1014. *Thiohydantoins. Part I. Ionisation and Ultraviolet Absorption.*

By J. T. Edward and S. Nielsen.

Absorption spectra of variously substituted 2-thiohydantoins in ethanol, water, and aqueous sodium hydroxide have been determined. Ionisation of 2-thiohydantoins in alkaline solution leads to characteristic changes in absorption, which may be used for distinguishing 1- from 3-substituted 2-thiohydantoins. They are also distinguishable by their pK_a' values.

Increasing use is made of procedures in which the *N*-terminal[1] or *C*-terminal[2] amino-acid residues of polypeptides are detached as 2-thiohydantoin derivatives. In this connection we have examined the ultraviolet absorption of various thiohydantoins. It is already known[3-5] that they have a strong absorption band in the region 260—270 mμ (main peak) and a band, usually weaker, at 220—230 mμ (subsidiary peak), but no detailed studies have been made of the effect on absorption of changes in structure.

In Table 1 are given the absorption characteristics of twenty-one thiohydantoins. It is evident that a change in solvent from ethanol to water results in small shifts of one or both peaks. Because of low solubilities in water, the spectra of some compounds were determined in water containing 10% (v/v) of ethanol; these should be almost the same as the spectra in water (cf. Table 2).

Substitution of the 2-thiohydantoin nucleus by alkyl groups at $N_{(1)}$ or $N_{(3)}$ causes small bathochromic shifts in the subsidiary peak. Substitution by a phenyl group at $N_{(1)}$ causes a bathochromic shift of 10—17 mμ in both peaks, but at $N_{(3)}$ is without appreciable

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[1] *Ann. Reports*, 1953, **50**, 275.

[2] *Ibid.*, 1955, **52**, 277.

[3] du Vigneaud and Melville, "The Chemistry of Penicillin," Princeton Univ. Press, 1949, pp. 269 *et seq.*

[4] Carrington and Waring, *J.*, 1950, 354.

[5] Kjaer and Erikson, *Acta Chem. Scand.*, 1952, **6**, 448; Dahlerup-Petersen, Linderstrøm-Lang, and Ottesen, *ibid.*, p. 1135; Jeffreys, *J.*, 1954, 2221.

TABLE 1. *Absorption characteristic of substituted 2-thiohydantoins in different solvents.*

	Ethanol		Water		Aqueous NaOH †	
Substituent	$\lambda_{max.}$ (mμ) *	$\varepsilon_{max.}$	$\lambda_{max.}$ (mμ) *	$\varepsilon_{max.}$	$\lambda_{max.}$ (mμ) *	$\varepsilon_{max.}$
(2-Thiohydantoin) [a]	264	17,000	259	16,700	260	24,300 [r]
	222	9,100	223	9,500	214	4,000
1-Methyl [b]	265	15,300	261	14,000	258	33,700 [r]
	230	11,400	230	13,500		
3-Methyl [c]	263	17,000	259	15,800	—	— [r]
	232	10,000	235	11,900		
5-Methyl [d]	266	17,600	261	16,700	259	24,600 [r]
	224	8,400	224	9,800	210	5,000
5-*iso*Propyl [e]	266	20,100	262	17,400	260	25,900
	226	8,900	226	8,800		
1-Phenyl [f]	281	11,300	269	11,100	263	25,500 [r]
	236	17,200	235	23,500		
3-Phenyl [g]	266	14,600	262	14,700	—	— [r]
	(229	8,400)	229	8,700		
5-Phenyl [h]	270	18,100	271	15,300 [p]	271	20,500 [r]
	(228	9,100)	234	8,400 [p]		
5-Benzyl [e]	267	18,200	264	16,900	—	— [r]
	224	7,500	225	8,500		
1 : 3-Dimethyl [i, j]	265	13,500	261	11,900	—	— [r]
	239	12,800	239	16,500		
3 : 5-Dimethyl [c]	265	14,900	263	14,800	—	— [r]
	233	8,500	235	9,500		
5 : 5-Dimethyl [k]	266	17,800	262	16,500	259	23,000
	223	8,700	223	9,600	210	6,000
5 : 5-Pentamethylene [k]	267	19,400	263	18,500	259	24,200
	224	8,400	224	10,300		
1-Methyl-3-phenyl [j]	267	14,000	261	12,800	—	— [r]
	232	10,400	236	11,800		
3-Ethyl-1-phenyl [l]	279	10,200	269	10,300 [p]	—	— [r]
	244	16,000	245	16,700 [p]		
1 : 3-Diphenyl [f]	280	11,700 [q]	278	11,300	378	8,070 [s]
					(278	8,300) [s]
3 : 5-Diphenyl [m]	271	17,600	269	12,900	—	— [r]
	(266	17,000)				
5 : 5-Diphenyl [n]	272	21,500	270	20,300 [p]	266	26,500
	(233	10,100)	(235	9,400) [p]		
3 : 5 : 5-Trimethyl [c]	265	17,300	262	17,000	258	5,000 [t]
	231	9,000	235	10,700	224	16,900 [t]
1-Methyl-5 : 5-pentamethylene [4] ...	266	19,000	262	16,100	255	27,800
	230	10,700	231	12,700	218	4,800
3-Methyl-5 : 5-pentamethylene [4] ...	266	16,500	264	14,900	259	5,000 [t]
	233	8,500	235	9,600	223	15,600 [t]

* Inflexions in parentheses.

[a] Johnson and Nicolet, *J. Amer. Chem. Soc.*, 1911, **33**, 1973. [b] Elmore, Ogle, and Toseland, *J.*, 1956, 192. [c] Marckwald, Neumark, and Stelzner, *Ber.*, 1891, **24**, 3278. [d] Johnson, *J. Biol. Chem.*, 1912, **11**, 97. [e] Jackman, Klenk, Fishburn, Tullar, and Archer, *J. Amer. Chem. Soc.*, 1948, **70**, 2884. [f] Wheeler and Brautlecht, *Amer. Chem. J.*, 1911, **45**, 446. [g] Aschan, *Ber.*, 1884, **17**, 420; Edman, *Acta Chem. Scand.*, 1950, **4**, 277. [h] Swan, *Austral. J. Sci. Res.*, 1952, **5**, *A*, 711. [i] Delépine, *Bull. Soc. chim. France*, 1903, **29**, 1198. [j] Cook and Cox, *J.*, 1949, 2342. [k] Carrington, *J.*, 1947, 684. [l] Brooker, Keyes, Sprague, Van Dyke, Van Lane, Van Zandt, and White, *J. Amer. Chem. Soc.*, 1951, **73**, 5326. [m] Kossel, *Ber.*, 1891, **24**, 4145. [n] Biltz, *Ber.*, 1909, **42**, 1792.

[p] Solvent was 9 : 1 (v/v) H_2O–EtOH. [q] Solution (4 ml.) acidified with one drop of conc. HCl. [r] Rapid hydrolysis indicated by disappearance of main peak; absorption characteristics, where given, obtained by extrapolation.

† Concentration 0·01N, except for: [s] 0·001N; [t] 1N.

TABLE 2. *Ultraviolet absorption of* 5 : 5-*dimethyl-2-thiohydantoin in various concentrations of aqueous ethanol.*

EtOH (% v/v)	100	90	70	50
$\lambda_{max.}$ (mμ) ($\varepsilon_{max.}$)	266 (17,800)	265 (18,000)	265 (18,000)	264 (18,500)
	233 (8,700)	223 (8,800)	224 (9,300)	233 (9,900)
EtOH (% v/v)	30	10	0	
$\lambda_{max.}$ (mμ) ($\varepsilon_{max.}$)	264 (17,400)	262 (17,000)	262 (16,500)	
	224 (9,700)	224 (9,600)	223 (9,600)	

effect.[6] This may be related to the fact that while the 1-phenyl group may approach coplanarity with the ·N·CS·NH·CO· chromophore by a small bending of the N–Ph bond, the 3-phenyl group is more severely hindered by the flanking carbonyl and thiocarbonyl groups (Fig. 1).[7] Substitution at $C_{(5)}$ by alkyl groups has only a minor effect on absorption; substitution by phenyl groups causes a small bathochromic shift, probably because of π–π electronic interaction across the saturated carbon atom.[8] When the phenyl group is separated from the ·NH·CS·NH·CO· chromophore by two saturated carbon atoms, as in 5-benzyl-2-thiohydantoin, its effect is slight.

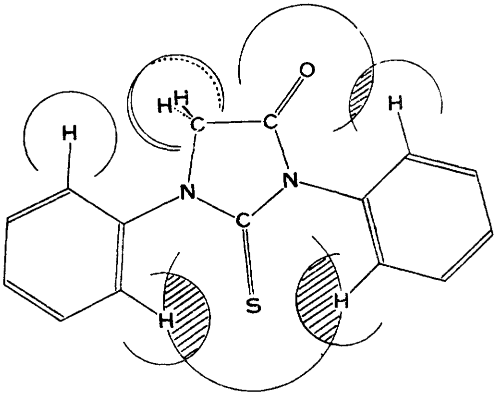

FIG. 1. *Scale drawing of* 1 : 3-*diphenyl*-2-*thiohydantoin, using bond-lengths and angles from crystallographic studies of related compounds* (*Corey,* J. Amer. Chem. Soc., 1938, **60**, 1598; *Davies and Blum,* Nature, 1954, **173**, 993) *and from Pauling* (*" The Nature of the Chemical Bond," Cornell University Press, Ithaca, 2nd ed.,* 1948, *pp.* 79, 160, 187); *van der Waals radii from Pauling except for hydrogen* (*Forbes and Mueller,* Canad. J. Chem., 1956, **34**, 1542; *cf. Crombie,* Quart. Rev., 1952, **6**, 101; *Braude and Sondheimer,* J., 1955, 3754).

In alkaline solution thiohydantoins ionise [3] but also undergo ring fission [9] at varying rates. However, the hydrolysis of the 2-thiohydantoins listed in Table 3 is so slow,[10] that the absorption characteristics of their ionised forms may be accurately determined. Ionisation at $N_{(3)}$–H, as in 1-methyl-5 : 5-pentamethylene-2-thiohydantoin (I; R = Me) results in a slight hypsochromic shift, but a big increase in intensity, of the main peak, and a hypsochromic shift and decrease in intensity of the subsidiary peak (Fig. 2). On the other hand, ionisation of $N_{(1)}$–H, as in 3-methyl-5 : 5-pentamethylene-2-thiohydantoin (II) results in a reduction of intensity of the main peak and in an increased intensity of the subsidiary peak, both at slightly shorter wavelength (Fig. 3). Thiohydantoins unsubstituted at $N_{(1)}$ and $N_{(3)}$, such as 5 : 5-pentamethylene-2-thiohydantoin (I; R = H), have in alkaline solution spectra similar in general to those of 1-substituted 2-thiohydantoins and hence must ionise, as expected,[11] at $N_{(3)}$–H.

TABLE 3. *Acid dissociation constants at* 18° ± 3°.

Compound	pK_a'
Hydantoin	9·16 *
5-*iso*Propyl-2-thiohydantoin	8·70
5 : 5-Dimethyl-2-thiohydantoin	8·71
5 : 5-Pentamethylene-2-thiohydantoin	8·79
5 : 5-Diphenyl-2-thiohydantoin	7·69 †
3 : 5 : 5-Trimethyl-2-thiohydantoin	10·80
1-Methyl-5 : 5-pentamethylene-2-thiohydantoin	9·25
3-Methyl-5 : 5-pentamethylene-2-thiohydantoin	11·23

* Zief and Edsall (*J. Amer. Chem. Soc.*, 1939, **61**, 423) report $pK_a' = 9{\cdot}12$ at 25°.
† In 9 : 1 (v/v) H_2O–EtOH.

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[6] Cf. Crombie and Hooper, *J.*, 1955, 3010.

[7] Braude and Waight, " Progress in Stereochemistry," ed. Klyne, Butterworths, London, 1954, p. 126.

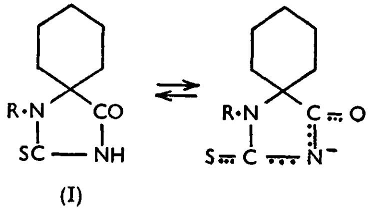
[8] Simonetta and Winstein, *J. Amer. Chem. Soc.*, 1954, **76**, 18; Baddeley, *Ann. Reports*, 1954, **51**, 167.

[9] Kenner, Khorana, and Stedman, *J.*, 1953, 673.

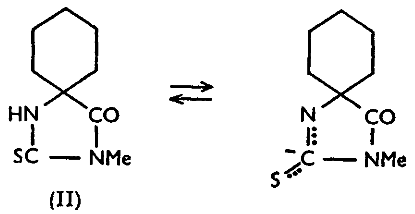
[10] Cf. Ingold, Sako, and Thorpe, *J.*, 1922, 1177.

[11] Pickett and McLean, *J. Amer. Chem. Soc.*, 1939, **61**, 423; Stuckey, *J.*, 1947, 331.

Unlike the other 1 : 3-disubstituted 2-thiohydantoins of Table 1, 1 : 3-diphenyl-2-thiohydantoin (III) proved moderately stable in very dilute sodium hydroxide solution, in which it had an absorption peak at 378 mμ (Table 1). We tentatively ascribe this peak to the enolate ion (IV), the formation of which would be assisted by the electron-withdrawing effects of the two phenyl groups.[12] Ionisation was also indicated in ethanol solution, with a well-defined peak at 390 mμ as well as a flat peak at 279 mμ; the former peak increased in intensity with dilution [13] but disappeared completely when one drop of concentrated hydrochloric acid was added to 4 ml. of the ethanolic solution. In aqueous solution the absorption was normal (Table 1).

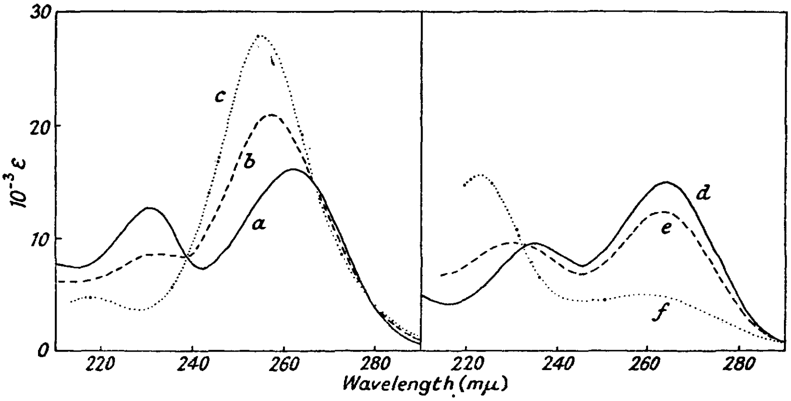


(I)



(II)

FIG. 2. FIG. 3.

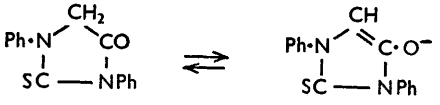


FIGS. 2 *and* 3. *Absorption spectra of* 1-*methyl*-5 : 5-*pentamethylene*-2-*thiohydantoin* (*Fig.* 2) *and* 3-*methyl*-5 : 5-*pentamethylene*-2-*thiohydantoin* (*Fig.* 3) *in aqueous solution.*

a, *d*, pH 5·5; *b*, pH 9·21; *c*, pH 12; *e*, pH 10·73; *f*, pH 14.

The apparent dissociation constants of several 2-thiohydantoins in water, and (for solubility reasons) of 5 : 5-diphenyl-2-thiohydantoin in 10% aqueous ethanol (Table 3), were determined by the well-established spectrophotometric procedure.[14,15] The use of the latter solvent instead of water should increase the pK_a' by about 0·1.[16] It is evident

(III)



(IV)

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[12] van der Krogt and Wepster, *Rec. Trav. chim.*, 1955, **74**, 161; Brown, McDaniel, and Häfliger, " Determination of Organic Structures by Physical Methods," ed. Braude and Nachod, Academic Press, New York, 1953, p. 567.

[13] Cf. Gillam and Stern, " Electronic Absorption Spectroscopy," Arnold, London, 1953, p. 8.

[14] Flexser, Hammett, and Dingwall, *J. Amer. Chem. Soc.*, 1935, **57**, 2103.

[15] Butler, Ruth, and Tucker, *ibid.*, 1955, **77**, 1486.

[16] Cavill, Gibson, and Nyholm, *J.*, 1949, 2466.

that 5-alkyl-substituted 2-thiohydantoins have a pK_a' of about 8·7 in water. Values of 8·9 and 9·1 have been reported for the pK_a' of 2-thiohydantoin and 5-methyl-2-thiohydantoin, respectively, in aqueous methanol (exact concentration unspecified, but less than 50%).[3] Hence a pK_a' of about 8·5 may be expected for the former compound in water.

The acid-strengthening effect of a 5-phenyl group [12,15] and the acid-weakening effect of a 1-methyl group [17] are in accord with analogous cases in the literature.

EXPERIMENTAL

Absorption measurements were carried out with a Unicam S.P. 500 or Beckman DU spectrophotometer, and 1 cm. matched quartz cells. pH measurements were made with a Radiometer pH meter No. 23 and glass and calomel electrodes. The thiohydantoins were prepared by the methods described in the literature, and purified to constant m. p.; in some cases purity was checked by paper chromatography.[18] A method more convenient than those reported [19] for preparing 1-substituted 2-thiohydantoins is given below.

1-*Phenyl*-2-*thiohydantoin*.—The hydrochloride of *N*-phenylglycine was prepared by evaporating the water from a mixture of the amino-acid (1·5 g.) and concentrated hydrochloric acid (1 ml.). Ammonium thiocyanate (0·8 g.) was added, and the mixture heated at 155° for 15 min. The melt was warmed with 3% hydrochloric acid (3 ml.), and the solid removed. On crystallisation from ethanol it afforded 1-phenyl-2-thiohydantoin (0·9 g.), m. p. 187—189°.[20] This gave with nitroprusside–piperazine [21] the characteristic purple colour of 2-thiohydantoins unsubstituted at position 5.[18]

Measurements of Ionisation Constants.—The spectra of the thiohydantoins were determined in distilled water (pH 5·5—6), in various buffer solutions (ionic strength about 0·01M), and in sodium hydroxide solution concentrated enough to ensure complete ionisation (M for 3-substituted compounds; 0·01M for others) (cf. Figs. 2 and 3). The pK_a' values were calculated from the change in absorption values at several different wave-lengths, according to the usual procedure,[14] and averaged.

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[17] Walter, Ryan, and Lane, *J. Amer. Chem. Soc.*, 1956, **78**, 5560.
[18] Edward and Nielsen, *Chem. and Ind.*, 1953, 197.
[19] Refs. *b* and *f* of Table 1; Komatsu, *Mem. Coll. Sci. Kyoto Imp. Univ.*, 1914, **1**, 69.
[20] Ref. *f* of Table 1.
[21] Edward and Waldron, *J.*, 1952, 3621.