## 35. *Degradative Studies on Peptides and Proteins. Part II.* Synthesis and Properties of 3-Benzoyl-1-phenyl-2-thiohydantoin.*

By D. T. Elmore and P. A. Toseland.

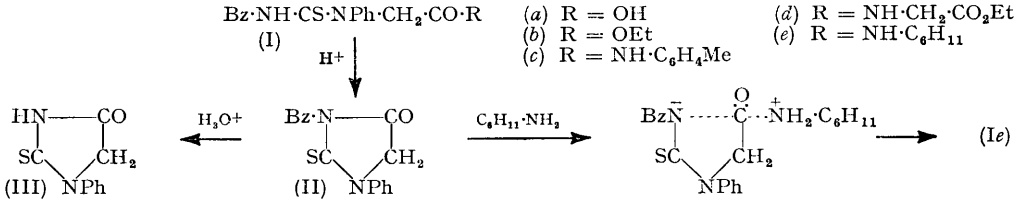
*N*-Benzoylthiocarbamoyl-*N*-phenylglycine (I*a*) and its ethyl ester (I*b*) and *p*-toluidide (I*c*), and *N*-benzoylthiocarbamoyl-*N*-phenylglycylglycine ethyl ester (I*d*), have been converted under suitable conditions of acid catalysis into 3-benzoyl-1-phenyl-2-thiohydantoin (II), a compound previously inadequately described by Douglass and Dains.[1] Hydrolysis of the 3-benzoyl group is brought about by aqueous acid, while *cyclo*hexylamine opens the ring to give *N*-benzoylthiocarbamoyl-*N*-phenylglycine *cyclo*hexylamide (I*e*).

In Part I,[2] we mentioned that, under favourable conditions, acid-catalysed cyclisation and degradation of *N*-acetyl- and *N*-benzoyl-thiocarbamoyl-peptides and their derivatives may yield 3-acetyl- or 3-benzoyl-2-thiohydantoins as intermediates in the formation of the parent 2-thiohydantoin. The only 3-acyl-2-thiohydantoin previously described, so far as we are aware, is 3-benzoyl-1-phenyl-2-thiohydantoin (II).[1] It was claimed that *N*-benzoylthiocarbamoyl-*N*-phenylglycine (I*a*) and its ethyl ester (I*b*) were cyclised by sulphuric acid; no experimental details were recorded and the product was characterised only by nitrogen analysis and m. p. [it should be noted that the m. p.s of (I*a*), (I*b*), and (II) all lie within a few degrees]. Since other 3-acyl-2-thiohydantoins have been obtained only

* Part I, *J.*, 1954, 4533.

[1] Douglass and Dains, *J. Amer. Chem. Soc.*, 1934, **56**, 719.
[2] Elmore and Toseland, *J.*, 1954, 4533.

by use of careful techniques (forthcoming paper), and have been found to be highly reactive, we decided to repeat this work.




Methyl *N*-benzoyldithiocarbamate did not react with *N*-phenylglycine under the usual conditions;[2] this may be attributed to the electron-withdrawing behaviour of the *N*-phenyl group. An additional consequence of this effect is that this amino-acid probably shows little tendency to exist as dipolar ions in aqueous solution, although the p*K* of the imino-group does not appear to have been determined; as a result it is quite soluble in non-aqueous solvents. We found that *N*-benzoylthiocarbamoyl-*N*-phenylglycine (I*a*) and its ethyl ester (I*b*) could be prepared satisfactorily from benzoyl *iso*thiocyanate in acetone as described by Douglass and Dains.[1] The acid (I*a*) was converted into 1-phenyl-2-thiohydantoin (III) by hot 2N-sulphuric acid, no other product being detected by paper chromatography. When allowed to stand at room temperature in 80% sulphuric acid, the acid (I*a*) was unchanged while the ester (I*b*) was recovered partly as such and partly as acid. On the other hand, 98% sulphuric acid at room temperature cyclised the acid to 3-benzoyl-1-phenyl-2-thiohydantoin (II), but better yields were obtained by treatment of the acid or ester with cold trifluoroacetic acid. It was also observed that the ester was largely cyclised when stored in the solid state at room temperature for some weeks. Paper chromatography and determination of infrared spectra were indispensable analytical tools for following the course of these reactions.

3-Benzoyl-1-phenyl-2-thiohydantoin (II) was also obtained by stepwise degradation of peptides of *N*-phenylglycine.[2] It is interesting that peptides of *N*-phenylglycine may be synthesised without previous protection of the imino-group, a further consequence of the inappreciable formation of dipolar ions by this amino-acid. *N*-Phenylglycine *p*-toluidide and *N*-phenylglycylglycine ethyl ester were isolated in satisfactory yields (68 and 45% respectively) from the reaction of *N*-phenylglycine with *p*-toluidine and glycine ethyl ester respectively through the agency of tetraethyl pyrophosphite.[3] Benzoyl *iso*thiocyanate converted *N*-phenylglycine *p*-toluidide and *N*-phenylglycylglycine ethyl ester into their *N*-benzoylthiocarbamoyl derivatives (I*c* and *d*). The last two were not degraded in nitromethane or acetic acid saturated with dry hydrogen chloride,[4] but trifluoroacetic acid at room temperature gave good yields of the thiohydantoin. Trifluoroacetic acid has been found to be an excellent agent for procuring the cyclisation and degradation of other *N*-acylthiocarbamoyl-peptides, and, in view of its solvent properties for proteins,[5] may well find a wide use in this and the Edman method of stepwise degradation of peptides.

The structure of the thiohydantoin (II) has now been firmly established by complete microanalysis and by formation of this compound from the derivatives (I*c* and *d*). Further, its infrared spectrum is strikingly different from those of the acid (I*a*) and its ester and the hydrolysis product (III). In particular, this spectrum shows no band attributable to a N–H stretching mode; in addition, absence of hydrogen bonding results in all the bands' being very sharp. Two intense bands at 1730 and 1624 cm.$^{-1}$ are assigned to the heterocyclic and the exocyclic CO stretching frequency respectively. This designation is supported, first, by examination of the spectrum of the hydantoin (III), which possesses an intense band at 1725 cm.$^{-1}$ and, secondly, by the comparable spectral analysis of 1-acyl-2-thiohydantoins.[6] A very strong band at 1527 cm.$^{-1}$ in the spectrum of the compound

[3] Anderson, Blodinger, and Welcher, *J. Amer. Chem. Soc.*, 1952, **74**, 5309.
[4] Edman, *Acta Chem. Scand.*, 1950, **4**, 283; 1953, **7**, 700.
[5] Katz, *Nature*, 1954, **174**, 509.
[6] Randall, Fowler, Fuson, and Dangl, "Infra-Red Determination of Organic Structures," D. van Nostrand, Inc., New York, 1949, p. 177.

(II) is probably due to the N–C=S system.[7] The complexity of the spectrum precludes further analysis at this stage. The ultraviolet absorption of the benzoyl compound (II) is quite different from those of the hydantoin (III) and the acid (I*a*) and its ester, the most notable feature of the spectrum of (II) compared with that of (III) being the large bathochromic shift due to the benzoyl group. The spectra of the acid (I*a*) and its ester are very similar; the maximal absorption at 3330 and 3300 Å respectively is conspicuous, but it would be premature to discuss this at present.

3-Acyl-2-thiohydantoins may be regarded as unsymmetrical acid anhydrides, and it has been found that the direction of cleavage depends on the reagent. Aqueous acid removed the benzoyl group from the hydantoin (II), while a powerful nucleophilic reagent such as *cyclo*hexylamine effected cleavage of the 2-thiohydantoin ring, presumably by an $S_N2$ mechanism. Reaction occurred solely at $C_{(4)}$, to give *N*-benzoylthiocarbamoyl-*N*-phenylglycine *cyclo*hexylamide (I*c*); this compound was cyclised and degraded to the hydantoin (II) on treatment with trifluoroacetic acid. It is explicable why nucleophilic reagents do not attack $C_{(2)}$, since, although the $-M$ effect of sulphur is greater than that of oxygen, this is more than offset by the joint influence of $N_{(1)}$ and $N_{(3)}$. It is not clear, however, why the 3-acyl group is unaffected, particularly since 1-acyl-2-thiohydantoins are readily converted by basic reagents into the parent 2-thiohydantoins.

## Experimental

Infrared spectra were measured in potassium bromide discs with a Grubb-Parsons double-beam spectrometer and a rock-salt prism.

*N-Benzoylthiocarbamoyl-N-phenylglycine.*—This *compound* was prepared by the general method of Douglass and Dains [1] and had m. p. 167—168° (Found: C, 61·0; H, 4·2; N, 9·0; S, 10·5. $C_{16}H_{14}O_3N_2S$ requires C, 61·1; H, 4·5; N, 8·9; S, 10·2%). The infrared spectrum contained the following bands: 3300, 3190, 3075, 2920, 1656. 1606, 1505, 1493, 1451, 1427, 1377, 1316, 1300, 1273, 1255, 1237, 1217, 1192, 1183, 1125, 1088, 1070, 1025, 1003, 986, 969, 933, 908, 832, 819, 799, 751, 710, 693 cm.$^{-1}$. Light absorption in EtOH: $\lambda_{max.}$ 3330 (ε 25,400), $\lambda_{min.}$ 2890 (ε 3200), $\lambda_{max.}$ 2420 Å (ε 16,300).

*N-Benzoylthiocarbamoyl-N-phenylglycine Ethyl Ester.*—Application of the above method to *N*-phenylglycine ethyl ester afforded the *N-benzoylthiocarbamoyl derivative*, m. p. 158—159° with softening at 125°, in good yield (Found: C, 62·9; H, 5·2; N, 8·2; S, 9·4. $C_{18}H_{18}O_3N_2S$ requires C, 63·1; H, 5·3; N, 8·2; S, 9·4%). Bands in the infrared spectrum were at 3300, 3020, 2980, 2920, 1744, 1644, 1606, 1591, 1481, 1459, 1435, 1382, 1328, 1302, 1279, 1231, 1207, 1182, 1128, 1114, 1094, 1074, 1032, 997, 932, 923, 909, 873, 847, 837, 797, 782, 755, 710, 703, 687 cm.$^{-1}$. Light absorption in EtOH: $\lambda_{max.}$ 3300 (ε 25,400), $\lambda_{min.}$ 2910 (ε 5300), $\lambda_{max.}$ 2430 Å (ε 17,600).

*N-Phenylglycine p-Toluidide.*—*N*-Phenylglycine (4·17 g.), *p*-toluidine (3·21 g.), and tetraethyl pyrophosphite (6·8 g.) were heated in diethyl hydrogen phosphite (30 c.c.) at 90—100° for 90 min. The cooled solution was poured into water (200 c.c.), and the precipitated solid (6·5 g.) was recrystallised twice from ethanol; then it had m. p. 170—171°. Meyer [8] reports m. p. 171—172° and Bischoff and Hausdörfer [9] record m. p. 165°.

*N-Phenylglycylglycine Ethyl Ester.*—*N*-Phenylglycine (3·02 g.), glycine ethyl ester hydrochloride (2·54 g.), triethylamine (2·08 g.), and tetraethyl pyrophosphite (4·5 g.) in diethyl hydrogen phosphite (25 c.c.) were heated at 90—95° for 40 min., cooled, and poured into water (200 c.c.). Precipitated solid (0·8 g.), m. p. 254—257° (decomp.) (presumably 2:6-dioxopiperazine), was collected, and the solution was extracted with ethyl acetate. The extract was dried and evaporated under reduced pressure; addition of light petroleum (b. p. 40—60°) caused *N*-phenylglycylglycine ethyl ester (3·1 g.) to crystallise; it had m. p. 84° (Wessely [10] reports m. p. 88°).

*N-Benzoylthiocarbamoyl-N-phenylglycine p-Toluidide.*—Benzoyl *iso*thiocyanate and *N*-phenylglycine *p*-toluidide in dry acetone afforded the *N-benzoylthiocarbamoyl derivative* (90%). Recrystallisation from ethanol gave a pale yellow product, m. p. 151° (Found: C, 68·6; H, 5·1; N, 9·9; S, 8·3. $C_{23}H_{21}O_2N_3S$ requires C, 68·5; H, 5·3; N, 10·4; S, 7·9%).

[7] Ref. 6, p. 5.
[8] Meyer, *Ber.*, 1875, **8**, 1152.
[9] Bischoff and Hausdörfer, *Ber.*, 1890, **23**, 1997.
[10] Wessely, *Z. physiol. Chem.*, 1925, **146**, 72.

N-*Benzoylthiocarbamoyl*-N-*phenylglycylglycine Ethyl Ester.*—Benzoyl *iso*thiocyanate and *N*-phenylglycylglycine ethyl ester were caused to react in dry acetone. The mixture was poured into water (100 c.c.), and the resultant yellow oil was extracted into chloroform. Addition of light petroleum (b. p. 40—60°) to the dried extract yielded N-*benzoylthiocarbamoyl*-N-*phenylglycylglycine ethyl ester* (55%), m. p. 118—119°; recrystallisation from aqueous ethanol raised this to 125° (Found: C, 59·7; H, 5·2; N, 10·3. $C_{20}H_{21}O_4N_3S$ requires C, 60·1; H, 5·3; N, 10·5%).

*Behaviour of* N-*Benzoylthiocarbamoyl*-N-*phenylglycine and its Derivatives in Acids.*—(i) *N*-Benzoylthiocarbamoyl-*N*-phenylglycine (300 mg.) was heated under reflux in 2N-sulphuric acid (20 c.c.) and ethanol (10 c.c.). The acid (I*a*), the hydantoin (III), benzoic acid, and an unidentified, ultraviolet-absorbing substance were detected by paper chromatography of aliquot parts withdrawn at intervals. After 5 hr., the hydantoin (III) (170 mg.) was isolated from the hydrolysate; it had m. p. and mixed m. p. 175°.

(ii) *N*-Benzoylthiocarbamoyl-*N*-phenylglycine in 80% sulphuric acid (cf. Douglass and Dains [1]) was unchanged after 2 days at room temperature. The ethyl ester under the same conditions was partly hydrolysed to the free acid.

(iii) *N*-Benzoylthiocarbamoyl-*N*-phenylglycine (2 g.) was dissolved in 98% sulphuric acid; the solution was kept at room temperature for 4 days and poured into water (500 c.c.). The resultant oil was extracted into ethyl acetate, and the extract was washed with 5% sodium hydrogen carbonate solution, followed by water, and dried. Addition of light petroleum (b. p. 40—60°) to the warm solution caused 3-*benzoyl*-1-*phenyl*-2-*thiohydantoin* (0·84 g.) to separate as slightly purple crystals, m. p. 155—157° (Found: C, 64·9; H, 4·1; N, 9·2; S, 10·5. $C_{16}H_{12}O_2N_2S$ requires C, 64·8; H, 4·1; N, 9·5; S, 10·8%). Douglass and Dains [1] record m. p. 163° for their product of the same alleged structure. The infrared spectrum had bands at 2930, 2870, 1730, 1624, 1595, 1573, 1527, 1493, 1451, 1430, 1383, 1338, 1324, 1254, 1230, 1207, 1172, 1160, 1130, 1104, 1090, 1081, 1069, 1025, 1001, 951, 880, 824, 762, 722, 686, 678 cm.$^{-1}$. Light absorption in EtOH: $\lambda_{max.}$ 2980 (ε 14,600), $\lambda_{min.}$ 2930 (ε 14,200), $\lambda_{max.}$ 2870 (ε 14,800), $\lambda_{min.}$ 2760 (ε 13,600), $\lambda_{max.}$ 2590 Å (ε 20,600).

(iv) *N*-Benzoylthiocarbamoyl-*N*-phenylglycine *p*-toluidide and *N*-benzoylthiocarbamoyl-*N*-phenylglycylglycine ethyl ester were unaffected by dry hydrogen chloride in either nitromethane or acetic acid.

(v) All the foregoing *N*-benzoylthiocarbamoyl derivatives (I*a*, *b*, *c*, *d*) were cyclised in trifluoroacetic acid at room temperature. Experiments are summarised in the Table. The

| Compound | Time (hr.) | Yield of (II) (%) | M. p. | Compound | Time (hr.) | Yield of (II) (%) | M. p. |
|---|---|---|---|---|---|---|---|
| I*a* | 24 | 84 | 155—157° | I*c* | 2 | — | 155—157° |
| I*b* | 24 | 90 | 155—157 | I*d* | 0·25 | 92 | 156—157 |

product was isolated by pouring the reaction mixture into water. After recrystallisation from ethanol or dioxan, identity was established by determination of infrared spectra.

*Reaction of* 3-*Benzoyl*-1-*phenyl*-2-*thiohydantoin with* cyclo*Hexylamine.*—3-Benzoyl-1-phenyl-2-thiohydantoin (2 g.) in chloroform (20 c.c.) was warmed on the steam-bath for 10 min. with *cyclo*hexylamine (0·7 g.). The cooled solution was washed with dilute hydrochloric acid and evaporated under reduced pressure. The residue was crystallised from ethyl acetate–light petroleum (b. p. 90—120°). Pale yellow prisms (2·2 g.) of N-*benzoylthiocarbamoyl*-N-*phenylglycine* cyclo*hexylamide* separated, having m. p. 167°, depressed on admixture with starting material (Found: C, 66·9; H, 6·3; N, 10·5; S, 7·9. $C_{22}H_{25}O_2N_3S$ requires C, 66·9; H, 6·4; N, 10·6; S, 8·1%). This compound in trifluoroacetic acid afforded 3-benzoyl-1-phenyl-2-thiohydantoin (65%).

*Acid Hydrolysis of* 3-*Benzoyl*-1-*phenyl*-2-*thiohydantoin.*—3-Benzoyl-1-phenyl-2-thiohydantoin (11 mg.) in ethanol (0·5 c.c.) and 2N-hydrochloric acid (0·5 c.c.) was heated at 100°; aliquot parts were removed at intervals, evaporated to dryness, and analysed by chromatography in butan-1-ol saturated with water and in benzene–acetic acid–water (1 : 1 : 1). After 2·5 hr., most of the starting material had been converted into 1-phenyl-2-thiohydantoin. Traces of two unknown substances were also detected.

The authors are grateful to Professor R. D. Haworth, F.R.S., for his continued interest and encouragement, and to Imperial Chemical Industries Limited for financial assistance.

STAVELEY RESEARCH LABORATORIES,
THE UNIVERSITY, SHEFFIELD, 10.

[*Received, August 24th*, 1955.]