**1015.** *Thiohydantoins. Part II.[1] Alkaline Hydrolyses.*

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Kinetic studies indicate that the rate-determining step in the hydrolysis of 2-thiohydantoins to thioureido-acids is attack of a hydroxide ion on the un-ionised molecule. The effect of substitution of the 2-thiohydantoin ring on its rate of hydrolysis has been investigated.

In alkaline solution 5-substituted 2-thiohydantoins (I) (*a*) ionise (practically instantaneously) as weak acids to give the anions (II),[1,2] and (*b*) are hydrolysed (more slowly) to salts of the thioureido-acids (V),[3] probably *via* the intermediates (III)[4] and (IV). Because it seemed possible that the low yields[5] of 2-thiohydantoins in Schlack and Kumpf's procedure[6] for degrading proteins might be due in part to hydrolysis, we have studied the
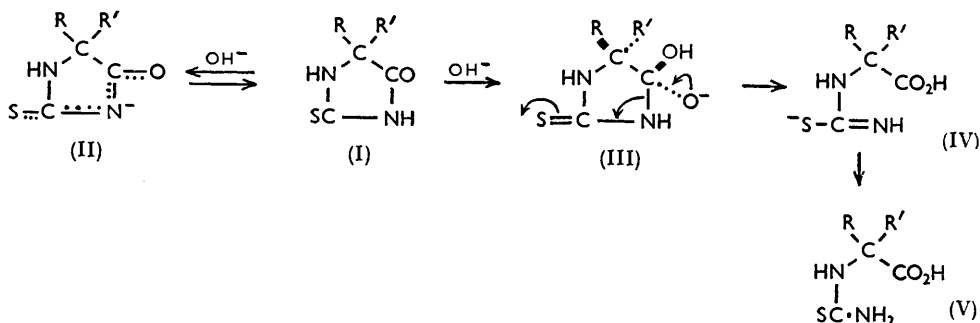
kinetics of this reaction for variously substituted compounds. The reaction was easily followed spectrophotometrically.[7] In alkaline solution the spectra changed from those characteristic of 2-thiohydantoins to those characteristic of thioureido-acids (Figs. 1 and 2). When the solutions were made strongly acid these changes were reversed.[3] The rates of hydrolysis were obtained by following the change in optical density with time at some wavelength in the region 260—270 mμ, the exact wavelength usually being that at which the change in absorption was maximal. Because the temperatures of the cells in the spectrophotometer were not controlled precisely (see p. 5083), the rates are only approximate, and this paper attempts only a preliminary survey of the mechanism of the hydrolysis.

To eliminate the complications due to ionisation,[1] the hydrolysis of 1 : 3-disubstituted 2-thiohydantoins was first studied (Table 1). These compounds were hydrolysed extremely

TABLE 1. *Alkaline hydrolysis of* 1 : 3-*disubstituted 2-thiohydantoins.*

Substituents	1 : 3-Dimethyl				3-Ethyl-1-phenyl		1-Methyl-3-phenyl	
pH	9·74	10·84	11·32	11·64	9·82	10·78	9·82	10·78
$10^2 k_e$ (min.$^{-1}$)	0·68	8·11	33·6	50·0	1·69	14·1	3·92	24·9
k_2 (l. mole^{-1} min.$^{-1}$)	124	118	128	114	256	234	593	413

rapidly in dilute alkali, and the reaction could be followed conveniently only in buffer solutions. In the large excess of buffer the rate of hydrolysis *v* was of the first order with

[1] Part I, preceding paper.
[2] du Vigneaud and Melville, " The Chemistry of Penicillin," Princeton University Press, 1949, p. 269.
[3] Ware, *Chem. Rev.*, 1950, **46**, 403.
[4] Bender and Ginger, *J. Amer. Chem. Soc.*, 1955, **77**, 348.
[5] Waley and Watson, *J.*, 1951, 2394; Edward and Nielsen, *Chem. and Ind.*, 1953, 197.
[6] Schlack and Kumpf, *Z. physiol. Chem.*, 1926, **154**, 125.
[7] Kenner, Khorana, and Stedman, *J.*, 1953, 673.

respect to thiohydantoin; however, the second-order character of the reaction was shown by the rough proportionality of the apparent first-order rate constant, k_e, to the hydroxide-ion concentration, as required by the equations:

$$v = k_e \text{ [Thiohydantoin]} \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$
$$= k_2 \text{ [Thiohydantoin][OH}^-] \quad . \quad . \quad . \quad . \quad . \quad (2)$$

where $k_2 (= k_e/[\text{OH}^-])$ is a second-order rate constant. The kinetics of alkaline hydrolysis of the amide linkage are generally of second order.[8]

Hydrolysis of 2-thiohydantoin (I; R = R' = H) in dilute alkali was slower and again appeared to be of first order with respect to the stoicheiometric concentration of thiohydantoin. However, the apparent first-order rate constants were changed only slightly
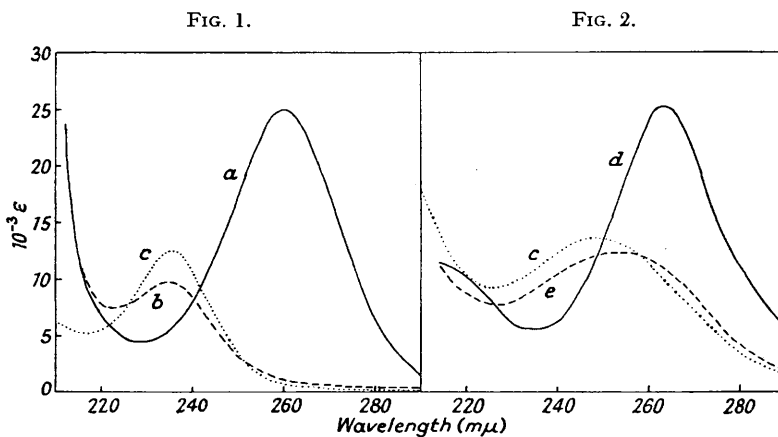

FIG. 1.   FIG. 2.

FIG. 1.   5-*Methyl-2-thiohydantoin in* 0·01N-*sodium hydroxide immediately* (*curve a*) *and* 48 *hours* (*curve b*) *after mixing; thiourea* (*curve c*) *in water*.

FIG. 2.   1-*Phenyl-2-thiohydantoin in* 0·01N-*sodium hydroxide immediately* (*curve d*) *and* 24 *hours* (*curve e*) *after mixing; phenylthiourea* (*curve c*) *in water*.

by a hundred-fold change in hydroxide-ion concentration (Table 2), indicating a zero order with respect to hydroxide ion. This kinetic order may be explained if it is assumed that only the un-ionised molecules (I) react with hydroxide ion.[9] The rate of hydrolysis is

TABLE 2.   *Hydrolysis of 2-thiohydantoin at* 21—22°.

[OH⁻] (mole/l.) ...	0·02	0·05	0·05 *a*	0·05 *b*	0·10	0·20	0·50	1·00	2·00
$10^2 k_e$ (min.⁻¹)	1·79	1·63	1·61	1·56	1·20	1·25	1·56	1·58	1·71

Plus [KCl] = *a* 0·25; *b* 1·00 mole/l.

again given by equation (2), but the thiohydantoin concentration is that of un-ionised molecules, and not the stoicheiometric concentration. It may then be shown [9] that

$$k_e = k_2 K_w / ([\text{H}^+] + K_a') \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

where K_w is the ionic product of water and K_a' the apparent dissociation constant for the reaction: (I) $\rightleftarrows$ (II) + H⁺. Since for the alkali concentrations of Table 2 H⁺ ≪ K_a',[1] equation (3) simplifies to:

$$k_e = k_2 K_w / K_a' \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

[8] Ingold, " Structure and Mechanism in Organic Chemistry," G. Bell, London, 1953, p. 784.
[9] Edward and Terry, *J.*, 1957, 3527.

whence follows the approximate independence of k_e of hydroxide-ion concentration. The small variation of k_e would then be due to the variation of K_a' with ionic strength.[10]

Taking the k_e (1.79×10^{-2} min.$^{-1}$) of the most dilute solution, and assuming a pK_a' of 8.5,[1] we can calculate a value of $k_2 = 5650$ l. mole^{-1} min.$^{-1}$ from equation (4) for the hydrolysis of 2-thiohydantoin. It is thus evident that, judged by the rate of hydrolysis of its un-ionised molecule, 2-thiohydantoin is extremely reactive and that it owes its comparative stability in alkaline solution to its ionisation.

TABLE 3. pH *of an aqueous* $0.01M$-*solution of 2-thiohydantoin on standing.*

Time (min.)	0	15	45	75	260	600	1400	2020	3000	4260
pH	(5.66)	5.12	4.89	4.79	4.41	4.26	3.98	3.82	3.70	3.70

TABLE 4. *Hydrolysis of 1- and 5-substituted 2-thiohydantoins.*

Substituent	1-Phenyl	5-Methyl	5-isoPropyl	5 : 5-Dimethyl
$10^4 k_e$ (min.$^{-1}$)	32 ^{a}	16 ^{b}	5.5 ^{b}	0.1 ^{b}
k_2 (l. mole^{-1} min.$^{-1}$)	201	320	110	2

In ^{a} 0.01N-; ^{b} 0.50N-sodium hydroxide.

TABLE 5. *Hydrolysis of 3-substituted 2-thiohydantoins.*

Substituent	3-Methyl		3-Phenyl			3 : 5-Dimethyl	
$[OH^-]$ (mole/l.)	0.001	0.010	0.001	0.002	0.010	0.001	0.010
$10^2 k_e$ (min.$^{-1}$)	3.0	5.5	5.3	6.8	10.7, 10.0	6.5	11.4
k_2 (l. mole^{-1} min^{-1})	60	60	106	102	118, 110	130	125

Equation (3) implies that 2-thiohydantoin should hydrolyse fairly rapidly even in very weakly alkaline solution; thus in changing the pH of the solution from 11.5 to 5.5 (*i.e.*, on reducing the hydroxide-ion concentration a million-fold), k_e should be reduced only a thousand-fold. In fact, even in distilled water (pH 5.5—6.0) hydrolysis occurred, as shown by the steady downward drift in pH of a $0.01M$-solution (Table 3), with formation of thioureidoacetic acid (V; R = R' = H). (A $pK_a' \approx 3.6$ may be assumed for this acid, taking account of the pK_a' of hydantoic acid [11] and of the comparative inductive effects of the ureido- and the thioureido-group.[12]) The pH became constant at about 3.70, presumably because the acid-catalysed ring closure (V ⟶ I) became sufficiently rapid for equilibrium (I ⇌ V) to be established. The pK_a' given above being assumed, this pH requires a thioureidoacetic acid concentration of about $4 \times 10^{-4}M$, corresponding to 4% of hydrolysis; the drop in ultraviolet absorption at 259 mμ indicated 3% of hydrolysis. As expected, the absorption of a $5 \times 10^{-5}M$-solution at this wavelength showed a very much larger drop, about 65% hydrolysis being indicated after 156 hr. At the same time the wavelength of the peak shifted from 259 to 265 mμ. This may be due to some concurrent oxidation (to be discussed in a later paper).
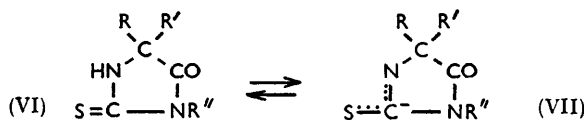
A slow hydrolysis probably explains the gradual decrease (over a period of one week) in the intensity of colour given by a standard solution ($6 \times 10^{-5}M$) of 5-*iso*butyl-2-thiohydantoin with the Folin–Denis reagent.[13] It appears desirable slightly to acidify standard solutions of 2-thiohydantoins to prevent this reaction.

[10] Cf. Simms, *J. Phys. Chem.*, 1928, **32**, 1121; 1929, **33**, 745.
[11] Zief and Edsall, *J. Amer. Chem. Soc.*, 1937, **59**, 2247.
[12] Kumler and Fohlen, *ibid.*, 1942, **64**, 1944.
[13] Folin and Denis, *J. Biol. Chem.*, 1912, **12**, 239; 1913, **14**, 95.

The thiohydantoin ring is stabilised by substitution at position 5 (Table 4).[14]   5 : 5-Di-methyl- and 5-*iso*propyl-2-thiohydantoin have pK_a' values of about 8·7;[1] this value has also been assumed for 5-methyl-2-thiohydantoin in calculating k_2 from k_e by equation (4). A pK_a' of 9·2 has been assumed for 1-phenyl-2-thiohydantoin, from analogy with the effect of substituting a phenyl group in thiourea.[1,15]   5 : 5-Diphenyl- and 5 : 5-penta-methylene-2-thiohydantoin were hydrolysed even more slowly than 5 : 5-dimethyl-2-thiohydantoin, little or no hydrolysis being observed in solutions in 0·01N-sodium hydroxide after several days.

3-Substituted 2-thiohydantoins (VI) are more weakly acidic (p$K_a' \approx 11$), ionising to give the anion (VII).[1]   With these compounds also, hydrolysis seems to involve reaction of the hydroxide ion with the un-ionised fraction only, as shown by the rough constancy of the values of k_2 (Table 5) calculated from equation (3), $K_a' = 10^{-11}$ being assumed for all three compounds.

It is evident that substitution of 2-thiohydantoin alters its rate of hydrolyses both by the effect on the reactivity of the neutral molecule, and by the effect on the ionisation constant, and hence on the proportion of the compound available for hydrolysis at a given pH.   The latter effect may be eliminated by comparing k_2 values.   The reciprocals of the k_2 values for the variously substituted 2-thiohydantoins, relative to the value for the parent compound are as follows:

1-Methyl-3-phenyl	11	3 : 5-Dimethyl	44	3-Methyl	94
5-Methyl	18	1 : 3-Dimethyl	47	5 : 5-Dimethyl	2820
3-Ethyl-1-phenyl	23	3-Phenyl	52	5 : 5-Pentamethylene	>10,000
1-Phenyl	28	5-*iso*Propyl	51	5 : 5-Diphenyl	>10,000

Because of the experimental errors and the various approximations in the theoretical treatment, too much weight should not be attached to the precise numerical values given here.   However, they indicate that, while substitution generally leads to decreased reactivity, the effect of geminal 5-groups is particularly strong,[14] probably because they oppose by non-bonded interactions the change from a trigonal (I) to a tetrahedral configur-ation (III) at $C_{(4)}$.[16]   Substitution at $N_{(3)}$ slightly stabilises the neutral molecule, but this effect is outweighed by the less complete ionisation of these compounds and so the net effect is to accelerate hydrolysis.

From these results it seems that hydrolysis accounts for only a small part of the losses of 2-thiohydantoins in the Schlack and Kumpf procedure.   Other possible degradative reactions will be discussed in subsequent papers.

EXPERIMENTAL

Materials [1] and general methods [1,9] have been described.   Readings were taken with either a Unicam S.P. 500 or Beckman DU spectrophotometer.   The cell compartment in the former instrument was cooled by running water, and no appreciable change in the temperature of a solution in the quartz cells was noted after 2—3 hr.   In the Beckman instrument the temper-ature of the solution generally increased by 1—2° over such a period.   Temperatures where not otherwise specified were in the range 17—23°.   Rate constants were obtained by the usual graphical method.

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[14] Cf. Ingold, Sako, and Thorpe, *J.*, 1922, 1177.
[15] Walter, Ryan, and Lane, *J. Amer. Chem. Soc.*, 1956, **78**, 5560.
[16] Brown, Brewster, and Schechter, *ibid.*, 1954, **76**, 467.