Thiohydantoins. XI. Kinetic Studies of the Alkaline Hydrolysis of 1-Acyl-2-thiohydantoins

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1-Acyl-2-thiohydantoins ionize in alkaline solution ($pK \sim 7$). In solutions more alkaline than $pH > 11$ they are rapidly hydrolyzed to 2-thiohydantoin and a carboxylic acid, by attack of a hydroxide ion on the conjugate base of the 1-acyl-2-thiohydantoin. Possible mechanisms to accord with the entropy of activation, which is less negative than usual for base-catalyzed amide hydrolyses, are discussed. 1-Benzoyl-2-thiohydantoin hydrolyzes more rapidly than 1-acetyl-2-thiohydantoin, possibly because the ground state of the former molecule is destabilized by steric effects.

Les acyl-1 thiohydantoïnes-2 s'ionisent en milieu alcalin ($pK \sim 7$). En milieu plus alcalin ($pH > 11$) ils s'hydrolisent rapidement aux acides carboxyliques et à thiohydantoïne-2, par l'attaque de l'ion hydroxide à la base conjuguée de l'acyl-1 thiohydantoïne-2. On réfléchit sur les mécanismes qui peuvent être en accord avec l'entropie de l'activation, qui est moins négative que les entropies de l'activation pour les amides plus ordinaires. Le benzoyl-1 thiohydantoïne-2 s'hydrolyse plus vite que l'acétyl-1 thiohydantoïne-2, peut-être parce que le premier est déstabilisé par les effets stériques.

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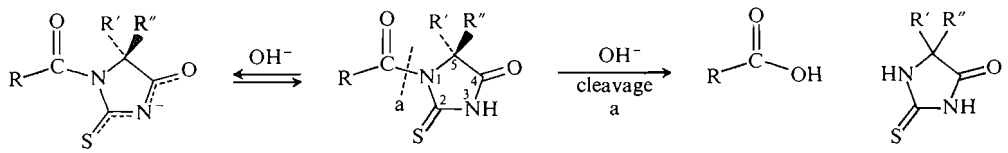
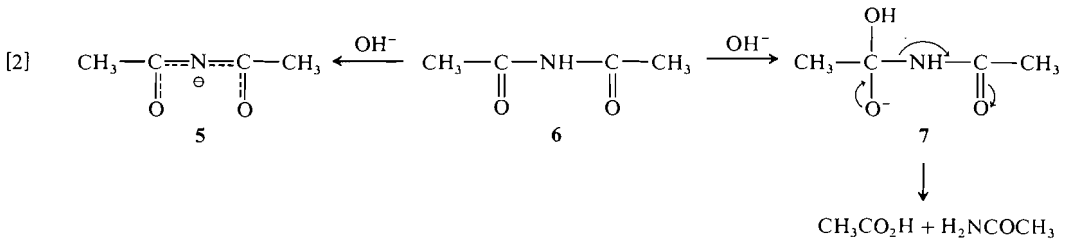
The study of the deacylation of 1-acyl-2-thiohydantoins (**1**) by acid or alkali (1) is of interest in connection with the procedure of Schlack and Kumpf (2) for the stepwise degradation of peptides or proteins. This procedure received only desultory attention for many years (3), but lately has been the focus of renewed interest (4). It consists of reacting the C-terminal amino acid of a polypeptide (RCO—NHCH$_1$R″—COOH) or protein with ammonium thiocyanate to give a 2-thiohydantoin derivative, attached to the remainder of the peptide chain by a 1-acyl linkage, as in **1** (R = polypeptide, R′ = H). This 1-acyl linkage can then be selectively cleaved by acid or alkali under unusually mild conditions (1) to give the polypeptide (**2**) minus its original C-terminal amino acid, the latter being now incorporated in a 2-thiohydantoin (**3**) (see eq. 1). This facility of hydrolysis is shared by other amides (5–7) and esters (8) having electron-attracting groups attached to the nitrogen or oxygen atom which separates from the acyl group in hydrolysis; there is a very rough correlation between the rate of the reaction and the stability of the anion A^- derived by ionization of the amino or alcohol moiety HA formed in the hydrolysis (as measured by the pK_{HA} of HA (9–11)).

The mechanisms for the alkaline hydrolysis of various amides differ with regard to the timing of various proton-transfers (see DeWolfe and Newcomb (12) for an excellent, brief review), and reference to them will be made later. However, we should first note that *p*-nitroacetanilide (pK_{HA} 13.8 (6)) and diacetylimine (**6**) (pK_{HA} 12.9 (7)) ionize as acids in dilute aqueous sodium hydroxide. Kinetic studies (6, 7) indicate that the hydrolysis of both of these compounds takes place by attack of hydroxide ion on the unionized compound (*e.g.*, **6**), and not on the anion (*e.g.*, **5**), presumably because the negative charge of the latter is delocalized over the amide

[1] 

4 **1** **2** **3**

[1]Holder of NRCC Studentship, 1968–1970.

[2] 

carbonyl group and decreases its electrophilic character drastically (see eq. 2).

1-Acyl-2-thiohydantoins also ionize as weak acids ($\mathbf{1} \rightarrow \mathbf{4} + H^+$), and have pK_a values of 6.5–7.0 (Table 1). Consequently, in the solutions more alkaline than p*H* 9, in which they begin to be hydrolyzed at appreciable rates, they must be more than 99% in the ionized form **4**. However, the negative charge of **4** is not delocalized over the 1-acyl carbonyl group which is attacked in hydrolysis, and so this charge might be expected to reduce its reactivity by a factor of about 10^2 (*cf.* ref. 13) or less. In such a case the greater reactivity of **1** would not be expected to compensate for the greater abundance of **4**. This expectation is realized: the rates for the hydrolysis of various 1-acyl-2-thiohydantoins in a large excess of buffer or alkali (at constant ionic strength) is first-order in hydroxide ion concentration, as shown by the invariance of the second order rate constants k_b ($=k_\psi/[OH^-]$) in Table 2. Consequently, hydrolysis must proceed mainly by attack of

TABLE 1. pK_{HA} values of 1-acyl-2-thiohydantoins in water at 25°

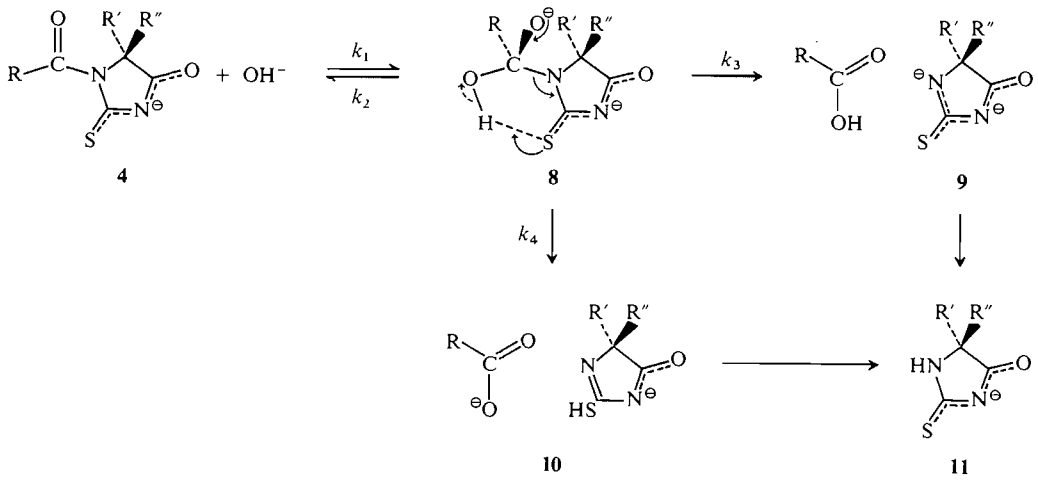
Compound	pK_{HA}
1-Acetyl-2-thiohydantoin*	7.03
1-Acetyl-5-methyl-2-thiohydantoin†	6.95
1-Acetyl-5,5-dimethyl-2-thiohydantoin‡	6.93
1-Benzoyl-2-thiohydantoin*	6.50
1-*p*-Anisoyl-2-thiohydantoin†	6.65
1-*p*-Nitrobenzoyl-2-thiohydantoin†	6.45

*Unpublished work by I. C. Wang.
†Reference 28.
‡Unpublished work by I. Lantos.

TABLE 2. The effect of p*H* and ionic strength on the rate of base-catalyzed hydrolysis of 1-acetyl-5,5-dimethyl-2-thiohydantoin at 25.4 °C

Buffer composition*	p*H*	Ionic strength	$k_\psi \times 10^4$ (s^{-1})	k_b ($l\ mol^{-1}\ s^{-1}$)
0.03 *M* K_2HPO_4, 0.01 *M* K_3PO_4	10.95†	1.0000	1.57 ± 0.05	0.176
0.0025 *N* NaOH	11.40‡	0.0025	2.04 ± 0.04	0.081
0.04 *M* K_2HPO_4, 0.04 *M* K_3PO_4	11.47†	1.0000	5.13 ± 0.08	0.174
0.08 *M* K_2HPO_4, 0.08 *M* K_3PO_4	11.56†	1.0000	6.25 ± 0.05	0.172
0.01 *M* K_2HPO_4, 0.03 *M* K_3PO_4	11.88†	1.0000	12.10 ± 0.15	0.159
0.01 *N* NaOH	12.00‡	0.0100	8.94 ± 0.23	0.089
0.01 *N* NaOH	12.00‡	0.0400	10.41 ± 0.01	0.104
0.01 *N* NaOH	12.00‡	0.0900	11.23 ± 0.23	0.112
0.01 *N* NaOH	12.00‡	0.1600	12.00 ± 0.20	0.120
0.0175 *N* NaOH	12.25‡	1.0000	25.60 ± 0.45	0.144
0.025 *N* NaOH	12.40‡	1.0000	41.40 ± 0.20	0.165
0.063 *N* NaOH	12.80‡	1.0000	102.40 ± 0.15	0.162
0.1 *N* NaOH	13.00‡	0.5000	136.50 ± 0.50	0.136
0.1 *N* NaOH	13.00‡	1.0000	148.00 ± 0.70	0.149
0.2 *N* NaOH	13.30‡	1.0000	302.00 ± 0.05	0.151
0.5 *N* NaOH	13.70‡	1.0000	764.00 ± 11.00	0.152
0.8 *N* NaOH	13.90‡	1.0000	1340.00 ± 13.00	0.169
1.0 *N* NaOH	14.00‡	1.0000	1740.00 ± 40.00	0.174

*Plus KCl to make up to ionic strength indicated.
†p*H* measured in quintuplicate (±0.04 p*H* units) with a Radiometer 202-C glass electrode. *vs.* a saturated calomel electrode on a Radiometer TTT-1 recording p*H* meter.
‡Calculated from $pH = 14.00 + \log [NaOH]$.



SCHEME 1

hydroxide ion on the anion **4** (*cf.* discussion in ref. 7).

This accords with observations of the effect of ionic strength on the rate of the reaction. For a reaction between two charged ions the observed rate will be given by:

$$[3] \quad \log k_b = \log k_0 + 1.02\, Z_a Z_b \sqrt{\mu}/(1+\sqrt{\mu})$$

where k_0 is the second order rate constant at zero ionic strength, and μ is the ionic strength (14*a*). In the present instance the ionic charges $Z_a = Z_b = -1$, so that k_b increases with ionic strength. The plot of $\log k_b$ for the hydrolysis of 1-acetyl-5,5-dimethyl-2-thiohydantoin *vs.* $\sqrt{\mu}/(1+\sqrt{\mu})$ gives a curve similar to that obtained for the base-catalyzed hydrolysis of a series of dicarboxylic acid half esters EtO—CO—$(CH_2)_n$—CO_2^- ($n = 0$–4) (15), with the slope approaching unity as $\mu \to 0$. This is shown in Fig. 1, which indicates that the second-order rate constant more than doubles on going from $\mu = 0$ to $\mu = 1$.

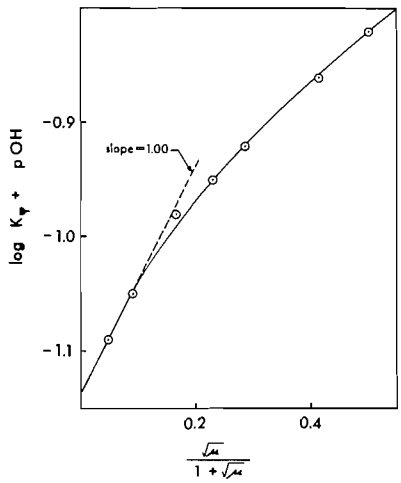


FIG. 1. A plot of $\log k_b$ ($\equiv \log k_\psi + pOH$) (in 1 mol^{-1} s^{-1}) against $\sqrt{\mu}/(1+\sqrt{\mu})$ for 1-acetyl-5,5-dimethyl-2-thiohydantoin at 25.4 °C.

The mechanisms which best accommodate these and other experimental facts discussed below are outlined in Scheme 1. Formation of the tetrahedral intermediate **8** (*cf.* refs. 5, 6, 12) will be hindered by bulky groups at the 5-position, particularly if hydrogen-bonding between the new hydroxyl group and the strongly basic sulfur atom, as shown in **8**, causes eclipsing of the groups at the 5-position with groups in the newly-tetrahedral atom. Steric hindrance will be especially severe for the solvated anionic oxygen. This may explain the retardation of alkaline hydrolysis by substitution, and more especially by geminal disubstitution, at the 5-position, as shown in Table 4. Substitution at this position is less effective in retarding acid-catalyzed hydrolysis, as can be seen by comparing the last two columns of Table 3, although it has usually been thought that steric effects on both base- and acid-catalyzed hydrolyses are the same (16). The

TABLE 3. Effects of C-5 substituents on the hydrolysis rates of 1-benzoyl-2-thiohydantoin

Substituent	$10^4 k_\psi$*	$10^4 k_\psi$†	Relative rates: Base	Relative rates: Acid
Unsubstituted	31.40±0.40	49.8±0.50	1.00	1.00
5-Methyl	12.70±0.21	35.5±0.60	0.41	0.71
5-Phenyl	10.20±0.66	22.8±0.36	0.33	0.46
5-Isopropyl	4.77±0.18	—	0.15	—
5-Isobutyl	6.64±0.15	18.4±0.54	0.21	0.37
5-sec-Butyl	4.65±0.16	17.9±0.40	0.15	0.36
5,5-Dimethyl	2.38±0.06	29.4±1.32	0.08	0.60

*Pseudo-first-order rate constants (s^{-1}) measured at 25.3 °C in a phosphate buffer at an ionic strength of 0.45 and a pH = 11.2.
†Pseudo-first-order rate constants (s^{-1}) measured at 25.3 °C in 58.0% sulfuric acid.

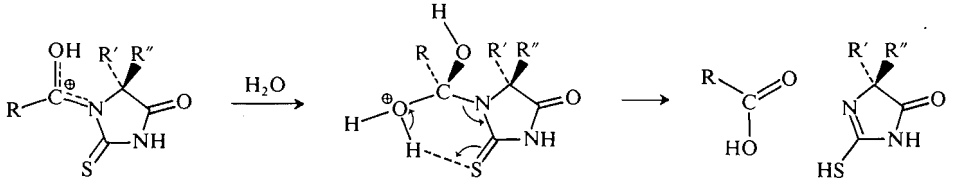
TABLE 4. Temperature dependence of the rate of alkaline hydrolysis of 1-acyl-2-thiohydantoins

Compound and buffer solution	Temperature (°C)	$10^4 k_\psi$ (s^{-1})	log A_1[a] ($l\ mol^{-1}\ s^{-1}$)	ΔS^{+}[b,c] (e.u.)	E_a (kcal mol^{-1})
1-Acetyl-2-thiohydantoin;	20.0	3.98±0.13	10.9±0.2	−11±2	18±2
pH = 10.95; μ = 1.0;	25.4	6.97±0.41			
0.03 M K_2HPO_4, 0.01 M K_3PO_4	30.4	11.50±0.15			
	35.6	18.20±0.90			
	41.5	30.80±1.40			
1-Benzoyl-2-thiohydantoin;	20.0	8.83±0.06	10.4±0.2	−13±2	16±2
pH = 10.95; μ = 1.0;	25.4	16.40±0.30			
0.03 M K_2HPO_4, 0.01 M K_3PO_4	30.4	25.60±0.06			
	35.6	39.10±1.20			
	41.5	65.80±0.50			
1-Acetyl-5,5-dimethyl-2-	11.0	3.65±0.02	10.1±0.2	−14±2	15±2
thiohydantoin	25.3	12.90±0.04			
pH = 11.95; μ = 0.55;	49.6	95.80±0.93			
0.1 M Na_2HPO_4, 0.075 M NaOH	58.1	177.00±2.10			

[a]Based on $k_b = k_\psi/[OH^-]$ in $l\ mol^{-1}\ s^{-1}$.
[b]The standard state for ΔS^+ utilizing k_b is 1 mol per liter and Δn^+ in the Arrhenius equation is assumed small since this is a bimolecular reaction in solution (14*b*).
[c]These values have been corrected for the effect of ionic strength at one temperature (25.3 °C).

difference in the present instance may reflect the difference in the proximity of the 5-substituents to the charged centers in **8** and in **12**, the tetrahedral intermediates for alkaline- and acid-catalyzed hydrolysis (17) respectively, and hence their different effects on the solvation required to stabilize these charged intermediates (see eq. 4).

The mechanism of Scheme 1 also takes account of measurements of the Arrhenius parameters for the base-catalyzed hydrolysis, which show its rapidity to be determined chiefly

[4] 

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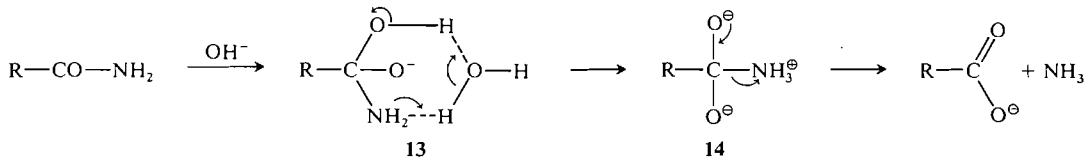

13 14

SCHEME 2

by favorable entropy factors. Plots of log $(k_\psi/[OH^-])^2$ against $1/T$ (shown in Fig. 2) give straight lines, the slopes of which give the activation energy ($E_a/2.3R$) in kcal mol^{-1} and the intercept, the logarithm of the frequency factor, log A_t, in 1 mol^{-1} s^{-1} (Table 4). This must be modified for the effect of ionic strength at one temperature to obtain meaningful entropy values.

The results in Table 4 show that the very rapid hydrolyses of 1-acetyl-2-thiohydantoin (**1**; R = Me, R′ = R″ = H) and of 1-benzoyl-2-thiohydantoin (**1**; R = Ph, R′ = R″ = H) are due to favorable entropy factors, as measured by the A_t values for the reactions, and not to low energies of activation. (For comparison,

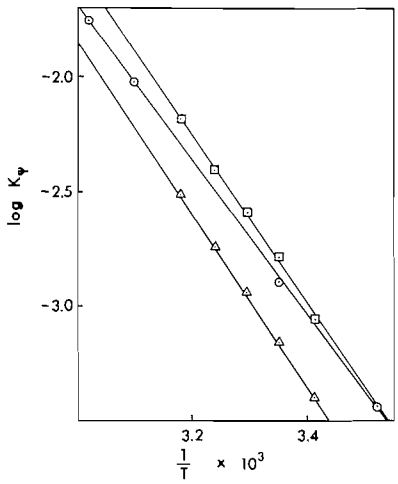

FIG. 2. Arrhenius plots for the hydrolysis of 1-acetyl-5,5-dimethyl-2-thiohydantoin (○), 1-benzoyl-2-thiohydantoin (□), and 1-acetyl-2-thiohydantoin (△) in phosphate buffer (see Table 5).

[2]Hydroxide ion concentration was calculated from pH measurements of buffer solutions at 25°. However, the hydroxide ion activity (or concentration) will not be constant for the same buffer composition but different temperatures, so the activation parameters must be regarded as only approximate. We are indebted to a referee for making this point.

log A_t for the alkaline hydrolysis of acetamide and benzamide is 4.4 and 4.2 l mol^{-1} s^{-1}, respectively, and E_a is 14.3 and 12 kcal^{-1} mol^{-1}, respectively (18)). The A_t values are derived from the overall rate constant k_b, which according to the steady-state principle applied to the mechanism of Scheme 1, should be given by

$$[5] \qquad k_b = \frac{k_1(k_3 + k_4)}{k_2 + k_3 + k_4}$$

In such a case the frequency factor A_t should depend on the individual frequency factors for the different reactions k_1, k_2, k_3, and k_4 (19). In general, the frequency factor is reduced by about 10^2 by electrostatic effects between two particles each bearing a charge of -1 (14*c*). If in the present instance the frequency factor for k_1 is so reduced, the high values of A_t must result from compensation by very high values for the frequency factors for k_3 and k_4, as compared with the frequency factor for the corresponding step in the hydrolysis of acetamide or benzamide (19). The latter is expected to be low because of stringent requirements for the ordering of solvent if, as seems probable (*cf.* refs. 5, 12), the mechanism for the hydrolysis is given by Scheme 2 with the formation of the zwitterion **14** (or something closely similar). On the other hand, the breakdown of the tetrahedral intermediate **8** for the hydrolysis of 1-acyl-2-thiohydantoins either does not require a proton-transfer (in the k_3 route), and leads to a dianion (**9**) with low solvation requirements (unlike **14**) because of the dispersal of the double charge, or else requires a proton-transfer (in the k_4 route) which can be effected internally without the necessity for ordering of solvent (as required in **13** → **14**).

While both routes (k_3 and k_4) of Scheme 1 are compatible with the evidence to date, it is difficult to know whether in fact both are kinetically significant, or whether the hydrolysis

TABLE 5. Ratio of pseudo-first-order rate constants for the hydrolysis of acetyl (k_ψ^a) and benzoyl (k_ψ^b) derivatives in alkaline and acid solutions

Compounds	k_ψ^a/k_ψ^b in base	k_ψ^a/k_ψ^b in acid
Ethyl acetate/ethyl benzoate	11.5(16)	384(16)
Acetamide/benzamide	4.1(18)	—
N-Acetylthiourea/*N*-benzoylthiourea	38.5*	22†
1-Acetyl-2-thiohydantoin/1-benzoyl-2-thiohydantoin	0.68‡	4.1§
1-Acetyl-5-methyl-2-thiohydantoin/1-benzoyl-5-methyl-2-thiohydantoin	0.95‡	4.8§
1-Acetyl-5,5-dimethyl-2-thiohydantoin/1-benzoyl-5,5-dimethyl-2-thiohydantoin	0.88‡	4.3§

*In aqueous 1 *N* NaOH at 25.3 °C.
†In 39.6% sulfuric acid at 49.1 °C.
‡In aqueous phosphate buffer, pH = 11.2, μ = 0.45.
§In 58.0% sulfuric acid at 25.3 °C.

proceeds by essentially only one route. Further investigations (*e.g.* of solvent isotope effects) would seem to be required.

A second factor promoting the rapid hydrolysis of 1-acyl-2-thiohydantoins in alkali is steric. This is shown up by an examination of the relative rates of hydrolysis of 1-acetyl- and of 1-benzoyl-2-thiohydantoin. In general, one expects amides and esters of acetic acid to be hydrolyzed more rapidly than the corresponding esters and amides of benzoic acid, and this is usually found true, as shown in Table 5. Thus ethyl acetate is saponified 11 times more rapidly than ethyl benzoate: the latter is stabilized by resonance interaction (admittedly weak (20)) between the benzene ring and the ester carbonyl group, and this resonance stabilization is lost in forming the tetrahedral intermediate. (The difference in rates becomes less for the amides, to the extent that the nitrogen atom releases electrons to the carbonyl group more readily than the oxygen atom, and so lessens the importance of phenyl–carbonyl resonance interaction.) However, surprisingly, the 1-benzoyl-2-thiohydantoins are found to hydrolyze in alkaline solution more *rapidly* than the corresponding 1-acetyl-2-thiohydantoins (Table 5).

Obviously a new factor is at work which is missing from the first three pairs of compounds of Table 5, and an examination of models suggests that this factor is steric. The 1-acyl group can be expected to be as much as possible coplanar with the thiohydantoin ring in order to maximize its amide-type resonance interaction with N-1 (21). For 1-acetyl-2-thiohydantoin, two coplanar conformations are possible: that in which the acyl carbonyl and the thiocarbonyl groups are aligned parallel; and that shown in Fig. 3, in which the acyl oxygen and thiocarbonyl sulfur are separated as much as possible. The former conformation involves very strong dipolar repulsions, and is expected to be strongly disfavored (22). However, for 1-benzoyl-2-thiohydantoin, the conformation which maintains the coplanarity of the acyl group with the thiohydantoin ring, and which avoids the dipolar repulsions, requires the benzene ring to be twisted about 90° out of the plane of the acyl group in order to avoid steric interference between the bulky sulfur atom and the ortho-hydrogens of the benzene ring (see Fig. 4).

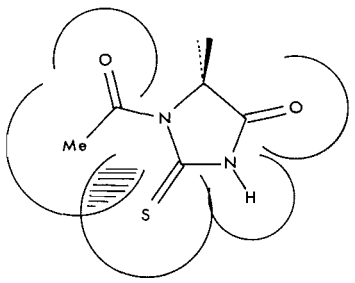

FIG. 3. Scale drawing of 1-acetyl-2-thiohydantoin, using bond-lengths and van der Waals radii from crystallographic studies of related compounds (32). The van der Waals radii for hydrogen and for the methyl group may be somewhat too large (33), and hence the overlap of methyl and sulfur would not seriously affect coplanarity of the acetyl group with the rest of the molecule.

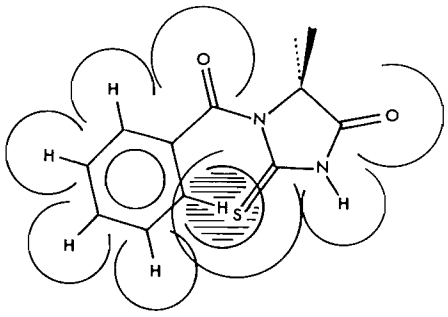

FIG. 4. Scale drawing of 1-benzoyl-2-thiohydantoin.

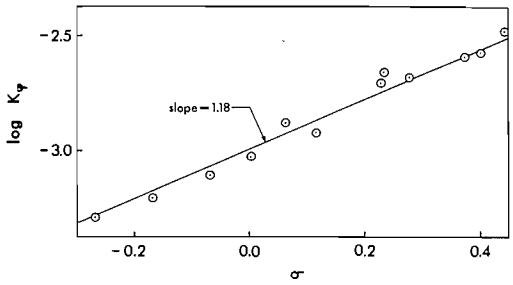

FIG. 5. A plot of log k_ψ against σ for *m*- and *p*- substituted 1-benzoyl-2-thiohydantoins (c.c.: 0.9902; s.d.: 0.05) in aqueous phosphate buffer, p*H* 10.75, $\mu = 0.42$, at 25.3 °C.

In fact, it seems likely that the steric congestion in 1-benzoyl-2-thiohydantoin is relieved by some rotation about *both* the phenyl—CO and the CO—N_1 bonds,[3] because the Hammett reaction constant ρ, given by the slope (1.18) of the plot of log k_ψ against σ for 12 *m*- and *p*-substituted 1-benzoyl-2-thiohydantoins (Table 7 and Fig. 5), has a value very close to that (1.055 (23)) reported for the alkaline hydrolysis of *m*- and *p*- substituted benzamides. This closeness is probably due to the operation of two opposing sets of factors. The lessening of electron-release from N-1 because of the juxtaposed thiocarbonyl, and because of twisting about the CO—N_1 bond, should increase ρ above 1.055; the lessening of resonance interaction between *m*- and *p*- substituents and the carbonyl group, by twisting about the phenyl—CO bond, should decrease ρ below 1.055 (24). The twisting about both Ph—CO and CO—N_1 should make the formation of the tetrahedral intermediate less costly (in terms of the loss of resonance energy), and should explain why the hydrolysis of 1-benzoyl-2-thiohydantoin goes faster than the hydrolysis of 1-acetyl-2-thiohydantoin.

It can be seen from Fig. 3 that any increase in size beyond that of the methyl group of 1-acetyl-2-thiohydantoin will lead to steric congestion between thiocarbonyl sulfur and the acyl group. This will be true for the acyl group derived from any amino acid, and so the 2-thiohydantoin derivative formed from the C-terminal amino acid of any peptide should be expected to be hydrolyzed in alkali at a greater rate than is 1-acetyl-2-thiohydantoin. It would seem, then, that the facility of Schlack and Kumpf's procedure for degrading peptides depends on favorable entropy and steric effects in the hydrolysis of the 2-thiohydantoin intermediates.

TABLE 6. Pseudo-first-order rate constants (k_ψ) for the hydrolysis of *m*- and *p*-substituted 1-benzoyl-2-thiohydantoins in alkaline phosphate buffer (p*H* 10.75, $\mu = 0.42$) at 25.3 °C

Substituent	$10^4 k_\psi$ (s^{-1})
p-Methoxy	5.12±0.15
p-Methyl	6.17±0.16
m-Methyl	7.61±0.23
(H)	9.31±0.18
p-Fluoro	13.30±0.30
m-Methoxy	11.90±0.30
p-Chloro	19.40±0.20
p-Bromo	21.90±0.28
p-Iodo	20.80±0.30
m-Chloro	25.90±0.45
m-Bromo	27.10±0.32
m-Trifluoromethyl	32.90±0.41

It is evident from Table 5 that the greater ease of acid-catalyzed hydrolysis of acetyl as compared with benzoyl derivatives extends to the hydrolysis of 1-acyl-2-thiohydantoins, in spite of the steric effects destabilizing the benzoyl derivatives. These effects would be expected to increase the rate of attack of water on the protonated 1-acyl-2-thiohydantoin to form the tetrahedral intermediate **12**; however, it is

[3]Alternatively, coplanarity of the benzene ring and the carbonyl group can be maintained if the alternative conformation having parallel carbonyl and thiocarbonyl groups is adopted. This conformation would explain the greater reactivity of 1-benzoyl- as compared with 1-acetyl-2-thiohydantoin, but is made less likely by the Hammett ρ value for the reaction.

TABLE 7. Physical properties of 5-substituted 1-acyl-2-thiohydantoins

Substituents	M.p. (°C)	Ultraviolet absorption bands* Type I λ_{max} (nm)	ε	Type II λ_{max} (nm)	ε	Type III λ_{max} (nm)	ε
1-Benzoyl-5-phenyl-	205–207†	372	130	282	9 600	242	21 900
1-Benzoyl-5-isobutyl-	169–170‡	365	220	273	11 600	242	20 200
1-Benzoyl-5,5-dimethyl-§	170–172	366	160	275	12 000	243	21 800
1-Acetyl-5-methyl-	167–168‖	366	28	277	18 800	233	14 300

*See ref. 3 for an explanation of band types.
†Kjaer reports m.p. 200–201 °C (29).
‡Cornforth reports m.p. 167–169 °C (30).
§Anal. Calcd. for $C_{12}H_{12}N_2O_2S$: C, 58.1; H, 4.8; N, 11.3. Found: C, 57.8; H, 5.0; N, 11.0.
‖Johnson reports m.p. 166° (31).

TABLE 8. Pseudo-first-order rate constants k_ψ (in s^{-1}) for hydrolysis of acetyl and benzoyl derivatives in an excess of acid or base

Compound	Base k_ψ	Acid k_ψ
N-Acetylthiourea	142.00±2.7*	8.69±0.18†
N-Benzoylthiourea	3.69±0.30*	0.39±0.01†
1-Acetyl-2-thiohydantoin	21.4 ±0.4‡	202.0 ±4.3§
1-Benzoyl-2-thiohydantoin	31.4 ±0.4‡	49.8 ±0.5§
1-Acetyl-5-methyl-2-thiohydantoin	12.0 ±0.5‡	171.0 ±1.5§
1-Benzoyl-5-methyl-2-thiohydantoin	12.7 ±0.2‡	35.5 ±0.6§
1-Acetyl-5,5-dimethyl-2-thiohydantoin	2.10±0.10‡	127.5 ±4.6§
1-Benzoyl-5,5-dimethyl-2-thiohydantoin	2.38±0.06‡	29.4 ±1.3§

*In 1 *N* NaOH at 25.3 °C.
†In 39.6% H_2SO_4 at 49.1 °C.
‡In phosphate buffer, $\mu = 0.45$, pH = 11.2 at 25.3 °C.
§In 58.0% H_2SO_4 at 25.3 °C.

apparent that this is more than offset by the decrease in the basicity of the 1-acyl group resulting from diminished Ph—CO and CO—N_1 resonance interaction (*cf.* refs. 25, 26).

Experimental

All compounds were made and purified according to the procedure of Johnson and Nicolet (27). Most were known compounds; physical properties are listed in Table 7 for compounds not described in ref. 17. Buffer solutions were made using Fisher reagent grade KCl, K_2HPO_4, K_3PO_4 and distilled water. pH values were measured at 25.4 °C using a TTT-1 automatic titrator and a Radiometer 202-C electrode *vs.* a saturated calomel electrode. pH values were found to remain constant during the reaction. For buffer solutions above pH of 12, standard B.D.H. sodium hydroxide (1 *N*) was used with KCl to prepare solutions; the pH values reported were calculated from the relation: pH = 14 + log [OH^-].

For the kinetic runs, 3.00 ml of buffer was allowed to come to thermal equilibrium in a stoppered u.v. cell contained in a thermostatted cell compartment. A methanolic solution (10 μl) of 1-acyl-2-thiohydantoin was then injected, using a Hamilton syringe with Chaney adaptor, the cell was vigorously shaken, and the absorbance (D) at constant wavelength was recorded by a Unicam SP 800 recording u.v. spectrophotometer fitted with scale expander and slave recorder attachments. Measurements were always made in the region 300–330 nm, because the 2-thiohydantoin has no appreciable absorption in this region, and so its further hydrolysis has no spectral consequences. Results were analyzed by a linear least squares program using log ($D_t - D_\infty$) *vs.* time. D_∞ was taken after 7–10 half-lives, and for several slow reactions Guggenheim's method was used. Correlation coefficients for individual runs were generally greater than 0.999 for 8 points, and separate runs were found to be reproducible to within 3–4%. The experimental data on which some of the entries of Table 6 are based are given in Table 8.

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