

The Reaction Between L-Cystine-bis-3-phenylhydantoin and Sodium in Liquid Ammonia.

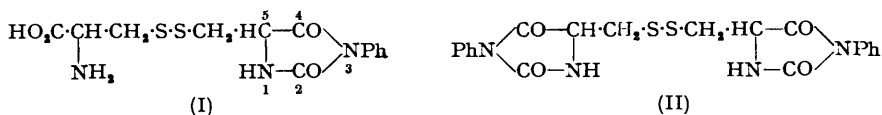
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Besides the expected $-S-S-$ and $Ph-CH_2-S-$ cleavage, L-cystine-3-phenylhydantoin (I) * and S-benzyl-L-cysteine-3-phenylhydantoin undergo a novel reduction at the 4-position when treated with sodium in liquid ammonia, giving substituted tetrahydro-2-oxoglyoxalines. Complete racemisation ensues: dissolution in liquid ammonia alone is sufficient to cause this. Hydantoins lacking a 3-phenyl substituent are neither reduced nor racemised. The structure of the benzylated reaction product, 4-S-benzylthiomethyltetrahydro-2-oxo-1-phenylglyoxaline, is confirmed by desulphurisation to the 4-methyl compound which is synthesised from alanine anilide.

Ultraviolet light absorptions of hydantoins, tetrahydro-2-oxoglyoxalines, and hydantoic acids are recorded in neutral, acid, and alkaline ethanol: there is a remarkable difference between the spectra of tetrahydro-2-oxoglyoxalines and 3-phenylhydantoins. Further, the latter are distinguished from unsubstituted hydantoins by rapid alkaline hydrolysis to 3-phenylhydantoic acids. Ketone stretching vibrations in these compounds agree broadly with the assignments by Randall *et al.* and are useful for identifications.

DURING an examination of certain peptide degradation products it became necessary to characterise the L-cystine-3-phenylhydantoin residue * in compounds of type (I) and (II).

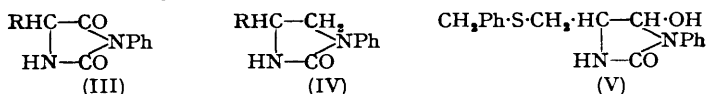


Oxidation of L-cystine-bis-3-phenylhydantoin (II) by performic acid (Toennies and Homiller, *J. Amer. Chem. Soc.*, 1942, **64**, 3054; Sanger, *Biochem. J.*, 1949, **44**, 126) was expected to yield cysteic acid-3-phenylhydantoin but the latter, in agreement with earlier reports (Andrews and Andrews, *J. Biol. Chem.*, 1933, **102**, 253), was unstable and unsuitable as a reference compound. As an alternative, the bishydantoin (II) was treated with sodium in liquid ammonia, to reduce the disulphide linkage, and then benzylated. However, the product was not S-benzyl-L-cysteine-3-phenylhydantoin as expected but a new optically inactive substance $C_{17}H_{18}ON_2S$, m. p. 135°. The same substance was isolated when authentic S-benzyl-L-cysteine-3-phenylhydantoin (Shiple and Sherwin, *J. Biol. Chem.*, 1923, **55**, 671) was treated with sodium in liquid ammonia and then rebenzylated. This communication is concerned with the structure and formation of the substance of m. p. 135° and includes spectroscopic data on related compounds.

When S-benzyl-L-cysteine-3-phenylhydantoin was merely kept in solution in liquid ammonia for 15 min. and the solvent allowed to evaporate, there was complete racemisation to the DL-compound, m. p. 154°, which was also prepared from S-benzyl-DL-cysteine and phenyl isocyanate in the usual way. On the other hand neither S-benzyl-L-cysteine-hydantoin itself nor L-cystine-bishydantoin was racemised under these conditions. Ease of racemisation is not associated with the presence of a 3-substituent alone since S-benzyl-L-cysteine-3-methylhydantoin retained its optical activity completely after 15 min. in liquid ammonia. The racemisation of hydantoins by sodium hydroxide was discovered by Dakin (*Amer. Chem. J.*, 1910, **44**, 48; cf. Bovarnick and Clarke, *J. Amer. Chem. Soc.*, 1938, **60**, 2426) but there are no records of the use of liquid ammonia for this purpose.

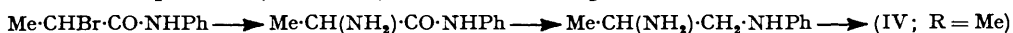
* For convenience, to preserve the amino-acid connexion, this type of nomenclature is retained in this paper. L-Cystine-bis-3-phenylhydantoin (II) can be named systematically di-(1-phenyl-L₃-hydantoin-4-ylmethyl) disulphide; L-cystine-3-phenylhydantoin (I) can be named L₃-2-amino-2-carboxyethyl 1-phenyl-L₃-hydantoin-4-ylmethyl disulphide; and S-benzyl-L-cysteine-3-phenylhydantoin can be named L₃-5-benzylthiomethyl-1-phenylhydantoin. ED.

Racemisation undoubtedly involves removal by the base of a proton from C₍₅₎, aided by the electron-attracting character of the 4-carbonyl group (equivalent to enolisation postulated by the earlier authors) and the great ease of this process in the 3-phenyl compounds (III) is attributable to increase in the acidity of the 5-hydrogen atom by the electron-attraction of the aryl residue. Reaction of sodium with the bishydantoin (II) in



liquid ammonia is thus probably with already racemised material. The infrared spectrum of the substance, m. p. 135°, indicated that the 4-oxo-group of the original hydantoin had been destroyed but the 2-oxo-vibration was present (see assignments below). The benzylthio-group was then removed by Raney nickel (Mozingo, Wolf, Harris, and Folkers, *ibid.*, 1943, 65, 1013); the product also showed only the 2-oxo-vibration and its ultraviolet light absorption (cf. Table 2) suggested that a residue of phenylurea type was present. When refluxed with barium hydroxide solution it gave carbonate and an amine, probably a primary amine since it gave a ninhydrin reaction. A tetrahydro-2-oxo-1-phenylglyoxaline system is therefore indicated; the desulphurisation product must be the 4-methyl derivative (IV; R = Me), and the substance, m. p. 135°, must be the 4-benzylthiomethyl derivative (IV; R = CH₂·S·CH₂Ph). These deductions were confirmed by synthesis of the methyl derivative (IV; R = Me).

2-Bromopropionanilide was aminated by Abderhalden and Brockmann's method (*Fermentforschung*, 1928, 10, 159) and the alanine anilide reduced by lithium aluminium hydride in refluxing tetrahydrofuran to 2-aminopropylaniline. This amine was converted by carbonyl chloride in toluene (Michler and Keller, *Ber.*, 1881, 14, 2181; Puschin and Mitic, *Annalen*, 1937, 532, 300) into the glyoxaline (IV; R = Me), identical [mixed m. p. and infrared spectrum (KBr discs)] with our earlier product.



During one reduction of 4-benzylthiomethyl-3-phenylhydantoin (not repeatable) a substance (m. p. 103—104°) was isolated which gave analyses for the hydroxy-compound (V). This represents an intermediate stage in reduction, and the ultraviolet (maximum 237 mμ; ε 13,000 in neutral and alkaline ethanol) and infrared spectra (no 4-oxo-absorption as in hydantoin; 2-oxo-band at 1671 cm.⁻¹) agree with this formulation. The tendency of the 4-keto-group to be reduced preferentially was shown by Wilk and Close (*J. Org. Chem.*, 1950, 15, 1020) who found 3-methyl-5-phenylhydantoin to yield 2:3-dihydro-1-methyl-2-oxo-4-phenylglyoxaline on treatment with lithium aluminium hydride.

Reduction of L-cysteine-bishydantoin with sodium in liquid ammonia, followed by benzylation, yielded S-benzyl-L-cysteine-hydantoin without racemisation or reduction of the 4-oxo-group: the reduction, like the racemisation, is clearly associated with the presence of a 3-phenyl substituent.

The high-intensity ultraviolet absorptions of a number of hydantoin and hydantoinic acids are summarised in Table 1. Phenylurea has maximal absorption at 239 mμ (ε 22,000 in neutral or alkaline ethanol) and incorporation into the 2-oxo-1-phenylglyoxaline system causes movement of this to somewhat longer wavelengths (cf. Table 2). When this group is incorporated in the acyclic phenylhydantoinic acid system (Table 1) the shift is less pronounced. Pickard and McKay (*Canad. J. Chem.*, 1953, 31, 896) have recently reported the light absorption of tetrahydro-2-oxo-1-phenylglyoxaline, in good agreement with our results (Table 2). In relation to the tetrahydro-2-oxo-1-phenylglyoxaline system, the light absorption of the 3-phenylhydantoin is surprising.* The only high-intensity

* We find that a similar spectral effect also occurs in (VI; R = Ph) (end-absorption, λ_{max.} 217 mμ; ε 10,500) and (VI; R = *p*-tolyl) (λ_{max.} 219 mμ; ε 10,000) as compared with the analogues (VII) (R = Ph: λ_{max.} 242 mμ, ε 13,000; and R = *p*-tolyl: λ_{max.} 246 mμ, ε 16,000).



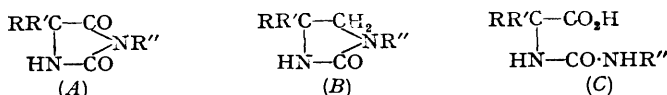
absorption is end-absorption, the maximum lying below 220 $m\mu$. The normal absorption of the phenylurea residue is thus substantially modified by the presence of a 4-oxo-group and some explanation may be found along the following lines (we are grateful to Dr. E. A. Braude for comment on this). Introduction of the 4-oxo-group causes association of unshared electrons of the nitrogen with the $N_{(3)}-C_{(4)}$ bond, as is normally the case with amides. Electrons are thus less available at $N_{(3)}$ and excitation to ${}^+N=\text{C}_6\text{H}_4^-$ or related excited states requires a higher energy than in the case of tetrahydro-2-oxo-1-phenylglyoxaline. Consequently the absorption maximum falls to shorter wavelengths. As is expected, the end-absorption of the hydantoin themselves is much weaker than that of the 3-phenyl derivatives.

The ultraviolet absorptions of both hydantoin and 3-phenylhydantoin are influenced by pH (Table 1). In 0.01N-alcoholic potassium hydroxide a maximum appears at 219–224 $m\mu$ for hydantoin and 5:5-dimethylhydantoin; in neutral or acid ethanol they have

TABLE 1. *Ultraviolet absorption* ^a (λ in $m\mu$) of hydantoin and hydantoinic acids.

Substance	Neutral ethanol	Acid ethanol	0.01N-Ethanol KOH			Alkaline solution acidified
			Immediate	After 4 hr.	After 24 hr.	
C; R=R'=R''=H	λ 215; 240 ϵ 2800; 165	215; 240 2600; 105	215; 240 3200; 55	215; 240 2700; 60	215; 240 3300; 75	215; 240 2400; 80
A; R=R'=R''=H	λ 215; 245 ϵ 400; 60	215; 245 360; 50	224 6200	—	224 6200	216; 245 350; 50
A; R=R'=Me, R''=H	λ 215; 246 ϵ 2100; 150	215; 246 2800; 135	219 6650	219 6300	219 5900	215; 246 1400; 40
C; R=CH ₂ Ph·S·CH ₂ , R'=R''=H	λ 220; 240 ϵ 5900; 320	220; 240 5300; 220	220; 240 9400; 760	220; 240 9400; 760	220; 240 9400; 760	220; 240 8400; 710
A; R=CH ₂ Ph·S·CH ₂ , R'=R''=H	λ 215; 245 ϵ 4700; 470	215; 245 8250; 1060	—	238 7800	238 8500	215; 245 4250; 240
[A; R=·S·CH ₂ , R'=R''=H] ₂	λ 215; 245 ϵ 6500; 1300	215; 245 5400; 1200	229–236 11,600	234 17,000	226 10,800	217; 245 4900; 400
C; R=R'=H, R''=Ph	λ 240 ϵ 19,400	240 19,000	240 19,600	240 19,600	240 19,600	240 18,800
A; R=R'=H, R''=Ph	λ 216; 245 ϵ 7400; 1050	216; 245 7400; 1050	216; 245 9500; 7030	242 19,300	242 19,300	242 18,600
C; R=CH ₂ Ph·S·CH ₂ , R'=H, R''=Ph ^e	λ 242 ϵ 23,800	242 22,800	242 23,400	242 23,400	242 23,400	242 23,400
A; R=CH ₂ Ph·S·CH ₂ , R'=H, R''=Ph ^e	λ 215; 245 ϵ 17,500; 1600	215; 245 17,500; 1600	238 18,400	238 18,100	238 18,100	244 12,800

^a Roman numerals indicate maxima; italic numerals indicate end-absorption measured arbitrarily at two wave-lengths. ^b A, B, C denote compounds as below. ^c λ_{\max} 239 (ϵ 22,800 in EtOH) when measured with the Medium Quartz instrument. ^d Substance [A; R = ·S·CH₂, R' = H, R'' = Ph]₂ had λ (infl.) 226 (ϵ 12,800) in neutral EtOH and λ_{\max} 237 (ϵ 33,000) after 0 and 24 hr. respectively in 0.01N-alkali; substance [C; R = ·S·CH₂, R' = H, R'' = Ph]₂ had λ_{\max} 239 (ϵ 38,000) in EtOH. (All measured on a Medium Quartz instrument.)

TABLE 2. *Ultraviolet absorption of tetrahydro-2-oxoglyoxalines.*

Compound ^a	λ_{\max} ($m\mu$)	ϵ	Compound ^a	λ_{\max} ($m\mu$)	ϵ
B; R=Me, R'=H, R''=Ph ^b	245	18,400	B; R=R'=H, R''=Ph ^{b, d}	245	19,050
B; " " "	246	19,600	B; R=R'=H, R''= <i>p</i> -tolyl ^{b, d}	247	19,500

^a See footnote to Table 1. ^b In EtOH. ^c In ethanolic 0.01N-potassium hydroxide. ^d Pickard and McKay, *Canad. J. Chem.*, 1953, **31**, 896.

only end-absorption. Cystine-bishydantoin and S-benzylcysteine-hydantoin, which also have only end-absorption in neutral or acid solutions, develop maxima at 234 and 237 $m\mu$ after 4 hours, though the absorption is not constant. When an alkaline solution of any of these four compounds is acidified, these maxima disappear, leaving end-absorption similar to that observed if they are dissolved directly in acid ethanol. Stuckey (*J.*, 1947, 331)

has explained such behaviour by postulating enolisation of the acidic 3-hydrogen atom (cf. Zeif and Edsall, *J. Amer. Chem. Soc.*, 1937, **59**, 2245; Pickett and McLean, *ibid.*, 1939, **61**, 423; Ware, *Chem. Rev.*, 1950, **46**, 403) and our results are consistent with his views: the maxima in alkaline solution are not due to hydrolysis to the corresponding hydantoic

TABLE 3. Infrared absorption (cm^{-1}) * of hydantoin and tetrahydro-2-oxoglyoxalines.

Compound †	2-CO	4-CO	Ph _I ‡	Ph _{II} ‡
A; R=R'=R''=H •	1697	1776	—	—
A; R=Me, R'=R''=H •	1694	1730	—	—
A; R=R'=Me, R''=H •	1706	1748	—	—
A; R=CH ₂ Ph·S·CH ₂ , R'=R''=H	1686	1739, 1773 ^b	1597	1488
[A; R=S·CH ₂ , R'=R''=H] ₂	1730 ^c	1754, 1776 ^b	—	—
A; R=R'=H, R''=Ph	1703	1718, 1767 ^b	1595	1497
A; R=Me, R'=H, R''=Ph	1692	1761	1592	1497, 1488
A; R=CH ₂ Ph·S·CH ₂ , R'=H, R''=Ph	1692	1724, 1773 ^b	1595	1497, 1488
B; R=Me, R'=H, R''=Ph	1700	—	1595	—
B; R=CH ₂ Ph·S·CH ₂ , R'=H, R''=Ph	1682	—	1598	—

* Spectra refer to paraffin mulls; a dash indicates that the vibration was absent.

† See footnote to Table 1.

• Randall, Fowler, Fuson, and Dangl, *loc. cit.* ^b It is uncertain which belongs to the 4-oxo-vibration. ^c This lies outside the range given by Randall *et al.* for a 2-oxo-group. ^d Randall *et al.* give 1600 and 1493 cm^{-1} .

acids as these too have merely end-absorption. Maxima given by the sulphur-containing hydantoin in alkali lie at longer wavelengths than would be expected if change involved only the hydantoin ring. The variation of maximal absorption and intensity remains unexplained. Decomposition is doubtless involved as these compounds are unstable to alkali.

The behaviour of 3-phenylhydantoin differs from that of hydantoin lacking this substituent. In neutral or acid ethanol the former show only end-absorption which changes to a high-intensity maximum soon after dissolution in 0.01N-alkali. But on acidification after 24 hours' storage in alkali the maximum remains unchanged in position. The position and intensity of absorption suggest that cleavage to the corresponding phenylhydantoic acid has occurred in alkaline solution. In support of this it was found that 3-phenylhydantoin was completely hydrolysed to 3-phenylhydantoic acid merely by treatment with one equivalent of alkali at 25°. This does not agree with Ware's generalisation (*loc. cit.*, p. 446) that substituted hydantoin are much more stable to hydrolytic agents than are the unsubstituted analogues. Figures in Table 2, and other data obtained during this work, indicate that the S-benzyl residue and the disulphide linkage make appreciable contributions to end-absorption in hydantoin, hydantoic acids, and related compounds.

Infrared measurements have accumulated for a number of compounds prepared during this work and some of these are summarised in Table 3. The vibrational assignments are based on investigations of Randall, Fowler, Fuson, and Dangl ("The Infra-red Determination of Organic Structures," Van Nostrand, New York, 1949) who consider that the 2-oxo-stretching vibration in a hydantoin lies at 1670—1710 cm^{-1} and that of the 4-oxo-group at 1720—1790 cm^{-1} . Our results are broadly interpreted by their assignments, though there are sometimes two carbonyl absorptions in the 4-oxo-region where only one would be expected. As with those of Randall *et al.*, our observations were made on paraffin mulls, to avoid solubility difficulties. Consequently, crystal-lattice effects such as intermolecular hydrogen bonding may be involved.

EXPERIMENTAL

Analyses were carried out in the microanalytical laboratories of Imperial College (Mr. F. H. Oliver). Ultraviolet absorptions were measured in EtOH, unless otherwise stated, by Mrs. I. Boston with a Unicam photoelectric instrument. Those marked * were measured by her with a Hilger Medium Quartz instrument. Some of the infrared data were obtained by Mr. L. Erskin using a Grubb-Parsons double beam spectrometer.

L-Cystine-bis-3-phenylhydantoin.—*L-Cystine* (2.0 g.) was dissolved in *N*-sodium hydroxide (18 ml.) and water (24 ml.). Phenyl isocyanate (2.0 g.) was added and the cooled suspension shaken until no sharp odour remained. The solution was filtered and acidification precipitated

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L-cystine-bisphenylhydantoic acid, m. p. 150°. The crude acid was heated under reflux for 2 hr. with 33% hydrochloric acid (50 ml.) and, on cooling, L-cystine-bis-3-phenylhydantoin crystallised (2.3 g., 62%). After crystallisation (ethanol) it had m. p. 125—127°, $[\alpha]_D^{21} - 98^\circ$ (*c*, 0.21 in EtOH) (Found: C, 54.0; H, 4.5. Calc. for $C_{20}H_{18}O_4N_4S_2$: C, 54.5; H, 4.1%). Shiple and Sherwin (*J. Biol. Chem.*, 1923, **55**, 671) give m. p. 117°; Gortner and Hoffman (*ibid.*, 1927, **72**, 433) give 122—123°.

When L-cystine (2.0 g.) was treated with only one mol. of phenyl isocyanate (0.9 g.) as described above, the product, m. p. 123° (1.6 g., 43%), was still essentially L-cystine-bis-3-phenylhydantoin.

Oxidation of L-Cystine-bis-3-phenylhydantoin with Performic Acid.—The hydantoin (0.88 g.) was dissolved in formic acid (15 ml.), 30% hydrogen peroxide (1 ml.) added, and the mixture set aside for 1 hr. The solvent was removed *in vacuo* and the oily residue dissolved in ethanol; on addition of water a flocculent mass was precipitated (100 mg.; m. p. 120—128°). Recrystallisation from ethanol–light petroleum (b. p. 60—80°) and concentration of the filtrate gave gluey material, m. p. 75—78°.

Reduction and Benzoylation of L-Cystine-bis-3-phenylhydantoin.—The hydantoin (0.8 g.) was dissolved in liquid ammonia (200 ml.), and sodium added in small quantities until the blue colour was just permanent. Benzyl chloride (0.8 ml.) was added dropwise, the ammonia evaporated, and the residue treated with 2*N*-hydrochloric acid (15 ml.). Extraction with ethyl acetate gave 4-benzylthiomethyltetrahydro-2-oxo-1-phenylglyoxaline (substance A) (0.5 g.) which, crystallised from this solvent, had m. p. 135—136°, $[\alpha]_D^{20} 0^\circ$ (*c* 2 in EtOH) [Found: C, 68.1; H, 5.8; N, 9.5; S, 10.0%; *M* (Rast), 329. $C_{17}H_{18}ON_2S$ requires C, 68.4; H, 6.1; N, 9.4; S, 10.7%; *M*, 298].

S-Benzyl-L-cysteine-3-methylhydantoin.—S-Benzylcysteine (11 g.) was dissolved in water (30 ml.) containing sodium hydroxide (2.2 g.). Methyl isocyanate (4.0 g.; prepared according to Boehmer, *Rec. Trav. chim.*, 1936, **55**, 379, in 60% yield) was added, and the suspension shaken. After 15 min. the solution was acidified with hydrochloric acid, and the oily precipitate isolated with chloroform. The oil was refluxed (30 min.) with 20% hydrochloric acid (100 ml.) and sufficient ethanol to effect dissolution. The product which separated on cooling had m. p. 115° (8.2 g.), raised on recrystallisation to 117—118°, $[\alpha]_D^{21.5}$ was $-35^\circ \pm 1^\circ$ (*c* 1.00 in EtOH) (Found: C, 57.5; H, 5.4. $C_{12}H_{14}O_2N_2S$ requires C, 57.6; H, 5.6%).

The hydantoin (0.5 g.), dissolved in liquid ammonia (30 ml.), was set aside for 15 min. On evaporation the product had m. p. 117° alone or mixed with the starting material. After one crystallisation from ethanol it had $[\alpha]_D^{21.5} - 35^\circ \pm 1^\circ$ (*c* 1.00 in EtOH).

S-Benzyl-L-cysteine-3-phenylhydantoin.—The crude phenylhydantoic acid (m. p. 147°) was prepared from S-benzyl-L-cysteine (2.0 g.) and phenyl isocyanate (2.0 g.) in the usual way and heated under reflux for 90 min. with 20% hydrochloric acid (100 ml.) containing sufficient acetone to effect dissolution. The acetone was evaporated and the oily suspension extracted with warm ethyl acetate. Evaporation and crystallisation from ethanol produced S-benzyl-L-cysteine-3-phenylhydantoin (2.0 g., 68%), m. p. 120°, $[\alpha]_D^{21} - 72.8^\circ$ (*c* 0.88 in EtOH). Shiple and Sherwin (*loc. cit.*) give m. p. 118—119.5°.

Racemisation of S-Benzyl-L-cysteine-3-phenylhydantoin by Liquid Ammonia.—The L-hydantoin was kept in solution in liquid ammonia for 15 min., and the ammonia then evaporated as speedily as possible, leaving S-benzyl-DL-cysteine-3-phenylhydantoin, m. p. 154°, $[\alpha]_D^{20} 0^\circ$ (*c* 1 in EtOH) (Found: C, 65.3; H, 5.0; N, 9.5. $C_{17}H_{18}O_2N_2S$ requires C, 65.3; H, 5.2; N, 9.0%).

An authentic specimen was prepared from S-benzyl-DL-cysteine (2.0 g.) by the method outlined for the L-isomer. Crystallisation from ethanol gave the DL-hydantoin (2.2 g., 75%), m. p. and mixed m. p. 154°. Under similar conditions L-cystine-bis-3-phenylhydantoin was completely racemised in liquid ammonia but the product was a glass.

Reduction and Benzoylation of S-Benzyl-L-cysteine-3-phenylhydantoin.—The hydantoin (10.0 g.) was dissolved in liquid ammonia (1 l.), reduced, and benzoylated as described above. This gave substance A (4.0 g.), m. p. 135°, identical with that mentioned previously.

In one experiment, with hydantoin (1.0 g.), 4-benzylthiomethyltetrahydro-5-hydroxy-2-oxo-1-phenylglyoxaline (?) (V) (0.4 g.), m. p. 103—104°, $[\alpha]_D^{21} + 10^\circ$ (*c* 1 in EtOH), was isolated (Found: C, 65.1; H, 6.0; N, 8.8. $C_{17}H_{18}O_4N_2S$ requires C, 65.0; H, 5.7; N, 8.9%).

Desulphurisation of 4-Benzylthiomethyltetrahydro-2-oxo-1-phenylglyoxaline.—The substance (2.5 g.) was heated in 90% ethanol (150 ml.) with Raney nickel (40 g.) for 4 hr. The suspension was centrifuged, the nickel was washed with acetone, and the combined solutions were evaporated to dryness. The residue of tetrahydro-4-methyl-2-oxo-1-phenylglyoxaline, when crystallised from light petroleum (b. p. 40—60°)–ether, had m. p. 102—103° (0.94 g.). It was

also purified by sublimation (98°/10⁻⁴ mm.) (Found: C, 67.9; H, 7.0; N, 16.0%; *M*, 193. C₁₀H₁₃ON₂ requires C, 68.2; H, 6.9; N, 15.9%; *M*, 176).

This product (200 mg.) was heated under reflux with carbonate-free barium hydroxide (2 g. in 5 ml. of water) for 10 hr. The solution was acidified in a stream of nitrogen, and the evolved carbon dioxide collected as barium carbonate. Filtration from unchanged material, treatment with alkali, and extraction with ether gave the liquid amine which was not further purified. It gave a strong ninhydrin reaction.

DL-Alanine Anilide.— α -Bromopropionic acid was converted into its chloride (by thionyl chloride) and anilide (m. p. 99°, from ethanol) in the usual way (96% yield). The anilide (75 g.) was suspended in ammonia (1.5 l.; *d* 0.880), and ethanol (300 ml.) added. Liquid ammonia was cautiously poured in to saturate the mixture and the flask closed with a wired-on stopper and set aside at 20° for 5 days. Evaporation and distillation gave *DL*-alanine anilide (41 g., 76%), b. p. 133°/0.23 mm., n_D^{19} 1.5710. Abderhalden and Brockmann (*loc. cit.*) give b. p. 190—196°/15—16 mm.

N-2'-Aminopropylaniline.—Alanine anilide (6 g.) in dry tetrahydrofuran (50 ml.) was added to a slurry of lithium aluminium hydride (3 g.) in tetrahydrofuran (100 ml.) at such a rate that gentle boiling was maintained. The mixture was heated under reflux for 2 hr. Water was added and the mixture filtered. Evaporation of the filtrate and distillation of the residue gave *N-2'-aminopropylaniline* (3.4 g., 62%), b. p. 106°/0.4 mm., n_D^{20} 1.5638 (Found: N, 18.2. C₉H₁₄N₂ requires N, 18.7%). Its orange *picrate* had m. p. 155° (Found: N, 18.1. C₁₅H₁₇O₇N₅ requires N, 18.5%).

Synthesis of Tetrahydro-4-methyl-2-oxo-1-phenylglyoxaline.—A solution of *N-2'-aminopropylaniline* (1.9 g.) in toluene (30 ml.) was cooled to 0° and saturated with carbonyl chloride. An oil precipitated which redissolved when warmed. Solvent was removed *in vacuo*. The residual oil solidified when triturated with ether and light petroleum. Filtration gave the crude product (0.4 g., 18%) which was purified by sublimation, then having m. p. 101°, not raised by further purification.

Hydrolysis of 3-Phenylhydantoin.—The hydantoin (0.5 g.) was dissolved in water (50 ml.) containing sodium hydroxide (0.114 g., 1 equiv.) with shaking and set aside for 1 hr. The solution was acidified with hydrochloric acid, and the precipitate (0.47 g.) removed. It had m. p. 196° alone or mixed with phenylhydantoic acid, m. p. 197°.

L-Cystine-bishydantoin.—*L*-Cystine {2.0 g.; $[\alpha]_D^{25}$ -210° (*c* 0.080 in *N-HCl*)} was suspended in boiling water (10 ml.), and potassium cyanate (1.5 g.) was added. 10% Hydrochloric acid (25 ml.) was added and the mixture heated under reflux (30 min.). *L*-Cystine-bishydantoin (1.8 g., 74%) separated on cooling; it had no m. p. but decomposed in the range 310—360°; it had $[\alpha]_D^{20}$ -215° (*c* 0.333 in dimethylformamide). A second preparation under similar conditions gave a 69% yield. The product from this, when recrystallised from dimethylformamide, had decomp. 310—360° after shrivelling, $[\alpha]_D^{22}$ -113.3° (*c* 0.300 in dimethylformamide). Hess (*J. Amer. Chem. Soc.*, 1934, 56, 1421) reports that the substance has no m. p. but decomposes from 310° upwards; he does not record rotation. The above experiments indicate that partial racemisation occurs during the preparation or purification.

When kept in solution in liquid ammonia for 15 min. and then recovered by evaporation of the solvent, both specimens had rotations almost unaltered { $[\alpha]_D^{19}$ -213° (*c* 0.4 in dimethylformamide) and $[\alpha]_D^{20}$ -114° (*c* 0.342% in dimethylformamide) respectively}.

Reduction and Benzoylation of L-Cystine-bishydantoin.—The hydantoin (0.29 g.; $[\alpha]_D^{19}$ -213°) was dissolved in liquid ammonia (100 ml.) and sodium added until a blue colour persisted for 10 min. The excess of reagent was just destroyed with ammonium chloride, and benzyl chloride (0.3 g.) added dropwise. After 15 min. the solvent was evaporated and the residue treated with *N*-hydrochloric acid (15 ml.) and filtered. The filtrate was extracted with chloroform, and the extract evaporated. Crystallisation of the residue yielded *S*-benzyl-*L*-cysteine-hydantoin (0.35 g., 74%), m. p. 118°, $[\alpha]_D^{20}$ -37° (*c* 0.4 in EtOH) (Found: C, 55.7; H, 5.2. Calc. for C₁₁H₁₅O₂N₂S: C, 55.9; H, 5.1%). When the hydantoin of $[\alpha]_D^{20}$ -114° was used, the product (84% yield) had m. p. 118°, $[\alpha]_D^{20}$ -20°.

S-Benzyl-L-cysteine-hydantoin.—*S*-Benzyl-*L*-cysteine ethyl ester hydrochloride (2.6 g.) was dissolved in water (10 ml.) to which a solution of potassium cyanate (0.73 g.) in water (5 ml.) was added. The hydantoic acid was at once precipitated and was heated under reflux with hydrochloric acid until dissolved. Recrystallisation from ethanol gave the hydantoin (1.95 g., 88%), m. p. 118°, $[\alpha]_D^{20}$ -11° (*c* 0.82 in EtOH). On admixture with the specimen having rotation $[\alpha]_D^{20}$ -37°, it had m. p. 117°. A second specimen had m. p. 121°, $[\alpha]_D^{20}$ -12° (*c* 0.209 in EtOH).

S-Benzyl-DL-cysteine-hydantoin.—*S*-Benzyl-*DL*-cysteine (2.11 g.) was treated with potassium

cyanate (0.73 g.) in the usual way, to give the hydantoin (1.7 g., 72%), m. p. 118°, $[\alpha]_D^{20}$ 0°. Admixture with *S*-benzyl-L-cysteine-hydantoin having $[\alpha]_D^{20} - 11^\circ$ did not depress the m. p.

Gawron and Glaide (*J. Amer. Chem. Soc.*, 1949, 72, 3232) give m. p. 129—130° for the L- and 118° for the DL-form of *S*-benzylcysteine-hydantoin (but no rotations). It is clear from the above experiments that some racemisation of the L-form is inevitable during the cyclisation with acid (cf. Dakin, *J. Biol. Chem.*, 1942, 146, 237) but that products with $[\alpha]_D$ varying from -37° to 0° all have m. p. 118°. No substance of m. p. 129—130° has been encountered.

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