^{Zwi} = (D_{pH} - x_{Ani}p^{Ani} - x_{Non}p^{Non} - x_{Cat}p^{Cat})/x_{Zwi} \tag{5}$$

Thus, in the knowledge of distribution coefficients near the isolectric point, and the lipophilicity contribution of the three other microspecies, p^{Zwi} can be calculated.

Table 3 shows all the experimentally determined microscopic partition coefficients of the examined compounds.

Table 3. Logarithm of Microscopic Partition Coefficients of the Examined Compounds in the Octanol/Water System at 0.15 M Ionic Strength

compd	log p^{Non}	log p^{Zwi}	log p^{Cat}	log p^{Ani}
3	3.46	−1.03	−0.05	−0.07
4	3.98	−0.22	0.13	0.21
1	4.45		0.94	
2	4.75		0.90	

3. DISCUSSION AND CONCLUSIONS

3.1. Acid−Base Equilibria. The microconstants in Table 1 show that, owing to the significantly different inherent basicities of the amino and carboxylate protonation sites, the k^N and k_N^O microconstants indicate the major protonation pathway, and they are practically equal to the K_1 and K_2 macroconstants, respectively. The K_1 macroconstant (representing mainly the amino group protonation) of molecules with *cis*-D/E ring anellation is always higher than that of its *trans*-D/E ring anellation epimeric counterpart. This difference is 0.77 in the case of the two apovincaminic acids, while it is 0.62 for their ethyl ester derivatives.

In these molecules the orientation of the N(4) lone electron pair is the same as that of the C(16) ethyl group. The barrier to nitrogen inversion at N(4) is high enough to preclude convex−concave pyramidal interconversions. The anellation-dependent different rotation of the C(16)-ethyl group contributes to this significantly different basicity. Molecular modeling showed[20] that the ethyl group in the *trans*-isomer can approach much more closely the basic N(4) nitrogen than in the *cis*-isomer. Thus, it can repel the hydrated proton, so that protonation of the amino group of the *trans*-derivative occurs at higher bulk hydrogen ion concentration (lower pH).

Some earlier, related observations showed significant differences between the *cis*- and *trans*-isomers by other techniques as well. It was reported[17] that 3,16-*trans*- and 3,16-*cis*-compounds of the eburnane skeleton exhibit characteristic differences in their ^1H and ^{13}C NMR spectra and undergo different fragmentations, as shown by characteristic mass spectral differences.[28]

The difference in N-basicity of apovincaminic acids and their ester derivatives can readily be interpreted. In the pH range of the amino protonation, the carboxyl groups of the acids are predominantly deprotonated; i.e., they are negatively charged. Hence, they do not have a strong electron-withdrawing effect, unlike the uncharged ester groups in the respective ester derivatives.

Irrespective of the pH of the solution, there are 245 000 and 75 900 times as many zwitterionic microspecies of compounds **3** and **4**, respectively, as noncharged microspecies. In the pH range of 2.30−8.49 compound **3** mainly exists in the zwitterionic form, whereas for compound **4** this pH range is 2.20−7.71.

3.2. Lipophilicity. The noncharged forms of the *trans*-epimers of apovincaminic acid and its ethyl ester are more lipophilic than those with *cis*-anellation. There are two major factors that contribute to the higher lipophilicity of the *trans*-epimers. Molecular mechanics calculations and NMR experiments show that ring D in *cis*-epimers occupies a perpendicular position to the plane of the ring system, whereas it is parallel in the *trans*-epimers (Figure 3). Thus, the smaller hydrophobic
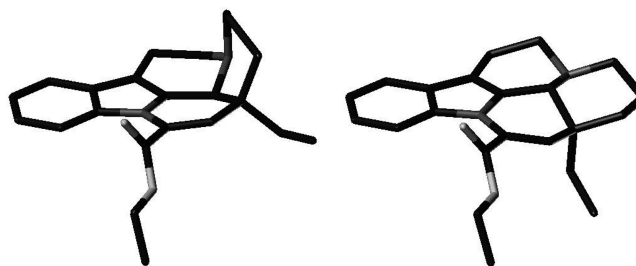

Figure 3. Three-dimensional models of compounds **1** (left) and **2** (right). Reprinted with permission from ref 20 (license number 2958581018889) (Copyright 2003, Elsevier).

surface area of *cis*-epimers makes them less lipophilic. Molecular mechanics calculations also show that the orientation of the C(16) ethyl group is dependent on the D/E ring anellation. Namely, during rotation the ethyl group in the *trans*-epimers can approach more closely the basic N(4) nitrogen than in the case of the *cis*-epimers. Thus, the ethyl group can hamper the water accessibility of N(4) to a greater extent and renders the *trans*-epimers less hydrophilic.[20]

The log(p^{Non}/p^{Zwi}) value is 4.49 for compound **3**, whereas this value is 4.20 for compound **4**. Thus, the noncharged microspecies of compound **3** is 30 900 times as lipophilic as its zwitterionic protonation isomer, whereas for compound **4** this ratio is around 15 800. These large log(p^{Non}/p^{Zwi}) values can be explained by the weakness of intramolecular effects that would enhance the lipophilicity of the zwitterionic microspecies.[6] The main reason for this is the rigid pentacyclic skeleton that excludes the formation of internal ionic bonds by conformational changes.

Using the mole fractions and microscopic partition coefficients of the noncharged and zwitterionic species, their pH-independent contribution ratio to the distribution coefficient can also be quantified. This $x_{Zwi}p^{Zwi}/x_{Non}p^{Non}$ value is 7.94 and 4.79 for compounds **3** and **4**, respectively. Thus, the contribution of the zwitterionic microspecies is much more important than that of the noncharged form to passive membrane penetration and other lipophilicity-related processes.

The need and validity of our correction factors is further proved by the following consideration. If the lipophilicity of the noncharged forms of the acids were equated to that of their esters, the product of $x_{Non}p^{Non}$ would exceed D, which is impossible even from a mathematical point of view, as shown by eq 2.

Our microscopic partition coefficients in Table 3 allow calculating and depicting the contribution of each microspecies to the distribution coefficient of compounds **3** and **4** at any pH value (Figure 4). The broad black line is the overall lipophilicity

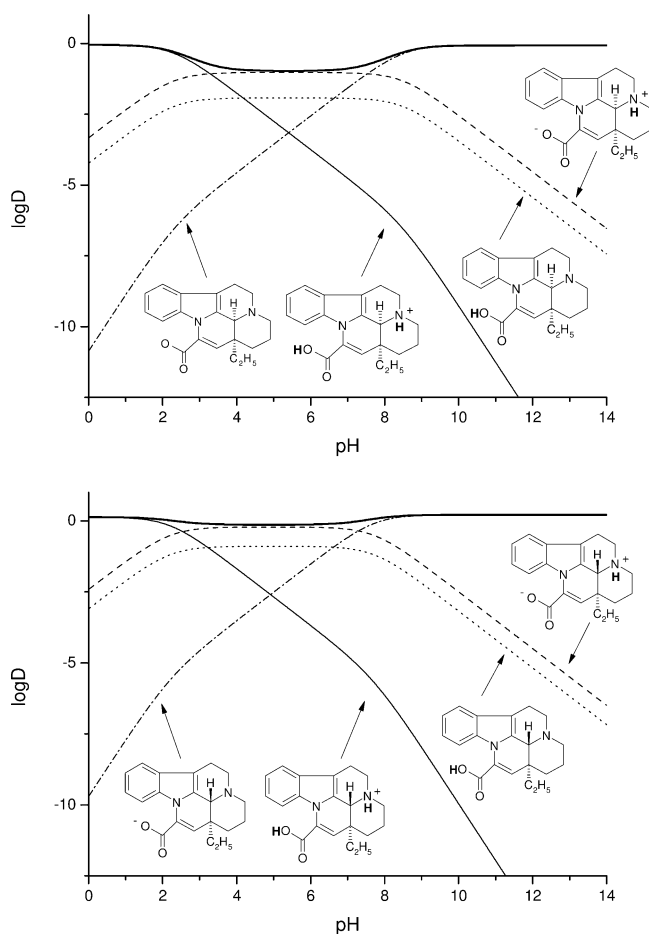profile of the molecule, the sum of the contributions of its four microspecies.

Figure 4. Contribution of the four microspecies of compounds **3** and **4** to the lipophilicity profile (with broad line) of these molecules.

The figure shows that in the pH range of 3.29−7.52 the contribution of the zwitterionic form is dominant for compound **3**, whereas this pH range is 2.55−7.28 for compound **4**. At lower or higher pH values the monoionic forms prevail in the contribution to the distribution coefficient. Although the noncharged form has a much higher inherent lipophilicity than any other forms, its contribution to the partition of the molecule is dominant at no pH value. The pH-independent contribution ratio of the zwitterionic to the noncharged form is visualized by the parallel lines on the graphs.

Our study presents for the first time the complete set of experimental microscopic partition coefficients of compounds where the contribution of the zwitterionic species to the overall lipophilicity is more important than that of the noncharged species. This is also evident from the shape of the overall lipophilicity profile of these molecules. Instead of the common bell-shaped profile, they display a U-shaped profile. In fact, for compound **4** the profile is nearly linear, showing a transition between the bell-shaped and U-shaped profiles.

We hope that the results of this study will help to dispel the still widespread notion that the lipophilicity of ionic, and especially zwitterionic, species is insignificant compared to that of the noncharged form.

4. EXPERIMENTAL SECTION

4.1. Materials. The eburnane alkaloids were provided by Chemical Works of Gedeon Richter Ltd. (Budapest, Hungary). All other reagents were of analytical grade (Reanal). All solutions were prepared from freshly boiled distilled water.

4.2. Partition cCoefficient Measurements by the Stir-Flask Method. The distribution coefficients were calculated from the absorbance of the molecules in the aqueous phase before and after partitioning at several octanol/water phase ratios as previously reported.[20] For the pH control buffers composed of phosphate and serine and standardized HCl and NaOH solutions were used with an ionic strength of 0.15 M.

■ AUTHOR INFORMATION

Corresponding Author

*Phone and fax: +36-1-2170891. E-mail: mazak.karoly@pharma.semmelweis-univ.hu.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Fujita, T. The extrathermodynamic approach to drug design. In *Comprehensive Medicinal Chemistry. The Rational Design, Mechanistic Study and Therapeutic Applications of Chemical Compounds*; Ramsen, C. A., Hansch, C., Sammes, P. G., Taylor, J. B., Eds.; Pergamon: Oxford, U.K., 1990; Vol. 4, pp 497−560.

(2) Avdeef, A. Physicochemical profiling. *Curr. Top. Med. Chem.* **2002**, *1*, 277−351.

(3) Testa, B.; Carrupt, P. A.; Gaillard, P.; Tsai, R. S. Intramolecular interactions encoded in lipophilicity: their nature and significance. In *Lipophilicity in Drug Action And Toxicology*; Pliska, V., Testa, B., van de Waterbeems, H., Eds.; VCH Publishers: Weinheim, Germany, 1996; pp 49−71.

(4) Liu, X. L.; Testa, B.; Fahr, A. Lipophilicity and its relationship with passive drug permeation. *Pharm. Res.* **2011**, *28*, 962−977.

(5) Hansch, C. On the future of QSAR. In *Medicinal Chemistry for the 21st Century*; Wermuth, C. G., Koga, N., König, H., Eds.; Metcalf Blackwell: Oxford, U.K., 1994; pp 281−293.

(6) Pagliara, A.; Carrupt, P. A.; Caron, G.; Gaillard, P.; Testa, B. Lipophilicity profiles of ampholytes. *Chem. Rev.* **1997**, *97*, 3385−3400.

(7) Taylor, P. J.; Cruickshank, J. M. Distribution coefficients of atenolol and sotalol. *J. Pharm. Pharmacol.* **1985**, *37*, 143−144.

(8) Takács-Novák, K.; Józan, M.; Hermecz, I.; Szász, G. Lipophilicity of antibacterial fluoroquinolones. *Int. J. Pharm.* **1992**, *79*, 89−96.

(9) Abraham, M. H.; Takács-Novák, K.; Mitchell, R. C. On the partition of ampholytes: application to blood-brain distribution. *J. Pharm. Sci.* **1997**, *86*, 310−315.

(10) Irwin, V. P.; Quigley, J. M.; Timoney, R. F. 1-Octanol-water partition coefficients of the anionic and zwitterionic species of diprotic zwitterionic cephalosporin antibiotics. *Int. J. Pharm.* **1988**, *43*, 187−188.

(11) Takács-Novák, K.; Szász, Gy. Ion-pair partition of quaternary ammonium drugs: the influence of counter ions of different lipophilicity, size and flexibility. *Pharm. Res.* **1999**, *16*, 1633−1638.

(12) Mandić, Z.; Gabelica, V. Ionization, lipophilicity and solubility properties of repaglinide. *J. Pharm. Biomed. Anal.* **2006**, *41*, 866−71.

(13) Mazák, K.; Kökösi, J.; Noszál, B. Lipophilicity of zwitterions and related species: a new insight. *Eur. J. Pharm. Sci.* **2011**, *44*, 68−73.

(14) Mazák, K.; Noszál, B. Lipophilicity of morphine microspecies and their contribution to the lipophilicity profile. *Eur. J. Pharm. Sci.* **2012**, *45*, 205−210.

(15) Weinshaar, R. E.; Bristol, J. A. Selective inhibitors of phosphodiesterases. In *Comprehensive Medicinal Chemistry. The Rational Design, Mechanistic Study and Therapeutic Applications of Chemical Compounds*; Ramsen, C. A., Hansch, C., Sammes, P. G., Taylor, J. B., Eds.; Pergamon: Oxford, U.K., 1990; Vol. 2, pp 501−514.

(16) Rischke, R.; Krieglstein, J. Effects of vinpocetine on local blood flow and glucose utilization seven days after forebrain ischaemia in the rat. *Pharmacology* **1990**, *41*, 153−160.

(17) Czibula, L.; Nemes, A.; Visky, Gy.; Farkas, M.; Szombathelyi, Zs.; Kárpáti, E.; Sohár, P.; Kessel, M.; Kreidl, J. Syntheses and cardiovascular activity of stereoisomers and derivatives of eburnane alkaloids. *Liebigs Ann. Chem.* **1993**, *3*, 221−229.

(18) Szántay, Cs.; Moldvai, M.; Vedres, A.; Incze, M.; Kreidl, J.; Czibula, L.; Farkas, J.; Juhász, I.; Gere, A.; Paróczai, M.; Lapis, E., Szekeres, A., Balázs Zajer, M.; Sarkadi, Á.; Auth, F.; Kiss, B.; Kárpáti, E.; Farkas, S. (Gedeon Richter Ltd.). Preparation of trans-apovincaminic acid ester derivatives as neurological drugs. PCT Int. Appl. WO 9723481, July 3, 1997; *Chem. Abstr.* **1998**, *127*, 135982.

(19) Mazák, K.; Nemes, A.; Noszál, B. Proton speciation and microspeciation of vinpocetine and related compounds in aqueous and biomimetic media. *Pharm. Res.* **1999**, *16*, 1757−1763.

(20) Mazák, K.; Vámos, J.; Nemes, A.; Rácz, Á.; Noszál, B. Lipophilicity of vinpocetine and related compounds characterized by reversed-phase thin-layer chromatography. *J. Chromatogr., A* **2003**, *996*, 195−203.

(21) Bjerrum, N. Dissociation constants of polybasic acids and their application to the calculation of molecular dimensions. *Z. Phys. Chem.* **1923**, *106*, 219−242.

(22) Noszál, B. Acid-base properties of bioligands. In *Biocoordination Chemistry: Coordination Equilibria in Biologically Active Systems*; Burger, K., Ed.; Ellis Horwood: Chichester, U.K., 1990; pp 18−55.

(23) Caron, G.; Reymond, F.; Carrupt, P. A.; Girault, H. H.; Testa, B. Combined molecular lipophilicity descriptors and their role in understanding intramolecular effects. *Pharm. Sci. Technol. Today* **1999**, *2*, 327−335.

(24) Johnson, C. A.; Westall, J. C. Effect of pH and KCl concentration on the octanol-water distribution of methylanilines. *Environ. Sci. Technol.* **1990**, *24*, 1869−1875.

(25) Escher, B. I.; Schwarzenbach, R. P. Partitioning of substituted phenols in liposome-water, biomembrane-water, and octanol-water systems. *Environ. Sci. Technol.* **1996**, *30*, 260−270.

(26) Reymond, F.; Gobry, V.; Bouchard, G.; Girault, H. Electrochemical aspects of drug partitioning. In *Pharmacokinetic Optimization in Drug Research*; Testa, B., van de Waterbeemd, H., Folkers, G., Guy, R., Eds.; Wiley-VCH Publishers: Zürich, Switzerland, 2001; pp 327−349.

(27) Bouchard, G.; Carrupt, P. A.; Testa, B.; Gobry, V.; Girault, H. H. The apparent lipophilicity of quaternary ammonium ions is influenced by Galvani potential difference, not ion-pairing: a cyclic voltammetry study. *Pharm. Res.* **2001**, *18*, 702−708.

(28) Szántay, Cs.; Nemes, A. The Eburnanine-Vincamine group. In *The Monoterpenoid Indole Alkaloids*; Saxton, J. E., Ed.; John Wiley & Sons Ltd.: Chichester, U.K., 1994; pp 437−486.