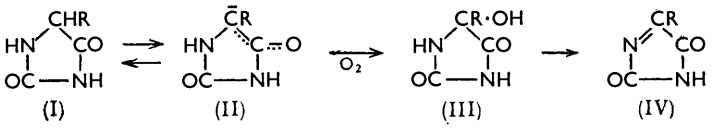
468. *Thiohydantoins. Part IV.* Oxidation in Alkaline Solution by Molecular Oxygen.*

By J. T. Edward and S. Nielsen.

In alkaline solution 2-thiohydantoins having a 5-hydrogen atom are oxidised by molecular oxygen to octahydro-1 : 5′-diglyoxalinyls.

The oxidation of 5-phenylhydantoin (I; R = Ph) with a variety of reagents affords octahydro-2 : 4 : 2′ : 4′-tetraoxo-5 : 5′-diphenyl-1 : 5′-diglyoxalinyl (" diphenylhydantil ") (VI; R = Ph).[1,2] Pinner [3] obtained this product by heating 5-phenylhydantoin in alcoholic potassium hydroxide, oxidation apparently being effected by molecular oxygen. We have found that in aqueous alkali at room temperature 5-phenylhydantoin absorbs

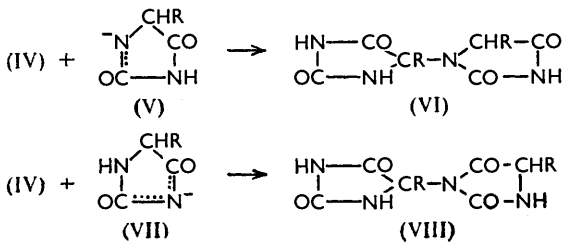


oxygen, to give diphenylhydantil. The reaction probably involves oxidation of the carbanion [4] (II; R = Ph) to the hydroxyamide [2] (III; R = Ph), which in the form of the pseudo-base (IV; R = Ph) can condense with the anion (V; R = Ph) derived from a second molecule of 5-phenylhydantoin. Condensation of the pseudo-base (IV; R = Ph) with the more abundant anion [5] (VII; R = Ph), which is usual in most reactions of

* Part III, *J.*, 1957, 5084.

[1] Gabriel, *Annalen*, 1906, **350**, 118.
[2] Holmberg, *Acta Chem. Scand.*, 1950, **4**, 821.
[3] Pinner, *Annalen*, 1906, **350**, 135.
[4] Cf. Walling, " Free Radicals in Solution," Wiley, New York, 1957, p. 464.
[5] Pickett and McLean, *J. Amer. Chem. Soc.*, 1939, **61**, 423; Stuckey, *J.*, 1947, 331.

hydantoins with electrophiles in alkaline solution,[6] is probably prevented in the present instance by the dipolar repulsions which might be expected in the product (VIII; R = Ph).



Unlike 5-phenylhydantoin, the 5-alkylhydantoins in alkaline solution are not oxidised by molecular oxygen, probably because of the much smaller concentration of the carbanions (II; R = alkyl) than of the aryl analogue (II; R = Ph).[7] However, the great electron-attraction of the thiocarbonyl group [8] might be expected to promote the ionisation of 2-thiohydantoins to carbanions analogous to (II). In fact, in alkaline solution these compounds, except when disubstituted at position 5, absorb oxygen readily (Table 1). We have attempted to elucidate the nature of the oxidation products of different types of 2-thiohydantoins.

TABLE 1. *Absorption of oxygen by solutions of substituted 2-thiohydantoins in sodium hydroxide.*

Substituent	—	1-Me	1-Ph	3-Ph	5-Me	5-Ph	5-CH_2Ph	3 : 5-Ph_2
O_2 absorption (atom/mole)	1·3	0·9	1·2	1·0	0·7	0·9	0·6	1·6

The oxidation of 5-monosubstituted and 3 : 5-disubstituted 2-thiohydantoins gave high-melting, relatively insoluble solids (Table 2) which are probably also octahydrodiglyoxalinyl derivatives (IX), although in only three cases (discussed below) were the structures investigated. The lower yields on oxidation of the 3 : 5-disubstituted 2-thiohydantoins are to be expected, because the competing hydrolysis of these compounds in alkaline solution is very rapid.[9]

TABLE 2. *Substituted octahydrodiglyoxalinyls* (IX) *obtained by oxidation of 2-thiohydantoins.*

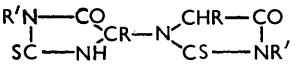
			Yield	Found (%)				Required (%)		
R	R′	M. p.	(%)	C	H	N	Formula	C	H	N
Me	H	270° [a]	65	37·0	3·7	21·4	$C_8H_{10}O_2N_4S_2$	37·2	3·9	21·8
Pr^i	H	212 [b]	76	45·8	5·9		$C_{12}H_{18}O_2N_4S_2$	45·8	5·8	
Bu^i	H	240 [b]	70	48·6	6·5		$C_{14}H_{22}O_2N_4S_2$	49·2	6·4	
Ph	H	250 [b]	50	56·1	3·3	14·5	$C_{18}H_{14}O_2N_4S_2$	56·5	3·7	14·6
Ph·CH_2	H	256 [b]	75	58·3	4·4		$C_{20}H_{18}O_2N_4S_2$	58·3	4·1	
Me	Me	250 [b]	6	41·7	4·7	19·2	$C_{10}H_{14}O_2N_4S_2$	43·0	4·9	18·9
Ph	Ph	260 [b]	14	66·9	3·8	10·2	$C_{30}H_{22}O_2N_4S_2$	67·2	4·1	10·4

[a] Crystallized from aqueous 2-methoxyethanol. [b] Crystallized from acetone.

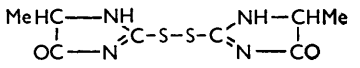
The compound obtained by oxidation of 5-phenyl-2-thiohydantoin afforded phenylglyoxylic acid on alkaline hydrolysis, as would be expected from the formulation (IX; R = Ph, R′ = H). In further agreement, its absorption spectrum (Table 3) was

[6] Ware, *Chem. Rev.*, 1950, **46**, 403.
[7] Cf. Bovarnick and Clarke, *J. Amer. Chem. Soc.*, 1938, **60**, 2426.
[8] Ingold, " Structure and Mechanism in Organic Chemistry," Bell, London, 1953, p. 77.
[9] Edward and Nielsen *J.*, 1957, 5080.

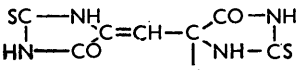
approximately the sum of the absorptions to be expected [10] for the two separate thiohydantoin moieties. Also, the increase in absorption at 268 mμ when the solution was made alkaline indicated the presence of two ionising $N_{(3)}H$ groups.[10] Finally, removal of



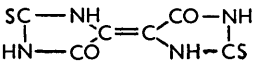
(IX)



(X)



(XI)



(XII)

sulphur from the compound with chloroacetic acid [11] afforded diphenylhydantil (VI; R = Ph).

Sjollema and Seekles [12] reported that in sodium hydrogen carbonate solution 5-methyl-2-thiohydantoin was oxidised by air to two compounds $C_8H_{10}O_2N_4S_2$, decomposing at about 240° and 270°, to which they assigned the structures (X) and (XI) respectively. We have obtained only one compound $C_8H_{10}O_2N_4S_2$, decomposing at about 270°, from

TABLE 3. *Absorption characteristics of substituted octahydrodiglyoxalinyls* (IX) *in aqueous acid and alkali* (*inflexions in parentheses*).

		In 0·002N-HCl		In 0·002N-NaOH	
R	R′	$\lambda_{max.}$ (mμ)	ε	$\lambda_{max.}$ (mμ)	ε
Me	H	(269)	28,000	263	47,000
		263	29,500	208	12,000
		226	15,000		
Me	Me	(268)	24,500	263	17,500
		263	26,000	230	20,000
		(235)	16,000		
Ph	H	278	30,500	268	42,000
		235	16,500		

aerial oxidation in alkaline solution. Its ultraviolet absorption in aqueous acid (Table 3) was that expected for two isolated 2-thiohydantoin moieties,[10] as in (IX; R = Me, R′ = H); the compound (XI) would be expected to show one absorption peak at a longer wavelength. Further, in alkaline solution the oxidation product showed the greatly enhanced absorption at 263 mμ characteristic of a thiohydantoin moiety having an ionizable $N_{(3)}H$ group; [10] this evidence eliminates the structure (X). Finally, the formation of pyruvic acid on alkaline hydrolysis of the oxidation product is compatible only with its formulation as (IX; R = Me, R′ = H).

The compound from 3 : 5-dimethyl-2-thiohydantoin showed in acid solution the ultraviolet absorption (Table 3) to be expected for the structure (IX; R = R′ = Me). The lower absorption at 263 mμ in alkaline solution indicated the hydrolysis of the 1 : 3 : 5-trisubstituted 2-thiohydantoin moiety of (IX); the extreme lability to alkali of such structures has been demonstrated previously.[9]

While alkaline hydrolysis of the compounds (IX; R = Ph and Me, R′ = H) afforded the expected α-keto-acids, hydrolysis for four hours in concentrated hydrochloric acid at 165° gave no α-keto-acid, but approximately two mols. each of phenylglycine and of alanine, as judged by spot intensities on paper chromatograms. On the other hand, acid hydrolysis of diphenylhydantil gave only one mol. of phenylglycine. Evidently the presence of the thiocarbonyl group caused the oxidised $C_{(5)}$-centre of compounds (IX) to be reduced at some stage in the hydrolysis; this may have been after the liberation of

[10] Edward and Nielson *J.*, 1957, 5075.
[11] Cf. Johnson, Pfau, and Hodge, *Amer. Chem. J.*, 1912, **34**, 1045.
[12] Sjollema and Seekles, *Rec. Trav. chim.*, 1925, **44**, 821.

α-keto-acid, as shown by the formation of alanine when pyruvic acid was heated with thiourea in hydrochloric acid under the same conditions.

The detachment of a terminal thiohydantoin residue from a polypeptide chain by mild alkaline hydrolysis, according to the procedure of Schlack and Kumpf,[13] will almost certainly result in some oxidation of the thiohydantoins. When the latter, contaminated by oxidation products, are identified by conversion into the parent amino-acid, it appears advisable for maximum yields to use acid [14] rather than alkali [15] for this second hydrolysis.

The oxidation of 2-thiohydantoin itself gave a black solid, forming a red solution in alkali. Because of its colour we tentatively formulate this compound as (XII); its formation by oxidation resembles that of indigo. This product was hydrolysed in hydrochloric acid in 3 hours at 155° to glycine, probably after preliminary fission to 2-thiohydantoin and thioparabanic acid, and to smaller amounts of two other ninhydrin-positive substances; in alkaline solution it absorbed oxygen and gave oxalic acid and thiourea.

Thiohydantoins substituted at $N_{(1)}$ absorbed oxygen in alkaline solution (Table 1), but no oxidation products were precipitated on subsequent acidification of the solutions.

Experimental

Whatman No. 1 filter paper was used for paper chromatography.

Oxidation of 2-Thiohydantoins.—(*a*) The 2-thiohydantoin (1 mmole) was placed in a small flask fitted with a dropping funnel and a magnetic stirrer and connected with a gas burette. The apparatus was flushed with oxygen, then 0·5N-sodium hydroxide (2 ml.) was added and stirring commenced. The thiohydantoin dissolved and absorption of oxygen, if any, began immediately and was complete within 24 hr. (Table 1). Solutions of 5 : 5-dimethyl- and 5 : 5-diphenyl-2-thiohydantoin absorbed no oxygen.

(*b*) Oxygen was bubbled overnight through a solution of 2-thiohydantoin (5 mmoles) in N-sodium hydroxide (5 ml.). The oxidation products separating on acidification were almost insoluble in all the common organic solvents, and crystallized only from large volumes of the solvents indicated (Table 2). They were soluble in aqueous sodium hydroxide, from which they were reprecipitated by acid.

Alkaline Hydrolysis of Octahydro-4 : 4′-dioxo-5 : 5′-diphenyl-2 : 2′-dithiono-1 : 5′-diglyoxalinyl (IX; R = Ph, R′ = H).—The octahydrodiglyoxalinyl (0·2 g.) was heated with 6N-sodium hydroxide (3 ml.) to 100° for 1 hr. The solution was acidified, filtered from a small quantity of starting material, and treated with a saturated solution of 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid (10 ml.). The yellow solid separating crystallized from aqueous acetone as prisms, m. p. 197—199° (decomp.) (Found: C, 49·1; H, 3·0; N, 16·3. Calc. for $C_{14}H_{10}O_6N_4$: C, 50·1; H, 3·0; N, 17·0%), identified as phenylglyoxylic acid 2 : 4-dinitrophenylhydrazone by mixed m. p. Both specimens migrated as a single zone of R_F 0·70 when chromatographed with butanol–water–ethanol–concentrated aqueous ammonia (40 : 49 : 10 : 1) (solvent A) as irrigating solvent.[16]

Acid Hydrolysis of Octahydro-4 : 4′-dioxo-5 : 5′-diphenyl-2 : 2′-dithiono-1 : 5′-diglyoxalinyl (IX; R = Ph, R′ = H).—The octahydrodiglyoxalinyl (0·5 g.) was heated with hydrochloric acid (5 ml.) in a sealed tube for 4 hr. at 165°. After removal of a tar, the solution was evaporated to dryness, the residue taken up in water, and the evaporation repeated twice. The residue, m. p. 236—240° (0·46 g.), was identified as the phenylglycine hydrochloride by paper chromatography [R_F 0·53 with propanol–water (7 : 3), 0·38 with butanol–acetic acid,[17] and 0·70 with phenol–ammonia [18]]. An authentic specimen run at the same time showed identical R_F values.

Reaction of Octahydro-4 : 4′-dioxo-5 : 5′-diphenyl-2 : 2′-dithiono-1 : 5′-diglyoxalinyl with Chloroacetic Acid.—The octahydrodiglyoxalinyl (1·6 g.) was refluxed for 6 hr. with 20% aqueous

[13] Schlack and Kumpf, *Z. physiol. Chem.*, 1926, **154**, 125.
[14] Waley and Watson, *J.*, 1951, 2394.
[15] Tibbs, *Nature*, 1951, **168**, 910; Baptist and Bull, *J. Amer. Chem. Soc.*, 1953, **75**, 1727.
[16] Metzler and Snell, *J. Biol. Chem.*, 1952, **198**, 353.
[17] Woiwood, *J. Gen. Microbiol.*, 1942, **3**, 312.
[18] Sanger and Tuppy, *Biochem. J.*, 1951, **49**, 463.

chloroacetic acid (60 ml.). The residue obtained on evaporation was washed with sodium hydrogen carbonate solution, and the insoluble solid (0·30 g.) dissolved in alkali and precipitated by acid. The solid, m. p. >320° (Found: C, 61·8; H, 4·1. Calc. for $C_{18}H_{14}O_4N_4$: C, 61·7; H, 4·0%), was identified as octahydro-2 : 4 : 2′ : 4′-tetraoxo-5 : 5′-diphenyl-1 : 5′-diglyoxalinyl by its infrared spectrum (KBr disc), which showed peaks (cm.$^{-1}$) at 1780s, 1700s, 1498w, 1451m, 1398s, 1368sh, 1292w, 1275w, 1250m, 1205w, 1165w, 1135w, 1080w, 1035w, 1015w, 955w, 876w, 845w, 792m, 757s, 738s, 699m, 670w, 642w, and 627m. An authentic specimen [3] showed the same spectrum.

Alkaline Hydrolysis of Octahydro-5 : 5′-dimethyl-4 : 4′-dioxo-2 : 2′-dithiono-1 : 5′-diglyoxalinyl (IX; R = Me, R′ = H).—The octahydrodiglyoxalinyl (0·58 g.) was treated for 2 days with 6N-sodium hydroxide (5 ml.) at 20°. The solution was then acidified and treated with 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid. The residue from an ethyl acetate extract of the solution, examined by paper chromatography with solvent A, gave zones corresponding to the 2 : 4-dinitrophenylhydrazones of pyruvic acid (R_F 0·40, 0·57) and formaldehyde (R_F 0·89).

Oxidation of 2-Thiohydantoin.—Oxygen was bubbled overnight through a solution of 2-thiohydantoin (1·54 g.) in N-sodium hydroxide (13·3 ml.). Purple-black crystals (0·20 g.), decomp. >230°, were then removed; a further quantity (0·15 g.) was obtained by neutralizing and concentrating the solution. *Octahydro-4 : 4′-dioxo-2 : 2′-dithiono-1 : 5′-diglyoxalinylidene* (XII) was obtained by crystallization from pyridine–methanol as reddish-black prisms, decomp. >230° (Found: C, 31·6; H, 1·8; N, 25·3. $C_6H_4O_2N_4S_2$ requires C, 31·6; H, 1·7; N, 24·5%). It gave a deep red solution with aqueous alkali.

Addition of calcium chloride to the filtrate from the second crop gave a precipitate of calcium oxalate (0·29 g.), recognized by its insolubility in dilute acetic but solubility in syrupy phosphoric acid, by affording carbon dioxide with potassium permanganate, and by a positive resorcinol test.[19] After removal of the oxalate the filtrate was evaporated to dryness, and the residue extracted with ethyl acetate and acetone. The combined extracts on concentration afforded colourless prisms of thiourea, m. p. and mixed m. p. 181° (0·05 g.) (*S*-acetylthiuronium chloride,[20] m. p. and mixed m. p. 120°), R_F 0·30, identical with thiourea, on paper chromatography with butanol saturated with water.

The solid (XII) (30 mg.) dissolved partially in 0·1N-sodium hydroxide (2·4 ml.); the solution absorbed oxygen (2 ml.). Addition of N-sodium hydroxide (1 ml.) then gave complete solution, and further uptake of oxygen (3 ml.). The oxidation caused the deep red colour of the solution to change to orange. The solution was acidified and treated with calcium chloride, giving a precipitate (6 mg.) which gave the characteristic tests for oxalate.

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Edgbaston, Birmingham, 15. [*Received, February 9th*, 1959.]

[19] Vogel, "Qualitative Chemical Analysis," Longmans Green, London, 1948, p. 306.
[20] Moore and Crossley, *J. Amer. Chem. Soc.*, 1940, **62**, 3273.