639. *Degradative Studies of Peptides and Proteins. Part V.* The Formation of 2-Acylimino-3-methylthiazolid-5-ones from N-Acylthiocarbamoylsarcosine Derivatives and their Behaviour towards Nucleophilic Reagents.*

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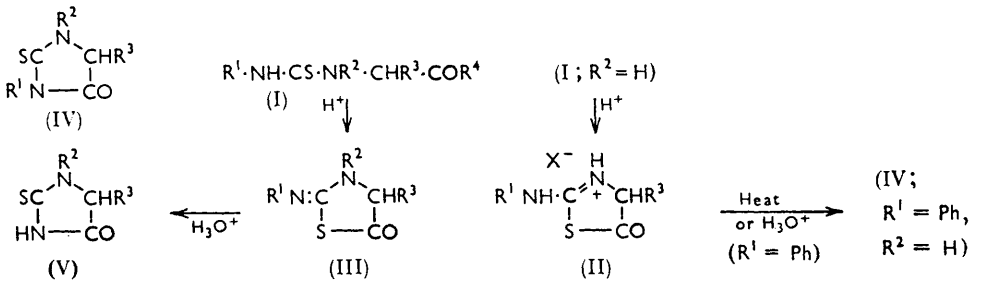
N-Acylthiocarbamoylsarcosine esters and amides undergo cyclisation and degradation in trifluoroacetic acid to 2-acylimino-3-methylthiazolid-5-ones. Scission of the heterocyclic ring is effected by nucleophilic reagents, although less readily than with the salts of 2-acylaminothiazol-5-ones. The former are converted into 2-thiohydantoins by hot, dilute acids.

Edman,[1] and Elmore and Toseland,[2] have shown that anhydrous acids cyclise and degrade *N*-phenyl- and *N*-acyl-thiocarbamoylpeptides (I; R^1 = Ph or acyl, R^2 = H) to salts of 2-anilino- and 2-acylamino-thiazol-5-ones (II; R^1 = Ph or acyl) respectively. In the Edman procedure, the *N*-terminal amino-acid is ultimately recovered as a 3-phenyl-2-thiohydantoin (IV; R^1 = Ph, R^2 = H), and it was shown that 2-anilino-4-isobutylthiazolonium hydrochloride (II; R^1 = Ph, R^3 = Bu^i, X = Cl) was converted into 5-isobutyl-3-phenyl-2-thiohydantoin (IV; R^1 = Ph, R^2 = H, R^3 = Bu^i) by either heat or warm, dilute acids. In the latter case, *N*-phenylthiocarbamoyl-leucine (I; R^1 = Ph, R^2 = H, R^3 = Bu^i, R^4 = OH) was an intermediate in the reaction.

* Part IV, *J.*, 1957, 2460.

[1] Edman, *Nature*, 1956, **177**, 667; *Acta Chem. Scand.*, 1956, **10**, 761.
[2] Elmore and Toseland, *J.*, 1957, 2460.

The presence of an additional substituent on the nitrogen atom of peptides containing proline, hydroxyproline, sarcosine, or *N*-phenylglycine as *N*-terminal residues (I; $R^2 \neq H$) precludes the formation of compounds of type (II). There are two possible courses for cyclisation. For *N*-acylthiocarbamoylpeptides, an analogous mechanism involving nucleophilic attack by sulphur on the carbon atom of the peptide linkage would give rise to 2-acyliminothiazolid-5-ones (III; R^1 = acyl). Alternatively, ring-closure could occur on the nitrogen, leading to 3-acyl-2-thiohydantoins (IV; R^1 = acyl). In an earlier



paper,[3] we favoured the second possibility and we concluded that *N*-benzoylthiocarbamoyl-*N*-phenylglycine (I; R^1 = Bz, R^2 = Ph, R^3 = H, R^4 = OH) and its derivatives were cyclised by trifluoroacetic acid to 3-benzoyl-1-phenyl-2-thiohydantoin (IV; R^1 = Bz, R^2 = Ph, R^3 = H), a structure previously advanced by Douglass and Dains.[4] We have now re-examined this problem, using *N*-acylthiocarbamoylsarcosine derivatives (I; R^1 = acyl, R^2 = Me, R^3 = H). Crystallographic analysis by Dr. H. Steeple, of the Department of Physics, Manchester College of Science and Technology, has proved conclusively that the compound obtained by cyclisation of *N*-benzoylthiocarbamoylsarcosine ethyl ester or *p*-toluidide (I; R^1 = Bz, R^2 = Me, R^3 = H, R^4 = OEt or NH·C_6H_4Me-*p*) is 2-benzoylimino-3-methylthiazolid-5-one (III; R^1 = Bz, R^2 = Me, R^3 = H). The molecule is virtually planar, as expected from a consideration of bond angles. Crystallographic data will be published in detail elsewhere. In view of the similarity between the derivatives from sarcosine and *N*-phenylglycine, we now believe that acid-catalysed cyclisation of *N*-benzoylthiocarbamoyl-*N*-phenylglycine and its derivatives affords 2-benzoylimino-3-phenylthiazolid-5-one (III; R^1 = Bz, R^2 = Ph, R^3 = H) and not 3-benzoyl-1-phenyl-2-thiohydantoin (IV; R^1 = Bz, R^2 = Ph, R^3 = H). Our earlier preference for the latter structure depended to some extent on the presence of a strong band at 1527 cm.$^{-1}$ in the infrared spectrum, which we attributed to the $>N-\underset{|}{C}=S$ system.[5] Neither of the 2-aroylimino-3-methylthiazolid-5-ones described herein absorbs over the region where thioureide bands are normally encountered.

N-2,4-Dichlorobenzoylthiocarbamoylsarcosine *p*-toluidide (I; R^1 = 2,4-$Cl_2C_6H_3$·CO, R^2 = Me, R^3 = H, R^4 = NH·C_6H_4Me-*p*) gave the corresponding 2-(2,4-dichlorobenzoylimino)-3-methylthiazolid-5-one (III; R^1 = 2,4-$Cl_2C_6H_3$·CO, R^2 = Me, R^3 = H). Attempts to cyclise *N*-benzoylthiocarbamoylhydroxy-L-proline ethyl ester [I; R^1 = Bz, R^2R^3 = CH_2·CH(OH)·CH_2, R^4 = OEt] in trifluoroacetic acid were unsuccessful, but it is probable that degradation of *N*-phenyl- or *N*-acyl-thiocarbamoyl-prolyl or -hydroxyprolyl peptides in anhydrous acids would involve this type of intermediate.

2-Aroylimino-3-methylthiazolid-5-ones with amines such as *p*-toluidine or cyclohexylamine gave the corresponding *N*-aroylthiocarbamoylsarcosine amide derivatives. In this respect, they are very similar to the 3-phenyl derivative [3] (III; R^1 = Bz, R^2 = Ph, R^3 = H) and the 2-acylaminothiazol-5-ones [2] (II) described previously. 2-Aroylimino-3-methylthiazolid-5-ones, however, unlike the latter, are stable to weaker nucleophiles such

[3] Elmore and Toseland, *J.*, 1956, 188.
[4] Douglas and Dains, *J. Amer. Chem. Soc.*, 1934, **56**, 719.
[5] Elmore, *J.*, 1958, 3489.

as water and alcohols. Undoubtedly, the replacement of a proton on the heterocyclic ring by an electron-repelling methyl group at $N_{(3)}$ decreases the fractional positive charge on the carbonyl-carbon atom and lowers its susceptibility to nucleophilic attack. Since the *N*-benzoylthiocarbamoyl derivatives of sarcosine ethyl ester and *N*-phenylglycine ethyl ester are cyclised by trifluoroacetic acid, whereas *N*-benzoylthiocarbamoylglycine ethyl ester is unchanged by this treatment, we may summarize the situation by the following expressions:

$$(\text{I};\ R^2 = \text{Me or Ph},\ R^4 = \text{OEt}) + H^+ \underset{k_2}{\overset{k_1}{\rightleftharpoons}} (\text{III}) + \text{EtOH} + H^+ \quad (k_1 \gg k_2)$$

$$(\text{I};\ R^2 = \text{H},\ R^4 = \text{OEt}) + H^+ \underset{k_4}{\overset{k_3}{\rightleftharpoons}} (\text{II}) + \text{EtOH} \quad (k_4 \gg k_3)$$

These inequalities may not always obtain owing to other factors, and this may explain the failure to cyclise *N*-benzoylthiocarbamoyl-L-hydroxyproline ethyl ester. Although the 2-aroylimino-3-methylthiazolid-5-ones are stable to alcohols and water, treatment with hot dilute acids affords 1-methyl-2-thiohydantoin (IV; $R^2 = Me$, $R^3 = H$). This parallels the behaviour of the 3-phenyl derivative (III; $R^1 = Bz$, $R^2 = Ph$, $R^3 = H \longrightarrow$ V; $R^2 = Ph$, $R^3 = H$) described previously.[3]

Attempts to isolate 2-acetylimino-3-methylthiazolid-5-one were unsuccessful. Surprisingly, *N*-acetylthiocarbamoylsarcosine *p*-toluidide (I; $R^1 = Ac$, $R^2 = Me$, $R^3 = H$, $R^4 = NH{\cdot}C_6H_4Me$-*p*) was stable to trifluoroacetic acid. The corresponding benzylamide was apparently cyclised and degraded under these conditions, a result which was expected in view of the stronger basicity of the benzylamide. The subsequent working up procedure, however, involved exposure to water and we surprisingly isolated an acidic product which gave analytical values for *N*-acetylthiocarbamoylsarcosine. The 2-acetylimino-3-methylthiazolidone thus appears to be more sensitive than the corresponding aroylimino-derivatives to nucleophiles. A less likely explanation is the alternative cyclisation to 3-acetyl-1-methyl-2-thiohydantoin and subsequent cleavage with water. Degradation of the benzylamide was also apparently effected by dry hydrogen chloride in nitromethane, but no crystalline material could be isolated. The presence of 2-acetylimino-3-methylthiazolid-5-one (or 3-acetyl-1-methyl-2-thiohydantoin) was confirmed by reaction with *p*-toluidine and the isolation of *N*-acetylthiocarbamoylsarcosine *p*-toluidide.

Experimental

Ultraviolet spectra were determined as before.[6] Infrared spectra were measured in pressed discs of potassium bromide, a rock-salt prism being used.

N-Benzyloxycarbonylsarcosine.—Methylaminoacetonitrile sulphate[7] (30 g.) in 5N-hydrochloric acid (250 c.c.) was heated under reflux for 3 hr. After evaporation under reduced pressure, the residue was dissolved in water (250 c.c.), and sufficient 2N-sodium hydroxide was added to bring the pH to 10—11. The solution was cooled to 0° and stirred while benzyl chloroformate (45 g.) and 2N-sodium hydroxide (135 c.c.) were added alternately portionwise during 45 min. Stirring was continued for 2 hr. without the ice-bath. The solution was extracted with ether, acidified, and extracted three times with chloroform. The extract was dried (Na_2SO_4) and evaporated, and the product (31·6 g.), m. p. 54°, was caused to crystallise by addition of light petroleum (b. p. 40—60°) and storage at 0° (Ben-Ishai and Katchalski[8] report m. p. 53—54°).

N-Benzyloxycarbonylsarcosine p-Toluidide.—A mixture of *N*-benzyloxycarbonylsarcosine (4·46 g.) and triethylamine (2·02 g.) in chloroform (15 c.c.) was cooled to −10°; ethyl chloroformate (2·17 g.) was added portionwise at −10° to −5°. After 30 min., *p*-toluidine (2·14 g.) in chloroform (10 c.c.) was added, and the mixture was allowed to warm to room temperature

[6] Elmore and Ogle, *J.*, 1957, 4404.
[7] Cook and Cox, *J.*, 1949, 2334.
[8] Ben-Ishai and Katchalski, *J. Amer. Chem. Soc.*, 1952, **74**, 3688.

overnight. Enough chloroform was added to obtain a clear solution, which was washed successively with saturated sodium hydrogen carbonate, water, dilute hydrochloric acid, and water, dried (Na_2SO_4), and evaporated. Addition of light petroleum (b. p. 40—60°) afforded the *product* (5·01 g.), which had m. p. 143·5—144·0° after recrystallisation from chloroform–light petroleum (b. p. 40—60°) (Found: C, 69·0; H, 6·6; N, 8·8. $C_{18}H_{20}O_3N_2$ requires C, 69·2; H, 6·5; N, 9·0%).

N-*Benzyloxycarbonylsarcosine Benzylamide*.—This *compound* (80%) was obtained by a similar method from benzylamine. Recrystallised from carbon tetrachloride–light petroleum (b. p. 40—60°), it had m. p. 116·5—117·0° (Found: C, 68·8; H, 6·6; N, 9·4. $C_{18}H_{20}O_3N_2$ requires C, 69·2; H, 6·5; N, 9·0%).

Sarcosine p-*Toluidide Hydrobromide*.—Treatment of *N*-benzyloxycarbonylsarcosine *p*-toluidide (9 g.) in acetic acid (40 c.c.) with 50% w/v hydrogen bromide in acetic acid (20 c.c.) at 100° for a few minutes, followed by cooling, addition of dry ether (400 c.c.), and storage at 0°, furnished this *salt* (7·33 g.), m. p. 262—263° (decomp.) (from ethanol) (Found: C, 46·4; H, 5·7; N, 10·7. $C_{10}H_{15}ON_2Br$ requires C, 46·3; H, 5·8; N, 10·8%).

Sarcosine Benzylamide Hydrobromide.—Prepared in almost theoretical yield by a similar method, this *compound* had m. p. 143·0—143·5° (from ethanol–ether) (Found: C, 46·1; H, 5·9; N, 11·3. $C_{10}H_{15}ON_2Br$ requires C, 46·3; H, 5·8; N, 10·8%).

N-*Acetylthiocarbamoylsarcosine* p-*Toluidide*.—The free base, obtained from treatment of sarcosine *p*-toluidide hydrobromide (2·6 g.) with ammonia in chloroform, was allowed to react with methyl *N*-acetyldithiocarbamate (1·49 g.) in ether (40 c.c.) at room temperature for 3 weeks. The precipitated *product* (2·12 g.) recrystallised from ethanol–light petroleum (b. p. 40—60°), then having m. p. 169° (Found: C, 56·1; H, 6·0; N, 15·7. $C_{13}H_{17}O_2N_3S$ requires C, 55·9; H, 6·1; N, 15·0%).

N-*Acetylthiocarbamoylsarcosine Benzylamide*.—Reaction of sarcosine benzylamide (from the hydrobromide) with methyl *N*-acetyldithiocarbamate in chloroform–ether (1 : 1) during 6 days at room temperature afforded N-*acetylthiocarbamoylsarcosine benzylamide* (49%), which had m. p. 144—145° after recrystallisation first from aqueous ethanol and then from benzene containing a little ethanol (Found: C, 55·7; H, 6·1; N, 15·3. $C_{13}H_{17}O_2N_3S$ requires C, 55·9; H, 6·1; N, 15·0%).

N-*Benzoylthiocarbamoylsarcosine* p-*Toluidide*.—The free base from sarcosine *p*-toluidide hydrobromide (2·6 g.) was treated with benzoyl isothiocyanate (1·63 g.) in ether (20 c.c.). The *product* (3·1 g.) had m. p. 155·5° after recrystallisation from ethanol (Found: C, 63·7; H, 5·6; N, 12·2. $C_{18}H_{19}O_2N_3S$ requires C, 63·3; H, 5·6; N, 12·3%).

N-2,4-*Dichlorobenzoylthiocarbamoylsarcosine* p-*Toluidide*.—Sarcosine *p*-toluidide and methyl *N*-2,4-dichlorobenzoyldithiocarbamate in ether–ethanol (4 : 1) during 5 days at room temperature gave this *compound* (73%), m. p. 172—173°, unchanged by recrystallisation from dioxan (Found: C, 52·9; H, 4·2; N, 10·0. $C_{18}H_{17}O_2N_3SCl_2$ requires C, 52·7; H, 4·2; N, 10·2%).

N-*Benzoylthiocarbamoylhydroxy*-L-*proline Ethyl Ester* (with J. R. Ogle).—A mixture of hydroxy-L-proline ethyl ester hydrochloride (1·22 g.) and triethylamine (0·62 g.) in acetone (10 c.c.) was shaken with benzoyl isothiocyanate (1·10 g.) for 1 hr., then poured into water (80 c.c.). The oily *product* (1·49 g.) solidified and was recrystallised as a monohydrate from ethyl acetate–light petroleum (b. p. 40—60°); it then had m. p. 83—84° (Found: C, 52·6; H, 5·9; N, 8·2; S, 9·4. $C_{15}H_{18}O_4N_2S,H_2O$ requires C, 52·9; H, 5·9; N, 8·2; S, 9·4%). Several attempts to remove the water of crystallisation from this compound gave amorphous materials which did not crystallise. The anhydrous ester was unchanged by treatment with trifluoroacetic acid overnight at room temperature.

2-*Benzoylimino*-3-*methylthiazolid*-5-*one*.—(i) A solution of *N*-benzoylthiocarbamoylsarcosine ethyl ester (3 g.) in trifluoroacetic acid (20 c.c.) was set aside overnight. After evaporation under reduced pressure, followed by addition of ether and evaporation, the *product* (2·24 g.) crystallised and had m. p. 158—159° (decomp.) [unchanged by recrystallisation from benzene–light petroleum (b. p. 90—120°)] and $\lambda_{max.}$ 2810 (ε 18,650), $\lambda_{min.}$ 2680 (ε 14,260), $\lambda_{max.}$ 2570 (ε 21,070) in methylene chloride (Found: C, 56·6; H, 4·6; N, 11·5. $C_{11}H_{10}O_2N_2S$ requires C, 56·4; H, 4·3; N, 12·0%).

(ii) A solution of *N*-benzoylthiocarbamoylsarcosine *p*-toluidide (300 mg.) in trifluoroacetic acid (3 c.c.) was left overnight and then poured into water. The product (190 mg.) was collected, washed, and recrystallised, and then had m. p. 157—158°. The two samples had

identical infrared spectra with bands at 1737, 1621, 1584, 1567, 1436, 1400, 1334, 1312, 1271, 1251, 1165, 1132, 1107, 1069, 1025, 965, 858, and 725 cm.$^{-1}$.

Ring-opening Reactions of 2-Benzoylimino-3-methylthiazolid-5-one.—(i) The thiazolid-5-one (468 mg.) in chloroform (5 c.c.) was allowed to react with cyclohexylamine (198 mg.) in the warm for 5 min. Addition of benzene caused N-*benzoylthiocarbamoylsarcosine cyclohexylamide* (460 mg.) to separate. After two recrystallisations from chloroform–light petroleum (b. p. 40—60°) and one from aqueous ethanol, it had m. p. 166·0—166·5° (Found: C, 61·2; H, 6·5. $C_{17}H_{23}O_2N_3S$ requires C, 61·2; H, 7·0%).

(ii) In a similar manner, reaction of the thiazolid-5-one (468 mg.) with *p*-toluidine (215 mg.) furnished *N*-benzoylthiocarbamoylsarcosine *p*-toluidide (400 mg.). After recrystallisation from ethanol, it had m. p. 155° and the same in admixture with the sample above. The infrared spectra were indistinguishable.

(iii) A solution of the thiazolid-5-one (50 mg.) in ethanol (2 c.c.) and 2N-hydrochloric acid (2 c.c.) was heated under reflux for 2 hr. After evaporation to dryness under reduced pressure, followed by partition between ethyl acetate and saturated sodium hydrogen carbonate, the organic phase was evaporated to give 1-methyl-2-thiohydantoin (12 mg.), m. p. 223·0—224·5° (decomp.), indistinguishable from an authentic sample by paper chromatography in cyclohexane–butan-1-ol–90% acetic acid (3 : 1 : 1).[6]

2-(2,4-*Dichlorobenzoylimino*)-3-*methylthiazolid*-5-*one.*—Degradation of 2,4-dichlorobenzoylthiocarbamoylsarcosine *p*-toluidide in trifluoroacetic acid afforded the *thiazolid-5-one* (92%), m. p. 157—158° [from benzene–light petroleum (b. p. 60—80°)], $\lambda_{max.}$ in CH_2Cl_2, 2840—2880 (ε 18,920), $\nu_{max.}$ 1730, 1634, 1568, 1479, 1435, 1396, 1336, 1289, 1260, 1240, 1151, 1134, 1105, 1046, 961, 883, 865, 848, 789, 779 cm.$^{-1}$ (Found: C, 44·0; H, 3·1; N, 9·4. $C_{11}H_8O_2N_2SCl_2$ requires C, 43·6; H, 2·7; N, 9·2%).

Ring-opening Reactions of 2-(2,4-*Dichlorobenzoylimino*)-3-*methylthiazolid*-5-*one.*—(i) Interaction of the thiazolid-5-one with cyclohexylamine in warm chloroform furnished N-2,4-*dichlorobenzoylthiocarbamoylsarcosine cyclohexylamide* (96%). Recrystallised twice from ethanol–light petroleum (b. p. 40—60°) and once from aqueous ethanol, it had m. p. 174—175° (Found: C, 50·7; H, 5·4; N, 10·3. $C_{17}H_{21}O_2N_3SCl_2$ requires C, 50·8; H, 5·3; N, 10·5%).

(ii) The thiazolid-5-one (100 mg.) was heated in ethanol (2 c.c.) and 2N-hydrochloric acid (2 c.c.) under reflux for 4 hr. Isolation and paper chromatography as described above revealed that a mixture of 1-methyl-2-thiohydantoin and starting material was present.

Attempted Preparations of 2-*Acetylimino*-3-*methylthiazolid*-5-*one.*—(i) *N*-Acetylthiocarbamoylsarcosine *p*-toluidide was recovered from solution in trifluoroacetic acid overnight.

(ii) A solution of *N*-acetylthiocarbamoylsarcosine benzylamide (837 mg.) in trifluoroacetic acid (1 c.c.) was left overnight and then poured into water. An extract thereof by ethyl acetate was washed with water, dried (Na_2SO_4), and evaporated. Addition of light petroleum (b. p. 40—60°) precipitated an oil, which rapidly, but only partly, solidified. The product (335 mg.), recrystallised from ethanol–light petroleum (b. p. 40—60°), had m. p. 149—152° with effervescence. It dissolved in saturated sodium hydrogen carbonate with effervescence and was presumably N-*acetylthiocarbamoylsarcosine* (Found: C, 37·7; H, 5·4; N, 15·0. $C_6H_{10}O_3N_2S$ requires C, 37·9; H, 5·3; N, 14·7%).

(iii) *N*-Acetylthiocarbamoylsarcosine benzylamide (560 mg.) in dry nitromethane (8 c.c.) was treated with dry hydrogen chloride for 10 min. Ether (8 c.c.) was added, and benzylamine hydrochloride (260 mg.), m. p. 244° (decomp.), was removed. The filtrate was evaporated under reduced pressure, and as much hydrogen chloride as possible was removed. *p*-Toluidine (430 mg.) in chloroform (5 c.c.) was added, and the solution was warmed and shaken for 5 min., washed successively with dilute hydrochloric acid, water, saturated sodium hydrogen carbonate, and water, and was then dried. Filtration and evaporation afforded *N*-acetylthiocarbamoyl *p*-toluidide (180 mg.), m. p. and mixed m. p. 169—170°, having the authentic infrared spectrum.

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