# Conformer-Specific Partition Coefficient: Theory and Determination

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The first conformer-specific partition coefficients are presented. Relationships between the macroscopic and the rotamer-specific partition coefficients are deduced. Constituents of the determination are shown to be the conformer (rotamer) mole fractions in both solvents and the macroscopic partition coefficient. Feasibility of the method is exemplified on amphetamine, a flexible molecule of psychostimulant activity. Partition coefficients of the most hydrophilic and most lipophilic amphetamine rotamers differ by a factor of 1.4. The conformer-specific partition coefficients are interpreted in terms of intramolecular interactions of the moieties, which are modulated by solvation effects on the rotamer-specific group settings.

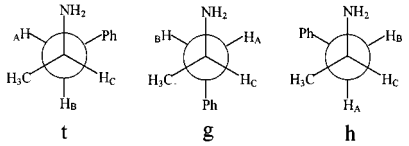
## Introduction

Conformational flexibility is a key property of small biomolecules. The consequent stereochemical adaptability enhances their versatility in membrane penetration, enzyme catalysis, receptor binding, and other important biological reactions. For example, the endogenous neurotransmitters (serotonine, dopamine, histamine, $\gamma$-aminobutyric acid, glutamic acid, acethylcholine, etc.) can take up a set of rotameric forms, which enables them to bind to several subtypes of receptors.[1] In contrast, their conformationally restricted exogenous counterparts can normally bind to one type of receptor only. This indicates that different conformers have their own physical, chemical, biological properties.[1,2]

The characterization of biomolecules and drug molecules in terms of conformer-specific physicochemical parameters has therefore become a recognized necessity[2,3] and could be accomplished for rotamer-specific basicity.[3−5] The lack of other conformer-specific parameters stems from the difficulties of their determinations. The short individual lifetime of rotamers makes them interconverting, coexisting species on the time scale of any spectroscopic, electrochemical, etc. techniques. The resulting observed analytical signals are therefore composite ones, in which neither the rotamer populations nor their intensive parameters are a priori known quantities.

The partition coefficient, *P*, the classical thermodynamic parameter, has recently gained widely acknowledged importance in biological chemistry, due to its power to predict the in vivo phase-transition behavior of biomolecules and drug molecules. Its current significance in drug design and development is shown by international conferences and books devoted entirely to the field of lipophilicity and log *P* determinations.[6]

To date, all the reported experimental log *P* values are macroscopic ones, irrespective of the solute conformations. This is although partition has been assumed to be a species-specific, conformation-dependent property for nearly 30 years. Hopfinger et al. used the solvent-dependent conformational analysis procedure (SCAP) to predict the octanol/water partition coefficient for 20 different compounds.[7] However, either most of these molecules were rigid or the constants referring to flexible molecules were of bulk type, encompassing an undefined number and concentration of conformers. In subsequent studies on hydroxyureas, systematic differences have been found between experimental and calculated log $P_{\text{octanol}}$ values, due to differences in conformational tendencies between the two groups of the molecules studied.[8]




t g h

**Figure 1.** Staggered conformers of amphetamine

The partition coefficient was first assigned to conformers in 1979.[2] Davies et al. defined the new species-specific parameter as the individual partition coefficient of distinct conformers. They hypothesized that membrane penetration of drugs is bound to particular conformers but they presented neither methodology nor experimental data. Four years later Pleiss et al. investigated a number of conformationally rigid phenethylamines,[9] and extended the *f*-fragment calculations of Rekker[10] and Leo[11] with correction factors to take conformation into consideration. A recent extensive study on diastereomers examined the influence of stereochemical factors on the log $P_{\text{octanol}}$ values.[12] Differences in lipophilicity between diastereomers were interpreted in terms of macroscopic properties, such as van der Waals volume, surface area, dipole moment, ionization constant, and H-bonding capacity. Conformer-specific partition coefficients of flexible molecules, however, have not yet been determined.

Here, we report the first conformer-specific partition coefficients. The theory and practice of the determination are exemplified on the coexisting rotamers of amphetamine, a small, flexible, psychostimulant drug.

## Theory

**Conformer-Specific Partition Coefficients.** The three staggered conformers of amphetamine are shown in Figure 1. The two bulkiest groups are in trans position in rotamer t, whereas they are in gauche in rotamers g and h. In rotamer h, the three bulky groups are adjacently situated.

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The macroscopic partition coefficient is, by definition, the ratio of the total solute concentrations in the organic and aqueous phases:

$$P = \frac{c_o}{c_w} \tag{1}$$

Since the bulk species are composed of rotamers, the macroscopic partition coefficient is a function of the rotamer concentrations as well:

$$P = \frac{[g]_o + [t]_o + [h]_o}{[g]_w + [t]_w + [h]_w} \tag{2}$$

where $[g]_o$, $[t]_o$, $[h]_o$ and $[g]_w$, $[t]_w$, $[h]_w$ are concentrations of the g, t, and h rotamers in the organic and aqueous phases, respectively.

Rotamer populations in either phase can be quantitated in terms of mole fractions. For example, $\alpha_{t_o}$, the mole fraction of rotamer t in the organic phase, and $\alpha_{h_w}$, the mole fraction of rotamer h in the aqueous phase, can be expressed as follows:

$$\alpha_{t_o} = \frac{[t]_o}{[t]_o + [g]_o + [h]_o} = \frac{[t]_o}{c_o} \tag{3}$$

$$\alpha_{h_w} = \frac{[h]_w}{[t]_w + [g]_w + [h]_w} = \frac{[h]_w}{c_w} \tag{4}$$

The conformer-specific partition coefficients can be defined analogously with the macroscopic (bulk) coefficient:

$$p_g = \frac{[g]_o}{[g]_w} \tag{5}$$

$$p_t = \frac{[t]_o}{[t]_w} \tag{6}$$

$$p_h = \frac{[h]_o}{[h]_w} \tag{7}$$

Introducing the rotamer concentrations from the conformer-specific partition coefficients into eq 2, and applying (4)-type aqueous rotamer mole fractions, a relationship between the macroscopic and the conformer-specific partition coefficients can be obtained:

$$P = \frac{p_g[g]_w + p_t[t]_w + p_h[h]_w}{[g]_w + [t]_w + [h]_w} = p_g\alpha_{g_w} + p_t\alpha_{t_w} + p_h\alpha_{h_w} \tag{8}$$

Equation 8 shows that the macroscopic partition coefficient is the weighted sum of the conformer-specific partition coefficients, where weighting factors are the corresponding aqueous rotamer mole fractions.

An analogous treatment yields another relationship involving rotamer mole fractions in the organic phase and reciprocal partition terms:

$$P^{-1} = \alpha_{g_o}p_g^{-1} + \alpha_{t_o}p_t^{-1} + \alpha_{h_o}p_h^{-1} \tag{9}$$

The formula for the calculation of rotamer-specific partition coefficients can be derived from eqs 5−7, by expressing rotamer concentrations from rotamer mole fractions in both phases:

$$p_t = \frac{\alpha_{t_o}c_o}{\alpha_{t_w}c_w} = \frac{\alpha_{t_o}}{\alpha_{t_w}}P \tag{10}$$

$$p_g = \frac{\alpha_{g_o}c_o}{\alpha_{g_w}c_w} = \frac{\alpha_{g_o}}{\alpha_{g_w}}P \tag{11}$$

$$p_h = \frac{\alpha_{h_o}c_o}{\alpha_{h_w}c_w} = \frac{\alpha_{h_o}}{\alpha_{h_w}}P \tag{12}$$

In general, eqs 10−12 show that rotamer-specific partition coefficients can be obtained in the knowledge of the macroscopic partition coefficient and the rotamer populations in the two solvents.

**Rotamer Populations.** Rotamer populations can be determined from vicinal $^1H-^1H$ NMR coupling constants for an ABC spin system.[13] Because of rapid interconversion among rotamers, the observed couplings are the weighted sums of the various gauche and trans coupling constants of individual rotamers, where weighting factors are the appropriate mole fractions:

$$^3J_{AC} = \alpha_tJ_T + \alpha_gJ_{Gg} + \alpha_hJ_{Gh_{AC}} \tag{13}$$

$$^3J_{BC} = \alpha_tJ_{Gt} + \alpha_gJ_T + \alpha_hJ_{Gh_{BC}} \tag{14}$$

$$\alpha_t + \alpha_g + \alpha_h = 1 \tag{15}$$

In eqs 13−15, $^3J_{AC}$ and $^3J_{BC}$ are the observed coupling constants between A−C and B−C protons, $\alpha_t$, $\alpha_g$, and $\alpha_h$ are rotamer populations, $J_T$ is the standard coupling constant belonging to the 180° dihedral angle, $J_{Gt}$, $J_{Gh_{AC}}$ and $J_{Gg}$, $J_{Gh_{BC}}$ are standard coupling values belonging to the 60° and 300° dihedral angles in rotamers t and h and g and h, respectively.

By combining eqs 13−15, we obtain

$$\alpha_t = \frac{(^3J_{AC} - J_{Gh_{AC}})(J_T - J_{Gh_{BC}}) - (^3J_{BC} - J_{Gh_{BC}})(J_{Gg} - J_{Gh_{AC}})}{(J_T - J_{Gh_{AC}})(J_T - J_{Gh_{BC}}) - (J_{Gt} - J_{Gh_{BC}})(J_{Gg} - J_{Gh_{AC}})} \tag{16}$$

$$\alpha_g = \frac{(^3J_{BC} - J_{Gh_{BC}})(J_T - J_{Gh_{AC}}) - (^3J_{AC} - J_{Gh_{AC}})(J_{Gt} - J_{Gh_{BC}})}{(J_T - J_{Gh_{AC}})(J_T - J_{Gh_{BC}}) - (J_{Gt} - J_{Gh_{BC}})(J_{Gg} - J_{Gh_{AC}})} \tag{17}$$

## Discussion

$^3J_{AC}$ and $^3J_{BC}$, the experimental coupling constants of amphetamine at 25 °C in water and chloroform, were measured by Makriyannis et al.,[14] and the rotamer populations were also evaluated by a method that was appropriate at that time. Nevertheless, that evaluation method considered all the gauche states equal and applied two imported parameters from model compounds, and the constants were irrespective of the solvent. Since Makriyannis' paper, a significantly improved, five-parameter, solvent-respective evaluation method was published by Altona et al.[15] In our process Makriyannis' experimental data and Altona's evaluation method have therefore been combined to obtain the most reliable rotamer populations at present. The standard coupling constants for amphetamine in both solvents

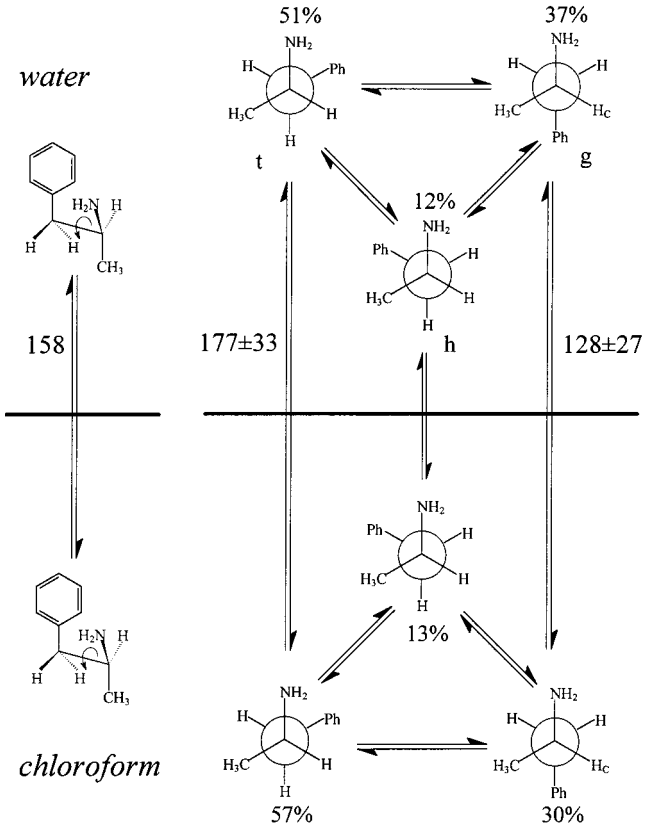


**Figure 2.** Scheme and data of amphetamine partition in the chloroform/water system, at the macroscopic and conformer-specific levels.

**TABLE 1: Observed and Standard Vicinal $^1H-^1H$ NMR Couplings of Amphetamine in $CDCl_3$ and $D_2O$**

| | obd couplings[a] | | std trans and gauche coupling constants[b] | | | | |
|---|---|---|---|---|---|---|---|
| solvent | $^3J_{AC}$ | $^3J_{BC}$ | $J_T$ | $J_{Gt}$ | $J_{Gg}$ | $J_{GhAC}$ | $J_{GhBC}$ |
| $CDCl_3$ | 8.06 | 5.5 | 11.73 | 2.72 | 3.59 | 2.47 | 3.33 |
| $D_2O$ | 7.7 | 6.23 | 11.83 | 2.89 | 3.54 | 2.64 | 3.28 |

[a] From ref 14. [b] Calculated by Altona's equation.[15]

**TABLE 2: Calculated Rotamer Populations of Amphetamine in $CDCl_3$ and $D_2O$**

| | calcd rotamer populations | | |
|---|---|---|---|
| solvent | $\alpha_t$ | $\alpha_g$ | $\alpha_h$ |
| $CDCl_3$ | $0.57 \pm 0.07$ | $0.30 \pm 0.05$ | $0.13 \pm 0.05$ |
| $D_2O$ | $0.51 \pm 0.07$ | $0.37 \pm 0.05$ | $0.12 \pm 0.05$ |

were calculated, using the reparametrized Haasnoot equation of Altona, resulting in values in Table 1, bearing 0.36 Hz rms error.[15] Rotamer populations were then determined according to eqs 15−17. Tables 1 and 2 list the coupling constants and rotamer mole fractions of amphetamine in $D_2O$ and $CDCl_3$.

The macroscopic partition coefficient, $\log P = 2.2$ of amphetamine in the chloroform/water system at 25 °C was determined by Modin et al.[16] From these data, rotamer-specific partition coefficients were calculated and are shown in Figure 2. Percentage rotamer populations are indicated near the species, the macroscopic and also the rotamer-specific partition coefficients can be seen next to the arrows. Uncertainties were calculated by the standard error-proliferation method of Gauss. The value $p_h = 171 \pm 97$ was also calculated. The high uncertainty is due to the low population of rotamer h in both solvents, which makes any distinctive comparisons apparent. This value is therefore omitted from Figure 2.

Values of the conformer-specific partition coefficients allow insight into the submolecular solvation effects on moieties of the rotamers. The orders of rotamer populations are identical in water and chloroform, indicating the primary importance of the intramolecular interactions of the moieties. The least populated rotamer is h, the sterically most hindered one. In contrast, the most abundant rotamer, t, contains the two bulkiest groups, the aromatic ring and the methyl moiety in trans position. The medium populated rotamer g has trans position for the bulkiest phenyl and the third bulkiest amino sites.

Within this order, solvation effects modulate the actual rotamer populations and the conformer-specific partition coefficients. Rotamer t of amphetamine is 1.4 times more lipophilic than rotamer g. Reasons to account for this phenomenon are the adjacent position of the polar amino group and the water-repellent aromatic ring, which hampers the water accessibility of the amino site. At the same time, the chloroform solvation is also disfavored due to the noncooperative trans position of the lipophilic methyl and phenyl moieties. Rotamer g contains a good water-accessible amino site, and also a preferential, gauche arrangement of the methyl and phenyl moieties for the chloroform solvation. The lower $p_g$ and higher $p_t$ values reveal that the major solvation effect governing the partitioning is the polar−polar interaction between the amino site and water.

The small difference between the partition coefficients of rotamer t and g ($\Delta\log P = 0.14$) is in agreement with the observation of Tsai et al.[14] on partition in the octanol/water system for diastereomers of one polar group. Greater differences in rotamer-specific partition coefficients can well be expected for molecules of two or more vicinal, polar moieties.

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