69. Kinetics and Mechanisms of Racemization: 5-Substituted Hydantoins (= Imidazolidine-2,4-diones) as Models of Chiral Drugs

by **Marianne Reist, Pierre-Alain Carrupt**, and **Bernard Testa***

Institut de Chimie Thérapeutique, BEP, Université de Lausanne, CH–1015 Lausanne-Dorigny

and **Søren Lehmann** and **Jan Jørn Hansen**

Department of Medicinal Chemistry, The Royal Danish School of Pharmacy, DK–2100 Copenhagen

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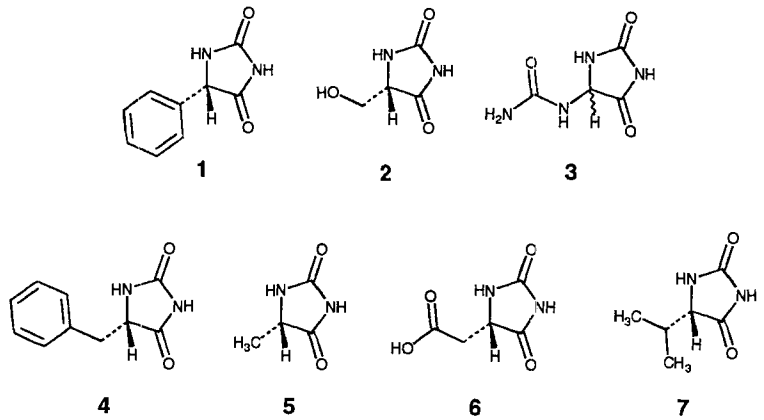
A chiral center in a drug molecule increases the complexity of synthetic, metabolic, pharmacological, and clinical studies, an additional problem being a possible lack of configurational stability. Here, we report detailed kinetic and mechanistic studies on the deuteration and racemization of seven 5-monosubstituted hydantoins (= imidazolidine-2,4-diones) used as model compounds. Using ^{1}H-NMR and chiral RP-HPLC, rates of reaction and thermodynamic parameters of activation were determined for the reactions of deuteration and racemization. Energies of deprotonation were obtained by molecular-orbital calculations performed at the AM1 level. It is demonstrated that the deuteration and racemization of 5-monosubstituted hydantoins follow general-base catalysis. The identical (within experimental errors) activation energies of deuteration and racemization indicate that the two reactions share a common reaction mechanism. The fact that the pseudo-first-order rate constants of deuteration are about half of those of racemization suggests that deuteration occurs with inversion of configuration. Very large differences in reaction rates were observed between the seven compounds, indicating the marked influence of substituents on chiral stability. These results, together with the small isotope effects observed, and the comparison between experimental activation energies and calculated energies of deprotonation, suggest a S_E2 push-pull mechanism for the racemization of 5-monosubstituted hydantoins.

Introduction. – In recent years, the interest in drugs possessing one or more stereogenic elements has increased markedly [1] [2]. A chiral center in a drug molecule increases distinctly the complexity of synthetic, metabolic, pharmacological, and clinical studies, an additional problem being a possible lack of configurational stability [3]. Thus, special attention to this problem is required, and it would be an asset in drug research to be able to rely on predictive rules as well as on fast methods to assess the configurational stability of chiral drug candidates. The present study of seven 5-monosubstituted hydantoins (= imidazolidine-2,4-diones), **1**–**7**, used as model compounds, aims at a better understanding of the influence of substituents and on the kinetics and mechanisms of racemization at the chirally substituted C-atoms of the type RR′R″C–H.

In the early stages of development of chiral drugs, the possibility of their chemical racemization should be investigated [3]. If chemical racemization is very fast, as is the case with oxazepam, resolution and separate examination of the enantiomers will be pointless or impossible. If, on the other hand, chemical racemization is not detectable or slow, the two separate enantiomers and the racemate will have to be evaluated for their pharmacokinetic and pharmacodynamic properties [4].



1 (*S*)-5-Phenylhydantoin
2 (*S*)-5-(Hydroxymethyl)hydantoin
3 (*RS*)-5-Ureidohydantoin
4 (*S*)-5-Benzylhydantoin
5 (*S*)-5-Methylhydantoin
6 (*S*)-5-(Carboxymethyl)hydantoin
7 (*S*)-5-Isopropylhydantoin

For chiral drug candidates of the type RR′R″C–H, it would be very helpful to have preliminary information on their rates of base-catalyzed racemization prior to obtaining the separate enantiomers. Such investigations should be performed with the racemates using ^{1}H-NMR [5–8] to monitor their rates of H/D exchange (*i.e.*, deuteration) [9]. Conditions for interpretable results are: *a*) previous studies must have established the existence of a common mechanism of racemization and deuteration in the series; *b*) the ratio of the rates of deuteration and racemization should be known.

Four limiting ratios of k_{deut} (rate constant of deuteration) over k_{rac} (rate constant of racemization) can be envisaged [10] [11]: *1*) if deuteration occurs with complete retention of configuration, the ratio k_{deut}/k_{rac} approaches infinity (isoinversion); *2*) if deuteration takes place with complete racemization, each carbanion is captured from either side with equal probability, and k_{deut}/k_{rac} equals unity; *3*) if deuteration occurs with inversion of configuration, its rate is half that of racemization (*i.e.*, equal to that of enantiomerization [9]), and the ratio k_{deut}/k_{rac} equals 0.5; *4*) if racemization takes place without deuteration, the ratio k_{deut}/k_{rac} approaches zero (isoracemization). Within the context of this study, we shall concentrate on cases *2* and *3*. When the rates of deuteration reflect those of racemization (*i.e.*, $k_{deut} = k_{rac}$, case *2*), the common reaction mechanism must be of an S_E1 type; and when they reflect those of enantiomerization (*i.e.*, $k_{deut} = k_{enant} = \frac{1}{2} k_{rac}$, case *3*), the common mechanism must be of an S_E2 type (*Fig. 1*). As noted in [9] [12], we define racemization as the macroscopic and statistical process of the irreversible transformation of one enantiomer into the racemic mixture, while enantiomerization refers to the microscopic and molecular process of the reversible conversion of one enantiomer into the other. The rate of enantiomerization is half of that of racemization, since the interconversion of one molecule reduces the enantiomeric excess by two molecules.

In the α-H exchange and base-catalyzed racemization of ketones, it is well recognized that the common mechanism is of the S_E1 type, with the rate-limiting formation of a

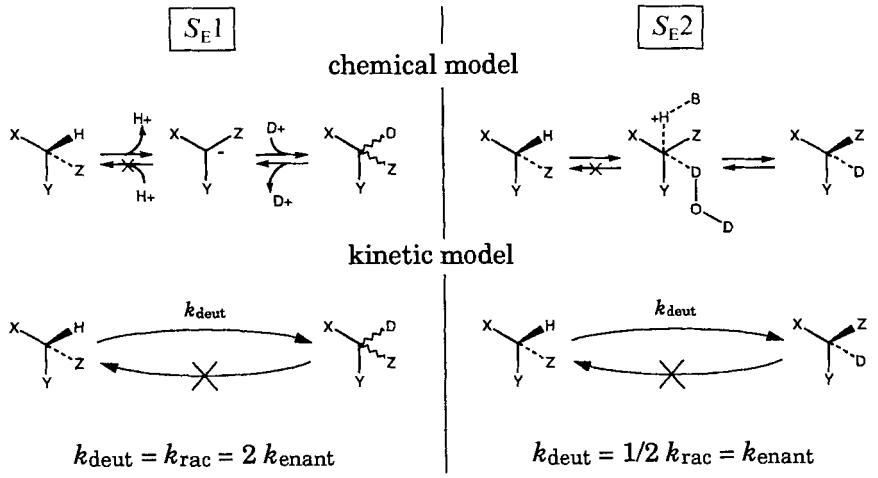


Fig. 1. *Mechanisms of H/D exchange and racemization: transition states, configuration of products, and kinetic models.* k_{deut}: rate constant of deuteration, k_{rac}: rate constant of racemization, k_{enant}: rate constant of enantiomerization.

carbanion intermediate stabilized by resonance [13–19]. In a previous study, we have examined the amino ketones amfepramone and (*RS*)-cathinone [20] and found evidence for deuteration with complete racemization (case *2*). The rates of racemization of amino ketones can thus be determined by ^{1}H-NMR in a straightforward manner using the racemates.

Besides amino ketones, various other patterns of substitution around asymmetric C-atoms are of interest in drug design, one being found in imidazolidine-2,4-diones. However, contradictory interpretations exist in the literature on the mechanism of racemization of 5-substituted hydantoins. *Dakin* [21] and *Dudley et al.* [22] suggested a mechanism of racemization involving keto-enol tautomerism in which concerted processes result in the formation of a neutral enol intermediate with loss of the chirality. *Bovarnick* and *Clarke* [23] proposed a mechanism in which rate-limiting abstraction of the proton at C(5) by a base forms a resonance-stabilized, short-lived carbanion intermediate. *Lazarus* [24] proposed a mechanism of general-base catalysis with a transition state having the proton at C(5) approximately halfway between the base and the chiral C-atom.

In the present study, evidence is presented for a S_E2 push-pull mechanism of racemization of 5-monosubstituted hydantoins. Firstly, a general-base catalysis is demonstrated. Secondly, the activation energies of deuteration and racemization are identical within experimental errors, thus confirming a common reaction mechanism. Thirdly, comparing the corresponding rates of reaction shows that deuteration occurs with inversion of configuration (*i.e.*, enantiomerization, case *3*). And finally, the marked influence of the various substituents on the stability of the chiral center and on the enthalpy and entropy of activation, together with the small isotope effects observed and the comparison of activation energies and calculated deprotonation energies, confirm the proposed reaction mechanism.

Results and Discussion. – *Syntheses.* The synthesis of six 5-monosubstituted (*S*)-hydantoins (compounds **1**, **2**, and **4**–**7**) was performed as described by *Dakin* [21] and *Suzuki et al.* [25] by condensation of the corresponding (*S*)-amino acids with KCN. This procedure is described to proceed with little or no racemization, at least for aliphatic 5-substituents [26]. This was confirmed here for (*S*)-5-benzylhydantoin (**4**), isolated with an enantiomeric excess (e.e.) of 99.0% as determined by chiral HPLC on a β-cyclodextrin column. In contrast, (*S*)-5-phenylhydantoin (**1**) was found to undergo partial racemization during synthesis (68% e.e. by chiral HPLC of the isolated product) in agreement with the facile racemization observed for this compound under acidic conditions [27].

The β-cyclodextrin column used for chiral HPLC could resolve only the enantiomers of 5-monosubstituted hydantoins containing an aromatic moiety (*e.g.* benzyl, 4-fluorobenzyl, phenyl, and 4-hydroxyphenyl) [28]. Hence, the presumably high (see above) enantiomeric purities of the aliphatically substituted (*S*)-5-isopropyl- (**7**), (*S*)-5-methyl- (**5**), (*S*)-5-(hydroxymethyl)- (**2**), and (*S*)-5-(carboxymethyl)hydantoin (**6**) could not be quantified by chiral HPLC. The specific rotations of these compounds, with the exception of **2**, were similar to those described in the literature.

Effect of Buffer Concentration on Rates of H/D Exchange and Racemization. It was first verified that the isotopic exchange of 5-monosubstituted hydantoins is subject to general-base catalysis. The influence of phosphate concentration on the H/D exchange of (*S*)-5-methylhydantoin (**5**) was investigated by ^{1}H-NMR at 37° and at four buffer concentrations having a pD value of 7.4 and a constant ionic strength of 1.1. The observed pseudo-first-order rate constants (in h^{-1}) of H/D substitution were linearly dependent on phosphate concentration (in M; individual results not shown). *Eqn. 1* describes this linear function for (*S*)-5-methylhydantoin:

$$k_{deut} = 0.052\ (\pm 0.002) \cdot c_{phosphate} - 0.0033\ (\pm 0.0006) \qquad (1)$$
$$n = 4,\ r^2 = 0.997;\quad s = 0.0005;\quad F = 3502$$

where n is the number of phosphate concentrations investigated, r^2 the squared correlation coefficient, s the standard deviation of the residuals, and F the *Fisher* test for significance of the equation (95% confidence limits are given in parentheses).

Previous studies have examined the influence of buffer concentration on the rate of racemization of (*S*)-5-phenyl- (**1**) and (*S*)-5-benzylhydantoin (**4**) [22] [24]. A number of buffers were tested (*e.g.*, phosphate, triethanolamine, arsenate, imidazole, carbonate, and *Tris*). For both compounds, the pseudo-first-order rate constant of racemization was found to increase linearily with increasing buffer concentration and with increasing pH [22] [24]. Together with our results, this confirms a mechanism involving general-base catalysis for the chiral inversion and H/D exchange of 5-monosubstituted hydantoins.

Comparison of Activation Energies of H/D Exchange and Racemization. The comparison between H/D substitution monitored by ^{1}H-NMR and racemization investigated by chiral HPLC was performed for (*S*)-5-phenyl- (**1**) and (*S*)-5-benzylhydantoin (**4**) because of their good resolution on the chiral stationary phase. Identical solutions (solvent mixture, pH or pD, ionic strength, concentrations of test compound and of sodium 3-(trimethylsilyl)(D_4)propionate) were investigated under identical conditions (four temperatures between 26° and 80°, sealed tubes) by both methods. To detect a possible solvent isotope effect, chiral HPLC experiments were performed in deuterated and in non-deuterated solvents.

The rate constants so obtained will be discussed in the next section. Before so doing, however, it is necessary to compare the activation energies derived from these rate constants to ascertain that deuteration and racemization proceed by the same mechanism. *Table 1* shows the activation energies of deuteration ($E_{a(deut)}$), racemization in non-deuterated solvents ($E_{a(racH)}$), and racemization in deuterated solvents ($E_{a(racD)}$), as calculated from *Arrhenius* plots. Within experimental errors, each compound had the same activation energy in all three reactions. This confirms a common mechanism of H/D substitution and racemization for 5-substituted hydantoins.

Table 1. *Comparison of Deuteration and Racemization of* **1** *and* **4**. Pseudo-first-order rate constants k and activation energies E_a.

	Substituent at C(5)	Temp. [°C]	k_{deut}[a]) [h^{-1}]	k_{racD}[a]) [h^{-1}]	k_{racH}[b]) [h^{-1}]
1	Ph	26	1.45 ± 0.10	1.95 ± 0.01	2.34 ± 0.05
		30	2.18 ± 0.17	3.20 ± 0.17	3.52 ± 0.03
		40	6.52 ± 0.85	8.38 ± 0.28	9.22 ± 0.03
		50	11.3 ± 1.9	21.1 ± 1.4	23.7 ± 3.0
			$E_{a(deut)}$ = 17.89 ± 0.98[c])	$E_{a(racD)}$ = 18.86 ± 0.30[c])	$E_{a(racH)}$ = 18.50 ± 0.36[c])
4	$PhCH_2$	50	0.060 ± 0.004	0.094 ± 0.016	0.119 ± 0.006
		60	0.244 ± 0.045	0.346 ± 0.031	0.462 ± 0.016
		70	0.577 ± 0.047	0.938 ± 0.070	1.098 ± 0.097
		80	1.41 ± 0.24	2.39 ± 0.13	2.854 ± 0.101
			$E_{a(deut)}$ = 23.19 ± 0.61[c])	$E_{a(racD)}$ = 24.39 ± 0.72[c])	$E_{a(racH)}$ = 23.60 ± 0.67[c])

[a]) k_{deut} and k_{racD} in a mixture of phosphate buffer (pD 7.4, 0.1M, ionic strength 0.22) and (D_6)DMSO in the proportion 1:1 (v/v).

[b]) k_{racH} in a mixture of phosphate buffer (pH 7.4, 0.1M, ionic strength 0.22) and DMSO in the proportion 1:1 (v/v).

[c]) E_a in kcalmol^{-1}.

Comparison of the Rate Constants of Deuteration and Racemization: Mechanistic Implications. Table 1 compiles the pseudo-first-order rate constants of deuteration (k_{deut}) and of racemization in light (k_{racH}) and heavy (k_{racD}) solvents at four different temperatures for (*S*)-5-phenyl- and (*S*)-5-benzylhydantoin. At first sight, the pseudo-first-order rate constants of isotopic substitution k_{deut} are approximately half of those of racemization k_{rac} for both substances. In fact, for (*S*)-5-phenylhydantoin (**1**) the ratio k_{deut}/k_{racD} is 0.68(±0.11), while the ratio k_{deut}/k_{racH} is 0.61(±0.10). For (*S*)-5-benzylhydantoin (**4**), the same ratios are 0.64(±0.05) and 0.51(±0.02), respectively. This means that, at the microscopic and molecular level, the H/D substitution occurs with inversion of configuration. Hence, we can exclude reaction mechanisms with symmetrical intermediates such as neutral enols or resonance-stabilized carbanions, since their deuteration would occur equally from either side, and, thus, the rate of deuteration would be identical with that of racemization [10].

A more likely mechanism involves H abstraction from the chiral center by a base, taking place simultaneously with the distal approach and D-bonding of a D donor (*e.g.*, a heavy water molecule). Two possible mechanisms for which isotopic substitution results in inversion of configuration can be suggested: *1*) an S_E1 mechanism with a very short-

lived asymmetrically solvated carbanion intermediate, and *2*) an S_E2 push-pull mechanism. In the former mechanism, short-lived carbanions are formed in highly solvating media with the solvent approaching preferentially from the face distal to the leaving group due to the shielding effect of the latter. The result is an asymmetrical solvation, and the predominant product will have an inverted configuration [10] [29].

In such S_E1 reactions, the type of steric behavior during isotopic exchange is believed to depend on the solvent and base used [11]. Thus, retention of configuration (*i.e.*, isoinversion) was observed in nondissociating nonpolar solvents, racemization in polar aprotic solvents like DMSO, inversion of configuration (*i.e.*, enantiomerization) in protic solvents such as dietyleneglycol, and isoracemization in aprotic solvents with aprotic bases such as tertiary amines [11] [17] [30–34]. In our investigations, the solvent was a mixture of phosphate buffer and DMSO (1:1 (*v/v*)) and the base was dibasic phosphate, hence a polar, protic solvent and a protic base. An S_E1 mechanism with a short-lived asymmetrically solvated carbanion causing inversion of configuration during isotopic exchange would, therefore, be possible.

A number of reactions can be represented satisfactorily in terms of independent bond-cleavage and bond-forming processes. These reactions follow the *Bell-Evans-Polanyi* (*BEP*) principle [35], the first corollary of which can be stated as follows. For a group of related *BEP* reactions, there is an approximately linear relation between the activation energy and the heat of reaction. Aliphatic electrophilic substitutions of the two-step (S_E1) type, with formation of the intermediate carbanion as rate-determining step, are expected to follow the *BEP* principle [35]. Thus, the heat of S_E1 reactions, *i.e.*, the difference between the enthalpies of the neutral molecule and the intermediate carbanion, which corresponds to the calculated deprotonation energy in the gas phase, is considered to be correlated with the activation energy of the substitution reaction. To verify whether H/D substitution and racemization of 5-monosubstituted hydantoins follow the *BEP* principle, we investigated the influence of seven different 5-substituents on the H/D-exchange reaction rates and activation parameters, and calculated the corresponding deprotonation energies.

Influence of Functional Groups on the Rate of H/D Substitution. To study the influence of the 5-substituent on the configurational stability of hydantoins **1**–**7**, H/D-substitution rates in a solvent mixture of phosphate buffer (pD 7.4, 0.1M, ionic strength 0.22) and (D_6)DMSO in the proportion of 1:1 (*v/v*) were determined for all compounds by ^{1}H-NMR at four or five different temperatures between 26° and 80°. The addition of 50% (D_6)DMSO as co-solvent was necessary to solubilize some of the compounds. No hydrolysis was observed under these conditions.

Semi-logarithmic rate plots of the rates of deuteration at 50° are shown in *Fig. 2*, while the calculated pseudo-first-order rate constants k_{deut} are compiled in *Table 2*. The rates of deuteration decreased in the order Ph > $HOCH_2$ > ureido > $PhCH_2$ > Me > $HOOCCH_2$ > i-Pr. Half-lives of deuteration at 50° varied from 3.8 min for (*S*)-5-phenylhydantoin (**1**) to 115.5 h for (*S*)-5-isopropylhydantoin (**7**), a 1800-fold difference. The deuteration and racemization of (*S*)-5-phenylhydantoin (**1**) was *ca.* 190 times faster than that of (*S*)-5-benzylhydantoin (**4**; *Table 1*). Interestingly, *Lazarus* [24] reported the same ratio in the rates of racemization of the two compounds in 100% aqueous phosphate buffers. This suggests that the addition of 50% DMSO as co-solvent does not have a significant influence on the relative rates of deuteration of the investigated compounds.

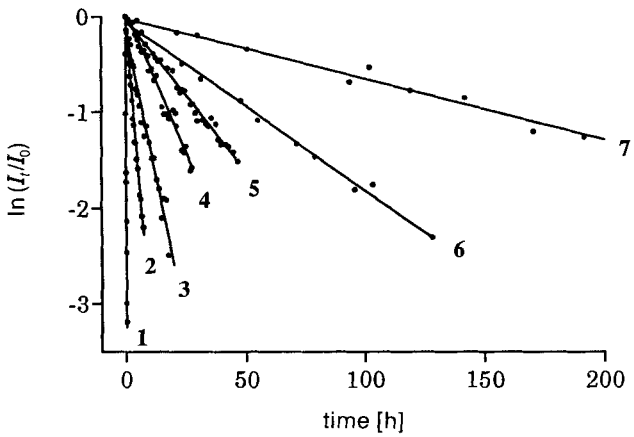


Fig. 2. *Pseudo-first-order rate plots for the deuteration of the investigated 5-monosubstituted hydantoins* **1**–**7** *at 50°.* I_t: integral of exchanging CH proton at time t; I_0: integral of exchanging CH proton at time 0.

Table 2. *Pseudo-First-Order Rate Constants and Activation Parameters of Deuteration, and Calculated Deprotonation Energies* (AM1) *of 5-Monosubstituted Hydantoins*

	Substituent at C(5)	k_{deut} at 50° [h^{-1}]	$E_{a(deut)}$ [kcal mol^{-1}]	$\Delta H^{\neq}$ [kcal mol^{-1}]	$\Delta S^{\neq}$ [e.u.]	$\Delta G^{\neq}$ [kcal mol^{-1}]	E_{calc} [kcal mol^{-1}]
1	Ph	11.3 ± 1.9	17.89 ± 0.98	17.27 ± 0.95	−16.3 ± 1.2	22.6 ± 2.8	333.4
2	$HOCH_2$	0.280 ± 0.055	18.96 ± 0.67	18.33 ± 0.67	−20.7 ± 1.1	25.0 ± 2.3	342.5
3	$NH_2(O)NH$	0.118 ± 0.006	23.16 ± 0.51	22.49 ± 0.49	−9.4 ± 0.3	25.5 ± 1.3	343.8
4	$PhCH_2$	0.060 ± 0.004	23.19 ± 0.61	22.53 ± 0.60	−10.3 ± 0.4	25.9 ± 1.6	346.8
5	Me	0.033 ± 0.001	21.04 ± 0.51	20.39 ± 0.50	−18.4 ± 0.7	26.3 ± 1.6	349.1
6	$HOOCCH_2$	0.017 ± 0.001	24.21 ± 0.78	23.53 ± 0.76	−10.1 ± 0.5	26.8 ± 2.1	421.7
7	i-Pr	0.0060 ± 0.0007	19.95 ± 0.75	19.28 ± 0.72	−25.2 ± 1.6	27.4 ± 2.8	348.9

Activation Parameters of Deuteration. Arrhenius plots for the H/D substitution of the investigated hydantoins are shown in *Fig. 3*. A comparison of the activation parameters (*Table 2*), as derived from *Arrhenius* equations and *Eyring* thermodynamic principles [36], shows that neither enthalpies ($\Delta H^{\neq}$) nor entropies of activation ($\Delta S^{\neq}$) are correlated with the order of reactivity. Differences in $\Delta H^{\neq}$ vary from 17.3 to 23.5 kcal mol^{-1} and differences in $\Delta S^{\neq}$ range from −9.4 to −25.2 e.u.

For 5-phenyl- (**1**) and 5-(hydroxymethyl)hydantoin (**2**) the determining factor in the rate of substitution is the comparatively low enthalpy of activation. For 5-ureido- (**3**) and 5-benzylhydantoin (**4**), which appear to have a relatively high $\Delta H^{\neq}$, the rate must be determined by the comparatively small negative entropy of activation. The difference in deuteration rates of 5-methyl- (**5**) and 5-isopropylhydantoin (**7**), which have a comparable $\Delta H^{\neq}$, can be explained by the considerably larger negative $\Delta S^{\neq}$ of the latter. Hence, the configurational stability of the investigated hydantoins depends on both the enthalpy and entropy of activation. This suggests that inductive and resonance effects on the stabilization of the carbanion transition state (which are reflected in a change of $\Delta H^{\neq}$) are only partially responsible for the observed influences of substituents on the rates of

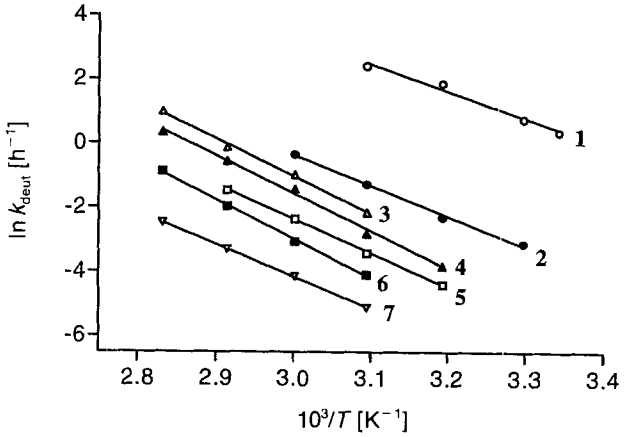


Fig. 3. Arrhenius *plots for the deuteration of the investigated 5-monosubstituted hydantoins* **1–7**

deuteration. Solvation and steric effects (which alter $\Delta H^{\neq}$ and $\Delta S^{\neq}$) must be similarly important.

A preliminary conclusion is that, because of the strong influence of entropy on the rates of deuteration and racemization, no general rules can be derived to predict substituent effects on the chiral stability of 5-monosubstituted hydantoins.

Correlation between the Energy of Activation and the Calculated Energy of Deprotonation: Mechanistic Implications. Table 2 shows the calculated energies of deprotonation (*i.e.*, the sum of the heats of formation of the carbanion and proton, minus the heats of formation of the neutral molecules) which were obtained by semi-empirical molecular-orbital calculations performed at an AM1 *Hamiltonian* level [37]. As can be seen in *Fig. 4*, no correlation between the activation energy $E_{a(deut)}$ and the calculated energy E_{calc} can be observed. This result implies that the reaction does not follow the *BEP* principle, and it

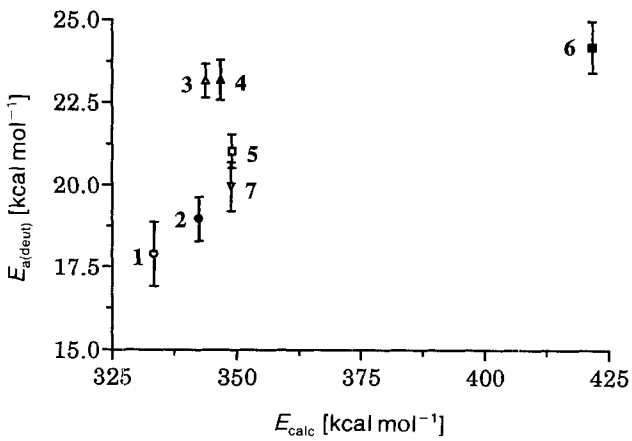


Fig. 4. *Lack of correlation between the activation energy of deuteration* $E_{a(deut)}$ *and the calculated deprotonation energy* E_{calc} *for the investigated 5-monosubstituted hydantoins* **1–7**

does not lend support to an S_E1 mechanism with a short-lived carbanion intermediate. Thus, an S_E2 push-pull mechanism becomes the likely route. To confirm this hypothesis, isotope effects were investigated.

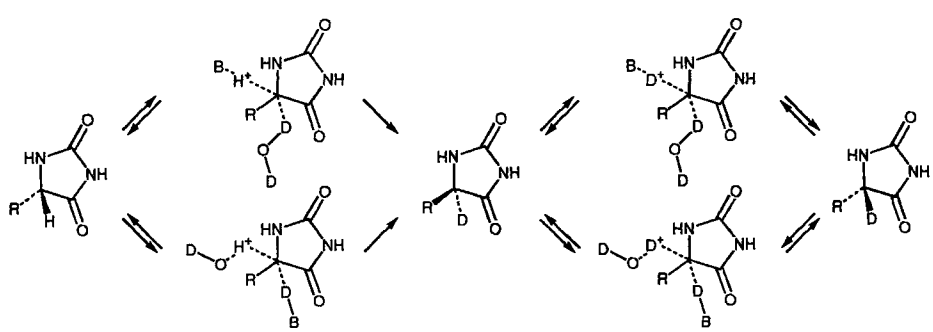
Isotope Effects: Mechanistic Implications. Comparing the rates of racemization in light and heavy solvents reveals a modest solvent isotope effect (*Table 1*). The ratios k_{racH}/k_{racD} were 1.13(±0.05) and 1.24(±0.08) for (*S*)-5-phenyl- (**1**) and (*S*)-5-benzylhydantoin (**4**), respectively. Such a minute solvent isotope effect offers another indication favoring an S_E2 push-pull mechanism in the racemization of 5-substituted hydantoins. Since enantiomerization is a reversible process, a deuterated molecule may invert again with cleavage of a C–D bond instead of a C–H bond. In an S_E1 mechanism, the C–H (or C–D) bond cleavage is the rate-determining step, and, therefore, a large solvent isotope effect is expected. Thus, *Wilson* [38] found an isotope effect of 3.0 to 4.4 for the racemization of α-acidic ketones, where racemization, deuteration, and halogenation occur with identical rates [13–19].

Conclusions and Implications. – The findings reported here have a number of implications for drug design and development. First, a further example is offered of general-base catalysis in the deuteration and racemization of chiral compounds [20] [22] [24]. A pharmacological implication is that data on chemical racemization obtained in the test tube will be poorly transposable to *in vivo* conditions, since a number of physiological bases may act in the body. The best model system to study chemical racemization may be blood plasma.

The strong dependence of configurational stability on both enthalpies and entropies of activation suggests that solvation and steric effects act together in influencing the rate of chemical racemization of 5-substituted hydantoins. Due to the influence of entropy on rate constants, no general rules can be derived for a quantitative prediction of substituent effects on racemization.

In the present study, evidence is presented for an S_E2 push-pull mechanism of the racemization and deuteration of 5-monosubstituted hydantoins, the latter reaction seemingly occurring with inversion of configuration at the molecular level (*Scheme*). This conclusion is based on a number of facts: *a*) the observed k_{deut}/k_{rac} ratio of *ca.* 0.5; *b*) the

Scheme. *Suggested General-Base-Catalyzed* S_E2 *Push-Pull Reaction Mechanism of H/D Exchange and Racemization for 5-Monosubstituted Hydantoins.* H or D abstraction from the chiral centre by a base (B, or DO^-) takes place simultaneously with the distal approach and D-bonding of a D donor (D_2O or BD). Both transition states occur with equal probability.



influence of functional groups on the rate of deuteration; *c*) the absence of correlation between the measured energies of activation and the calculated energies of deprotonation; and *d*) the small solvent isotope effects. In contrast, an S_E1 mechanism appears to be the rule for the racemization and deuteration of chiral α-amino ketones [20].

The use of ^{1}H-NMR was found to be an alternative method to chiral approaches for the determination of rates of racemization. This method can be very useful in the early stages of drug development, providing information on the chemical stability of drug candidates prior to preparing the separate enantiomers. Once the nature of the common mechanism of H/D substitution and racemization is known, reliable rates of racemization can be obtained by ^{1}H-NMR. If, in contrast, the ratio k_{deut}/k_{rac} is unknown, the relative rates of deuteration will nevertheless be of indicative value, since they can differ from the rates of racemization by a factor of two at the most, except in cases of isoinversion or isoracemization (see the *Introduction*).

Experimental Part

Materials. (*RS*)-5-Ureidohydantoin (= allantoin; **3**; *purum*) was purchased from *Fluka Chemie AG* (Buchs, Switzerland). The deuterated solvents were purchased from *Armar* (Döttingen, Switzerland), and their isotopic purities were: D_2O and (D_6)DMSO 99.8 atom-% D, sodium deuterium oxide 40% in D_2O > 99.5 atom-% D, and phosphoric acid 85% in D_2O > 99 atom-% D. All other chemicals and solvents used were obtained from *Fluka Chemie AG* or *Merck* (Darmstadt, Germany); they were of anal. grade and used without further purification. Phosphate buffers were prepared in D_2O as well as in H_2O. The pD (or pH) was adjusted with NaOD or D_3PO_4 (or NaOH or H_3PO_4), and constant ionic strengths were obtained by adding appropriate amounts of KCl. The pD and pH values were measured at r.t. (25°) using a *Metrohm 654* pH-meter (Herisau, Switzerland) coupled with a glass electrode and calibrated with standard aq. buffers. The pD values were obtained by adding a correction factor of 0.4 to the measured pH values [39–41].

Syntheses. Optically active hydantoins were prepared from the corresponding (*S*)-amino acids by condensation with KCN according to Method 1 of *Suzuki et al.* [25] with the following modifications: The preparation of (*S*)-5-(carboxymethyl)hydantoin (**6**) necessitated the addition of 2.2 equiv. KCN; (*S*)-5-methylhydantoin (**5**), (*S*)-5-(hydroxymethyl)hydantoin (**2**), and **6** only precipitated from the reaction mixture after evaporation to approximately half volume. The recrystallized hydantoins were pure by TLC on silica gel *60 F_{254}* pre-coated on aluminium sheets (*Merck*) developed with BuOH/AcOH/H_2O 4:1:1 and visualized by UV and by spraying with $KMnO_4$ or ninhydrin solns. The IR spectra, recorded from KBr disks on a *Perkin-Elmer 781* grating infrared spectrophotometer, and ^{1}H-NMR spectra, recorded from (D_6)DMSO solns. at 200 MHz on a *Bruker AC200F* instrument, showed the expected [25] [42] signals. Optical rotations were measured on a *Perkin-Elmer 241* polarimeter using thermostated microcuvettes with 10-cm light path, and enantiomeric purities (for 5-benzyl- and 5-phenylhydantoin) were determined as relative peak areas by chiral HPLC on a 244 × 4 mm *ChiraDex β*-cyclodextrin column (*Hewlett-Packard*) eluted at ambient temp. with 0.8 ml/min of 50 mM Na_3PO_4, pH 4.1, using a *Waters M590* pump, a *Rheodyne 7125* injector, and a *Waters M481* UV detector at 210 nm connected to a *Merck-Hitachi Chromato-Integrator D-2000*. Racemic 5-benzyl-, 5-(4-fluorobenzyl)-, 5-(4-hydroxyphenyl)-, 5-isopropyl-, 5-(hydroxymethyl)-, and 5-methylhydantoin (obtained as gifts from Prof. *C. Syldatk*, University of Stuttgart, Germany) were also used in the chiral HPLC investigations.

(S)-5-Phenylimidazolidine-2,4-dione (**1**): yield 89%. M.p. 173–174° (recryst. from H_2O). $[\alpha]_D^{25} = +80 \pm 3°$ (c = 1.08, EtOH) ([27]: $[\alpha]_D^{25} = +112$ (c = 0.20, EtOH)). Enantiomeric purity 68% e.e. by chiral HPLC.

(S)-5-(Hydroxymethyl)imidazolidine-2,4-dione (**2**): yield 56%. M.p. 187–190° (recryst. from EtOH) ([25]: 188°). $[\alpha]_D^{25} = -96 \pm 3$ (c = 1.31, H_2O) (calc. from [25]: $[\alpha]_D^{28} = -216$ (EtOH)).

(S)-5-Benzylimidazolidine-2,4-dione (**4**): yield 82%. M.p. 174–175° (recryst. from EtOH). M.p. 179–180° with partial melting at 174–175° when recryst. from H_2O ([24]: 173°; [25]: 182°). $[\alpha]_D^{24} = -103 \pm 3$ (c = 1.04, EtOH) (calc. from [25]: $[\alpha]_D^{24} = -96$ (EtOH)). Enantiomeric purity 99.0% e.e. by chiral HPLC.

(S)-5-Methylimidazolidine-2,4-dione (**5**): yield 27%. M.p. 172–174° (recryst. from H_2O) ([43]: 183–184°; [25]: 177°). $[\alpha]_D^{25} = -39 \pm 1$ (c = 1.09, EtOH) ([43]: $[\alpha]_D^{25} = -42$ (c = 0.2, EtOH); calc. from [25]: $[\alpha]_D^{28} = -42°$ (EtOH)).

(S)-2,5-Dioxoimidazolidine-4-carboxylic Acid (**6**): yield 49%. M.p. 215–218° (dec.; recryst. from H_2O) ([25]: 218°). $[\alpha]_D^{25} = -97 \pm 4$ (c = 0.51, H_2O) (calc. from [25]: $[\alpha]_D^{24} = -95°$ (H_2O)).

(S)-5-Isopropylimidazolidine-2,4-dione (**7**): yield 72%. M.p. 147–148° (recryst. from H_2O) ([43]: 151–153°; [25]: 145°). $[\alpha]_D^{25} = -95 \pm 3$ ($c = 1.10$, EtOH) ([43]: $[\alpha]_D^{25} = -92°$ ($c = 0.2$, EtOH); calc. from [25]: $[\alpha]_D^{28} = -94°$ (EtOH)).

Determination of H/D Substitution Rates by ^{1}H-NMR. All ^{1}H-NMR spectra and integrals were recorded on a *Varian VXR-200* NMR spectrometer operating at 200 MHz (*Varian Associates Inc.*, Palo Alto, California, USA). To study the general-base catalysis, rates of H/D substitution of 0.13M solns. of **5** prepared in phosphate buffers of four different concentrations (0.1M, 0.2M, 0.35M, 0.5M) were measured at constant temp. (37.0 ± 0.2°), constant pD (7.40 ± 0.005), and constant ionic strength (1.1). To investigate substituent effects on the configurational stability of the seven hydantoins, rates of H/D substitution were measured at four different temp. between 26 and 80° in a solvent mixture of phosphate buffer (pD 7.4, 0.1M, ionic strength 0.22) and (D_6)DMSO in the proportion 1:1 (*v*/*v*). The final hydantoin concentration varied between 0.1 and 0.16M, a concentration domain for which a preliminary experiment showed that hydantoin concentration did not influence the rate of H/D exchange. After addition of sodium 3-(trimethylsilyl)(D_4)propionate (5 mg) to calibrate the NMR spectra, each soln. was placed in a sealed NMR tube and kept in a water bath (*Haake D1*, *Haake GmbH*, Karlsruhe, Germany; temp. variation ±0.2°) at the appropriate temp. ^{1}H-NMR Spectra were recorded at regular time intervals depending on the rate of H/D substitution. For all compounds, the H/D exchange was monitored by integration of the signal of the exchanging CH proton coupled to the chiral C(5)-atom ad the signal of an appropriate unexchangeable reference H-atom. The observed pseudo-first-order rate constants of H/D substitution were obtained by plotting the natural logarithm of the decreasing integral as a function of time according to *Eqn. 2*:

$$\ln (A_t/B_t) = -k_{deut} \cdot t \tag{2}$$

where A_t was the integral of the exchanging CH proton at time t, B_t the integral of the unexchangeable reference protons at time t, and k_{deut} the observed pseudo-first-order rate constant of H/D substitution.

Determination of Rates of Racemization by Chiral HPLC. The HPLC system consisted of an autosampler of *Model 360*, a pump of *Model 420*, a diode array detector of *Model 440*, a MT2/DAD data system of *Model 450*, a plotter of *Model 800* (all *Kontron Instruments*, Zürich-Müllingen, Switzerland), and a *Haake D1-GH* thermostat (*Haake GmbH*, Karlsruhe, Germany). The enantiomers of **1** (resolution factor 1.9) and **4** (resolution factor 1.4) were separated on a *ChiraDex β*-cyclodextrin column 244 × 4 mm (*Merck*) of 5-μm packing and 100-Å pore size, using a mobile phase consisting of 50 mM Na_3PO_4 buffer, pH 4.1, a flow rate of 0.8 ml/min, a column temp. of 18°, and UV detection at 210 nm.

To compare the activation energies and the rate constants of isotopic exchange and chiral inversion, identical solns. (solvent mixture, pD or pH, ionic strength, hydantoin and sodium (3-(trimethylsilyl)(D_4)propionate concentration) as used for ^{1}H-NMR experiments were prepared for **1** and **4**, once with deuterated and once with non-deuterated solvents. Each soln. was placed in a sealed glass tube and kept in a water bath (*Haake D1*, Haake GmbH, Karlsruhe, Germany; temp. variation ±0.2°) at the appropriate temp. Aliquots of 10 μl were taken at regular time intervals depending on the rate of racemization, diluted with 1 ml of ice-cold mobile phase (ice-bath) to stop the reaction, and analyzed by chiral HPLC. The observed pseudo-first-order rate constants of racemization were obtained by plotting the natural logarithm of the decreasing enantiomeric excess of the (*S*)-enantiomer as a function of time according to *Eqn. 3*:

$$\ln\left(\frac{[S]_t - [R]_t}{[S]_t + [R]_t} \cdot 100\right) = -k_{rac} \cdot t \tag{3}$$

where $[S]_t$ is the concentration of the decreasing (*S*)-enantiomer at time t, $[R]_t$ the concentration of the increasing (*R*)-enantiomer at time t, and k_{rac} the observed pseudo-first-order rate constant of racemization.

All ^{1}H-NMR and chiral HPLC studies were performed in triplicate, and all reactions (*i.e.*, H/D substitutions and racemizations) were allowed to proceed for at least three half-lives. There was no detectable hydrolysis of any investigated hydantoin during the course of the substitution or racemization studies as evidenced by *a*) no additional signals in ^{1}H-NMR spectra, and *b*) no additional peaks in HPLC chromatograms.

Thermodynamic Parameters. Activation parameters were calculated from *Arrhenius* plots of ln (k_{deut}), *i.e.*, ln (k_{rac}) *vs.* $1/T$ (*Kelvin*): the activation energy was derived from the slope of the regression line ($-E_a/R$) and the preexponential factor A from the y intercept ln (A). Enthalpies of activation were obtained from *Eqn. 4*:

$$\Delta H^{\neq} = E_a - RT \tag{4}$$

and entropies of activation were calculated using the *Eqn. 5*:

$$\Delta S^{\neq} = R \cdot (\ln A - \ln (ekT/h)) \tag{5}$$

with k being the *Boltzmann* constant and h the *Planck* constant. Free energies of activation were derived from the *Gibbs-Helmholtz* equation [36].

Molecular-Orbital Calculations. The semiempirical quantum chemical method AM1 (Mopac 5.0, QCPE N° 509) was used for all calculations performed on a *Silicon Graphic 4D-320* workstation. All geometries were optimized with the keyword PRECISE [44]. Preparation of starting geometries and analyses of results were performed into the SYBYL package (*Tripos Associates*, St. Louis, MO, USA).

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