Molecular dynamics of hydantoins and barbiturates assessed by ^{1}H, ^{13}C and ^{15}N relaxation data

Petra Gruhlke,[a] Christa G. Seipelt,[a] Andreas Dölle,*[,a] Manfred D. Zeidler,[a] Paula Zaderenko,[b] Paloma Ballesteros[b] and Sebastián Cerdán[c]

[a] *Institut für Physikalische Chemie, Rheinisch-Westfälische Technische Hochschule, D-52056 Aachen, Federal Republic of Germany*

[b] *Departamento de Química Orgánica y Biología, Facultad de Ciencias, Universidad Nacional de Educación a Distancia, c/Senda del Rey s/n, E-28040 Madrid, Spain*

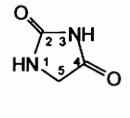
[c] *Instituto de Investigaciones Biomédicas, Consejo Superior de Investigaciones Científicas, c/Arturo Duperier 4, E-28029 Madrid, Spain*

The molecular dynamics of hydantoin, 5,5-dimethylhydantoin, 5,5-diphenylhydantoin, barbituric acid and 5-ethyl-5-phenylbarbituric acid in solution are determined to investigate the molecular basis for the activity of these neuroactive drugs. From dipolar ^{13}C and ^{15}N spin-lattice relaxation times the correlation times for the rotational dynamics of specific bond vectors are evaluated. Conclusions can be drawn concerning the anisotropy of the overall molecular rotational motion and the internal rotations of molecular segments. The translational molecular diffusion coefficients are obtained from self-diffusion measurements by the pulsed gradients method. The observed differences in the dynamics as well as structural differences may account for the variation in the neuroactivity of the studied compounds.

Introduction

5-Substituted barbituric acids and 5-substituted hydantoins possess important pharmacological properties, because of which they are well known as neuroactive drugs.[1] Their hypnotic and anticonvulsant activities have been related to neuronal Na^+ and Ca^{2+} channel interactions,[2] although the mechanisms of action are still poorly understood. In contrast, the 5-unsubstituted derivatives are inactive. In addition to conventional properties used in QSAR methodology (quantitative structure activity relationships),[3] we investigate here whether other molecular properties are also affecting the activity of the drugs. As model compounds we have considered unsubstituted hydantoin **1**, 5,5-dimethylhydantoin **2**, 5,5-diphenylhydantoin **3**, barbituric acid **4** and 5-ethyl-5-phenylbarbituric acid **5**. The aim of the present investigation is to study the molecular dynamics of these substances in solution, which could help to explain their different pharmacological activity.

Since the transport and action of the drugs takes place in the liquid phase, it is desirable to achieve a deeper understanding of their molecular dynamics in liquids and therefore of their activity. A particularly important experimental method for probing molecular dynamics in liquids is the measurement of nuclear magnetic relaxation times. From dipolar spin-lattice relaxation times reorientational correlation times are obtained which characterise the velocity of molecular rotational motions of internuclear spin–spin vectors.[4,5] By measurement of echo decays obtained with application of pulsed field gradients the self-diffusion constants of the molecules are accessible.[6] The diffusion constants characterise the translational motion of the diffusing molecules. Both methods are applied in the present study to describe the molecular dynamics of hydantoins and barbiturates.



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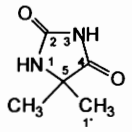

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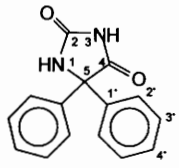

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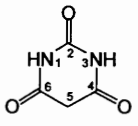

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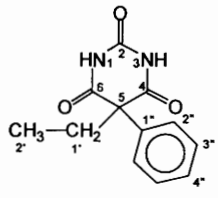

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Results

^{13}C NMR data

^{13}C Chemical shifts (δ) and their assignments, ^{13}C spin-lattice relaxation times (T_1), NOE factors (η) and dipolar spin-lattice relaxation times (T_1^{DD}) for compounds **1** to **5** dissolved in [$^2H_6$]DMSO at 310 K are listed in Table 1. The dipolar spin-lattice relaxation times were calculated using eqn. (1).[5] Since the

$$T_1^{DD}(^{13}C) = \frac{1.988}{\eta} T_1(^{13}C) \quad (1)$$

experimental error in the NOE factors is known to be relatively large, all aliphatic carbon atoms bearing directly attached protons were assigned the highest possible NOE factor η of 1.988 for calculation of the dipolar relaxation rates T_1^{DD}, *i.e.* it was assumed that these ^{13}C nuclei are relaxing fully *via* the dipolar relaxation pathway.

^{15}N NMR data

^{15}N Chemical shifts (δ) and their assignments, ^{15}N spin-lattice relaxation times (T_1), and NOE factors (η) for **1** and **3**–**5** dissolved in [$^2H_6$]DMSO at 310 K are given in Table 2.

Table 1 ^{13}C Results: chemical shifts (δ), assignments, spin-lattice relaxation times (T_1), NOE factors (η), dipolar spin-lattice relaxation times (T_1^{DD}), bond distances (r_{C-H}) and effective correlation times (τ_c) for compounds **1** to **5** at 310 K

Assignment	δ/ppm	T_1/s	η	T_1^{DD}/s[a]	r_{C-H}/pm	τ_c/ps
Hydantoin **1**						
2	157.6	11.8	1.03	22.8		
4	173.0	13.6	1.31	20.6		
5	46.5	1.08	1.88	1.08	109	22
5,5-Dimethylhydantoin **2**						
2	155.7	8.88	0.88	20.1		
4	178.6	12.9	0.82	31.2		
5	58.6	13.2	1.72	15.3		
1′	23.9	0.92	1.97	0.92	111	19
5,5-Diphenylhydantoin **3**						
2	155.6	3.25	0.31	21.0		
4	174.3	3.90	0.24	32.2		
5	69.9	11.3	0.81	27.8		
1′	139.3	4.06	0.23	34.6		
2′	127.8	0.95	1.45	1.30	110	38
3′	126.1	0.96	1.42	1.33	110	37
4′	127.4	0.35	0.77	0.91	110	54
Barbituric acid **4**						
2	150.7	5.14	1.33	7.70		
4	166.8	5.90	1.24	9.44		
5	38.4	0.54	1.99	0.54	111	48
5-Ethyl-5-phenylbarbituric acid **5**						
2	149.8	3.49	0.76	9.16		
4	171.5	4.20	0.61	13.7		
5	60.2	7.73	1.55	9.89		
1′	28.7	0.22	1.76	0.22	112	125
2′	9.6	1.19	1.98	1.19	111	15
1″	138.5	4.70	0.23	41.0		
2″	128.9	1.18	1.58	1.49	110	33
3″	126.2	1.20	1.83	1.31	110	38
4″	128.1	0.39	1.78	0.44	110	112

[a] For calculation of the T_1^{DD} values of aliphatic ^{13}C nuclei with directly attached protons a full NOE factor of 1.988 was used.

Table 2 ^{15}N Results: chemical shifts (δ), spin-lattice relaxation times (T_1), NOE factors (η), bond distances (r_{N-H}) and effective correlation times (τ_c) for compounds **1**, **3**, **4** and **5** at 310 K

δ/ppm	T_1/s	η	r_{N-H}/pm	τ_c/ps[a]
[1-^{15}N]Hydantoin **1**				
−296.5 (N-1)	4.20	−4.85	99.4	39
[1,3-$^{15}N_2$]-5,5-Diphenylhydantoin **3**				
−233.9 (N-3)	0.86	−4.65	101	211
−271.7 (N-1)	0.94	−4.56	102	205
[1,3-$^{15}N_2$]Barbituric acid **4**				
−227.8 (N-1,3)	1.61	−4.68	102	119
[1,3-$^{15}N_2$]-5-Ethyl-5-phenylbarbituric acid **5**				
−226.9 (N-1,3)	1.05	−4.57	102	183

[a] For calculation of the T_1^{DD} values when evaluating the correlation times a full NOE factor of −4.93 was used, *i.e.* the dipolar spin-lattice relaxation times were set equal to the experimental ones.

Table 3 Self-diffusion constants D for hydantoin **1**, 5,5-diphenylhydantoin **3**, barbituric acid **4** and 5-ethyl-5-phenylbarbituric acid **5** at 310 K

Compound	**1**	**3**	**4**	**5**
$D/10^{-8}$ m^2 s^{-1}	10.6 ± 0.2	4.15 ± 0.06	8.0 ± 0.2	4.60 ± 0.06

Self-diffusion data

For compounds **1** and **3**–**5** the self-diffusion constants D at 310 K are given in Table 3.

Reorientational correlation times

The dipolar spin-lattice relaxation rate $(1/T_1^{DD})_i$ of nucleus X_i (^{13}C or ^{15}N) resulting from interaction with n_H protons j is connected in the extreme narrowing regime to molecular rotations by eqn. (2),[4,5] with the permeability constant of the

$$\left(\frac{1}{T_1^{DD}}\right)_i = \left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_X^2 \gamma_H^2 \hbar^2 n_H}{r_{ij}^6} (\tau_c)_i \qquad (2)$$

vacuum μ_0, the gyromagnetic ratios γ_X and γ_H of X (^{13}C or ^{15}N) and ^1H, respectively, and r_{ij} the length of the internuclear vector between i and j. The effective reorientational correlation time $(\tau_c)_i$ is a measure for the velocity of rotational motion of the corresponding internuclear X–H vectors.

The effective correlation times of the ^{13}C–^1H and ^{15}N–^1H vectors for those heteroatoms connected to directly bonded protons are included in Tables 1 and 2 together with the bond distances r_{X-H} used for their calculation. The T_1^{DD} values used for calculation of the correlation times of the N–H vectors were set equal to the measured T_1 values because the magnitudes of the measured NOE values were within the experimental error in agreement with the value of −4.93 for fully dipolar relaxation.

Table 4 Anisotropy ratios σ^a and ratios $\sigma_i{}^b$ for hydantoin **1**, 5,5-diphenylhydantoin **3**, barbituric acid **4** and 5-ethyl-5-phenylbarbituric acid **5** at 310 K

Compound	1	3	4	5
σ	1.8	3.9	2.5	1.6
σ_i		2.5		9.5

[a] $\sigma = \tau_c(N\text{–}H)/\tau_c(C\text{–}H)$. [b] From the ratio $\tau_c(C\text{–}H)^{para}/\tau_c(C\text{–}H)^{ortho,meta}$ analogous to ref. 7.

Discussion

Relaxation data

The NOE factors of aliphatic ^{13}C nuclei with directly bonded protons have, within the experimental error of about 10%, the full value of 1.988. The same reasoning applies to the $\{^1H\}$–^{15}N NOE factors: they also show within experimental error the value for fully dipolar relaxation (−4.93). The NOE factors of the ^{13}C nuclei without directly bonded protons or in the aromatic rings are considerably smaller than the maximum value, indicating that these nuclei do not relax only *via* the dipole–dipole mechanism. Since the reorientational motion of the studied molecules is relatively slow, the only additional relaxation mechanism that has to be taken into account for contributing to the relaxation of the aromatic carbon atoms is chemical shift anisotropy (CSA).

Translation and rotational dynamics

The translational diffusion is slowed down by a considerable amount when the self-diffusion constants of the unsubstituted hydantoin **1** or barbituric acid **4** are compared with the values of the derivatives **3** and **5**, respectively.

A corresponding increase is found for the effective correlation times of rotational motion, *i.e.* the rotational dynamics become slower for the substituted compounds. The correlation times for the ^{15}N–^1H vectors (see Table 2), the ^{13}C–^1H vectors of C-5 in the unsubstituted compounds **1** and **4**, and the ^{13}C–^1H vector of C-4′ in **3** or C-4″ in **5** (see Table 1), respectively, are a measure of the velocity of the overall reorientational motion. The overall molecular reorientations of the unsubstituted compounds **1** and **4** are considerably faster than those of their substituted derivatives **3** and **5**.

In the unsubstituted molecules the reorientational motion of the N–H bond vectors is slower than that of the C–H bonds. This indicates the presence of anisotropic reorientation. It can be explained by the formation of hydrogen bonds between the hydrogen atoms bonded to the nitrogen atom and surrounding molecules. Since the orientation of the bonds between C-4′ in **3** or C-4″ in **5** and the directly attached hydrogens corresponds to that of the bonds between C-5 and the hydrogens, the correlation times of the former can be compared with those of the ^{15}N–H vectors in the same molecule to yield the anisotropy of rotational motion. For the substituted compounds the reorientational motion of the N–H vectors is also slower than that of the C-4′–H or C-4″–H vectors, respectively, as can be seen from the ratios σ of the corresponding effective correlation times in Table 4.

The ratios σ_i between the correlation times of C-4′ or C-4″ and those of the other two carbons in the phenyl rings indicate the amount of internal rotation about the C-5–C-1′ or C-5–C-1″ bonds, respectively.[7] The values of σ_i, which are also included in Table 4, illustrate that the internal phenyl ring rotation in the 5-ethyl-5-phenylbarbituric acid **5** is much faster than in the 5,5-diphenylhydantoin **3**. The reason for this behaviour is the smaller steric hindrance by the ethyl group in **5** compared with that by the geminal phenyl group in **3**. The small correlation time for the methyl ^{13}C nucleus in the ethyl group of **5** (see Table 1) is an indicator of the fast internal rotation in this molecular segment.

The results clearly illustrate that significant differences exist in the dynamics of the studied compounds. In particular, the self-diffusion coefficients (see Table 3) of the pharmacologically active compounds **3** and **5** are very similar and significantly smaller than those of the inactive compounds **1** and **4**. Similar conclusions can be drawn from the rotational correlation times: the overall rotational dynamics of the active compounds are again much slower. The anisotropy ratios σ do not show a clear correlation with the pharmacological activities. From the results presented, it is possible to propose that the translational diffusion coefficients and the reorientational correlation times are appropriate parameters to be related to the pharmacological activity of the studied hydantoins and barbiturates.

Usually, only structural molecular parameters of drugs, such as their electronic or topological structure, are used to explain their activity. Macroscopic parameters, *e.g.* partition coefficients, molar refractivity, are also used to relate physicochemical properties to biological activity. In the present study, however, we suggest that in addition to structural molecular properties, molecular dynamics can be employed as an additional or complementary tool to relate molecular properties to the activity of drugs.

Experimental

Synthesis

The compounds hydantoin,[8] 5,5-diphenylhydantoin,[9a] barbituric acid[9b] and 5-ethyl-5-phenylbarbituric acid[9c] were prepared following known procedures. To obtain the ^{15}N labelled compounds [^{15}N]glycine with 100% and [^{15}N]urea with 15% enrichment from Isomed (Madrid, Spain) were used. The products were identified by melting point, elemental analysis, ^1H and ^{13}C NMR spectroscopy. The remaining compounds were obtained commercially.

NMR measurements

^{13}C NMR measurements were performed on a Bruker AM 360 spectrometer {B_0 = 8.4 T, $\nu_0(^{13}C)$ = 90.56 MHz, internal standard and lock [$^2H_6$]DMSO} and ^{15}N measurements on a Bruker AC 200 spectrometer {B_0 = 4.7 T, $\nu_0(^{15}N)$ = 20.28 MHz, internal lock [$^2H_6$]DMSO, external standard nitromethane}. Measurements of the self-diffusion constants using the pulsed gradients method were carried out on a Bruker CXP 300 spectrometer [B_0 = 7.047 T, $\nu_0(^1H)$ = 300.13 MHz]. Samples were prepared by dissolving 1.8 mol dm^{-3} of the corresponding compound in [$^2H_6$]DMSO and degassing by six freeze–pump–thaw cycles. Chemical shifts of the ^{13}C resonances were assigned with the help of known increments.[10] Measurements of the spin-lattice relaxation times were carried out with ^1H broadband decoupling and repeated five times, those for the NOE factors ten times. All measurements were performed at 310 K. The reproducibility of the resultant T_1 was better than 5%, that for the NOE factors better than 10% and that of the self-diffusion constants better than 3%.

Molecular geometries

To calculate the reorientational correlation times, the distance between the corresponding nuclei has to be known. The distances were obtained by optimising the molecular structures with the HYPERCHEM software package by Hypercube, Inc. (Waterloo, Canada) using the MM+ force field.

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