N-*Thiocarbamoylglycine and its Ethyl Ester.*

By D. T. Elmore, P. A. Toseland, and H. J. V. Tyrrell.

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The compound described in the literature as ethyl thiohydantoate (*N*-thiocarbamoylglycine ethyl ester) (I; R = OEt) is glycine ethyl ester thiocyanate (II; R = OEt). The authentic ester (I) has been prepared by two methods and its structure has been proved. Acid catalysis induced cyclisation of *N*-thiocarbamoylglycine (I; R = OH) and its ethyl ester (I; R = OEt) to 2-thiohydantoin. The latter also resulted from the action of ammonia on the ester (I; R = OEt); the mechanism of the reaction between ethyl *iso*thiocyanatoacetate and ammonia is discussed briefly in relation to this observation.

Harries and Weiss (*Ber.*, 1900, **33**, 3418; *Annalen*, 1903, **327**, 355) and Johnson (*J. Amer. Chem. Soc.*, 1913, **35**, 780) found that interaction of glycine ethyl ester hydrochloride and potassium thiocyanate in boiling ethanol afforded a compound, m. p. 65°, which they concluded was *N*-thiocarbamoylglycine ethyl ester (I; R = OEt). The surprising fact that the latter did not produce 2-thiohydantoin with hydrochloric acid was reported by both groups of workers, although Klason (*Ofv. Kongl. Vet. Akad.*, 1890, 87) and Johnson (*loc. cit.*) agreed that a mixture of potassium thiocyanate and glycine ethyl ester hydrochloride at 140—150° without solvent formed 2-thiohydantoin directly.

(I) $NH_2 \cdot CS \cdot NH \cdot CH_2 \cdot CO \cdot R$ $\qquad$ $R \cdot CO \cdot CH_2 \cdot NH_3^+ \ SCN^-$ (II)

We obtained the compound, m. p. 65°, as described by Harries and Weiss and by Johnson (*locc. cit.*) and attempted to promote cyclisation in nitromethane saturated with dry hydrogen chloride (Edman, *Acta Chem. Scand.*, 1950, **4**, 283). Glycine ethyl ester hydrochloride was the sole product. At first, we suspected that the difference in behaviour towards acids between the compound, m. p. 65°, and other *N*-thiocarbamoyl derivatives of animo-acids and peptides (Ware, *Chem. Rev.*, 1950, **46**, 403; Edman, *loc. cit.*; Elmore and Toseland, *J.*, 1954, 4533) might be due to a difference in fine structure between *N*-monosubstituted and *NN'*- disubstituted thioureas. Thus, Clow (*Trans. Faraday Soc.*, 1938, **34**, 457) has adduced evidence that the former have a greater tendency than the latter to exist predominantly in the *iso*thiourea form. Other experiments which we performed seemed at first consonant with this interpretation. On the one hand, treatment of the compound, m. p. 65°, with alkyl halides and, on the other, the reaction between *N*-cyanoglycylglycine ethyl ester and ethanethiol in presence of dry hydrogen chloride, which might be expected

to produce *iso*thiourea derivatives, afforded only hydrohalides of glycine ethyl ester and glycylglycine ethyl ester respectively. There was no reaction between *N*-cyanoglycylglycine ethyl ester and ethanethiol in absence of acidic catalysts. Glycylglycine ethyl ester hydrochloride and potassium thiocyanate interacted in ethanol to give a compound, m. p. 105°, which behaved like the compound, m. p. 65°; it reverted to a dipeptide ester hydrohalide when treated with either dry hydrogen chloride in nitromethane or alkyl halides in hot ethanol.

Examination of the infrared spectra of the two compounds showed some remarkable features. In particular, the absorption in the 3 μ region was complex, and a strong band at about 2060 cm.$^{-1}$ was present. The latter was incompatible with structure (I; R = OEt), the only reasonable course being to attribute it to a thiocyanate ion (Miller and Wilkins, *Analyt. Chem.*, 1952, **24**, 1253). On this basis, these compounds would be glycine ethyl ester thiocyanate (II; R = OEt) and glycylglycine ethyl ester thiocyanate (II; R = NH·CH_2·CO_2Et) respectively. This interpretation is confirmed by the complex absorption in the 3 μ region, which is similar to that observed in these laboratories for other, similar amine salts, and by the development of a characteristic thiocyanate colour with ferric chloride solution. The thiocyanate ion was determined quantitatively by titration with silver nitrate. Further, it was found that these compounds were obtained from the reaction of potassium thiocyanate with either glycine ethyl ester hydrochloride or glycylglycine ethyl ester hydrochloride in warm ethanol for a few minutes, conditions which were unlikely to cause isomerisation to *N*-thiocarbamoyl derivatives. The failure of the above compounds to cyclise under acidic conditions and their behaviour towards alkyl halides is now readily explicable. Moreover, the toxicity of compound, m. p. 65°, towards rats and rabbits (Leonard, *Arch. intern. Pharmacodynamie*, 1929, **35**, 314) may possibly be attributed to the thiocyanate ion.

Authentic *N*-thiocarbamoylglycine ethyl ester (I; R = OEt) was obtained by esterification of *N*-thiocarbamoylglycine (I; R = OH) using either the azeotropic-distillation or the Fischer–Speier technique. Both the acid (I; R = OH) and the ester (I; R = OEt) had normal infrared spectra and readily cyclised to 2-thiohydantoin in aqueous hydrochloric acid. In fact, it was found necessary to exercise care during esterification in order to avoid formation of 2-thiohydantoin directly.

Johnson and Hemingway's interpretation (*J. Amer. Chem. Soc.*, 1916, **38**, 1550) of the reaction between ethyl *iso*thiocyanatoacetate (SCN·CH_2·CO_2Et) and ammonia, resulting in the formation of 2-thiohydantoin, is now questionable. It was suggested that intermediate formation of *N*-thiocarbamoylglycine ethyl ester (I; R = OEt) did not occur, but that the first product was *iso*thiocyanatoacetamide which then cyclised. We have found that *N*-thiocarbamoylglycine ethyl ester (I; R = OEt) is rapidly converted into 2-thiohydantoin in ethanolic ammonia so that any of the intermediates (I; R = OEt), (I; R = NH_2) or *iso*thiocyanatoacetamide may be involved in the reaction between ammonia and ethyl *iso*thiocyanatoacetate. It is felt, however, that in view of the reactivity of *iso*thiocyanates, *iso*thiocyanatoacetamide is the least likely of these.

Experimental

Infrared spectra were obtained on a Grubb-Parsons double-beam spectrometer. The solid specimens were prepared in potassium bromide discs.

Glycine Ethyl Ester Thiocyanate.—The reaction between equimolar quantities of potassium thiocyanate and glycine ethyl ester hydrochloride in refluxing ethanol during 4—5 hr. (Harries and Weiss, also Johnson, *locc. cit.*) afforded a product which was coloured and rather difficult to crystallise. When the solution of reactants was warmed for 5—20 minutes and then filtered, and ether was added to the filtrate, the *salt* (52%) crystallised readily; it had m. p. 65° (Found: C, 37·4; H, 6·3; N, 17·3. $C_5H_{10}O_2N_2S$ requires C, 37·0; H, 6·2; N, 17·3%). The infrared bands were at 3346 (s), 2963 (vs), 2608 (m), 2060 (vs), 1752 (vs), 1606 (m), 1505 (s), 1433 (s), 1420 (s), 1395 (m), 1330 (sh), 1306 (sh), 1255 (vs), 1110 (m), 1054 (m), 1013 (m), 907 (m), 859 (m) cm.$^{-1}$. This compound gave glycine ethyl ester hydrochloride, m. p. 142°, when treated with dry hydrogen chloride in nitromethane. Reaction with ethyl iodide, *iso*propyl bromide, or *n*-butyl iodide afforded the corresponding hydrohalide.

Glycylglycine Ethyl Ester Thiocyanate.—This *compound*, obtained from glycylglycine ethyl ester hydrochloride and potassium thiocyanate in a similar manner, had m. p. 105° (Found : C, 38·0; H, 5·9; N, 19·1; SCN^-, 25·7. $C_7H_{13}O_3N_3S$ requires C, 38·3; H, 6·0; N, 19·2; SCN^-, 26·5%) and the following infrared bands : 3380 (vs), 3140—2870 (vs, unresolved), 2720 (s), 2610 (s), 2530 (m), 2400 (w), 2060 (vs), 1740 (vs), 1644 (vs), 1577 (vs), 1493 (vs), 1468 (s), 1456 (s), 1430 (vs), 1413 (vs), 1383 (vs), 1355 (m), 1324 (m), 1304 (m), 1253—1232 (vs, unresolved), 1137 (m), 1114 (s), 1108 (s), 1100 (m), 1048 (m), 1025 (m), 1008 (s), 976 (w), 943 (w), 904 (s), 862 (m) $cm.^{-1}$. Treatment with dry hydrogen chloride in nitromethane or with alkyl halides afforded the corresponding peptide ester hydrohalide.

N-*Cyanoglycylglycine Ethyl Ester.*—A solution of glycylglycine ethyl ester (1·6 g.) in anhydrous dioxan (20 c.c.) was slowly added with stirring to a solution of freshly prepared cyanogen bromide (1·06 g.) in dioxan (20 c.c.) containing suspended sodium hydrogen carbonate (1·5 g.). The mixture was shaken at room temperature for 12 hr. and filtered. Evaporation of the filtrate under reduced pressure left an orange-yellow oil which crystallised under ether at 0°. Recrystallised from ethanol–ether, the *compound* (0·76 g.) had m. p. 82° (Found : C, 45·1; H, 5·8; N, 22·2. $C_7H_{11}O_3N_3$ requires C, 45·4; H, 6·0; N, 22·7%). It polymerised when kept. The residue from the first filtration was extracted with hot ethanol (50 c.c.) and filtered. On cooling, *glycylglycine ethyl ester hydrobromide* (0·74 g.), m. p. 182°, separated (Found : C, 30·2; H, 5·5; N, 11·7. $C_6H_{13}O_3N_2Br$ requires C, 29·9; H, 5·4; N, 11·6%).

Attempted Condensation of N-*Cyanoglycylglycine Ethyl Ester with Ethanethiol.*—*N*-Cyanoglycylglycine ethyl ester (200 mg.) was added to a solution of ethanethiol (70 mg.) in dry dioxan (10 c.c.), and the solution was saturated with dry hydrogen chloride at 0°. The solution was kept at room temperature for 12 hr. and evaporated. The residue (120 mg.), crystallised from ethanol, had m. p. 181°, undepressed on admixture with glycylglycine ethyl ester hydrochloride. *N*-Cyanoglycylglycine ethyl ester did not react with ethanethiol at temperatures up to 40° in up to 10 days in absence of acid catalyst.

N-*Thiocarbamoylglycine.*—2-Thiohydantoin (5·8 g.) was heated in a refluxing solution (200 c.c.) of hydrated barium hydroxide (15 g.) for 1 hr. (cf. Klason, *loc. cit.*). The solution was cooled and adjusted to pH 2 with dilute sulphuric acid, and barium sulphate was filtered off. The filtrate was continuously extracted with ether for 2 days and the *N*-thiocarbamoylglycine (2·7 g.), which separated from the ethereal solution, was twice recrystallised from ethanol, then having m. p. 179° (Found : C, 27·0; H, 4·4; N, 21·3; S, 23·7. Calc. for $C_3H_6O_2N_2S$: C, 26·9; H, 4·5; N, 20·9; S, 23·9%). Light absorption in 95% EtOH : $\lambda_{max.}$ 241 mμ. The infrared spectrum had the following bands : 3340 (vs), 3350 (vs), 3120 (vs), 2920 (s), 2780 (s), 2655 (s), 2555 (m), 1700 (vs), 1685 (sh), 1621 (vs), 1560 (vs), 1543 (vs), 1446 (s), 1416 (s), 1381 (s), 1361 (s), 1347 (s), 1290 (m), 1255 (vs), 1235 (sh), 1182 (s), 1043 (m), 962 (vs), 896 (m), 747 (m), 737 (m), 685 (m) $cm.^{-1}$. *N*-Thiocarbamoylglycine was warmed in 2N-hydrochloric acid and then kept overnight at room temperature; a quantitative yield of 2-thiohydantoin, m. p. 227—229° (decomp.), was obtained.

N-*Thiocarbamoylglycine Ethyl Ester.*—(i) *N*-Thiocarbamoylglycine (1 g.) was suspended in ethanol–benzene (50 c.c.; 1 : 1) containing toluene-*p*-sulphonic acid (0·1 g.). The solution was slowly distilled during 2 hr., more ethanol–benzene being added as required. Most of the solvent was removed under reduced pressure and the product was extracted into benzene (25 c.c.) and washed with 5% sodium hydrogen carbonate solution. The benzene was evaporated off and the *ester* (0·84 g.) crystallised from a little water, m. p. 78°.

(ii) A solution of *N*-thiocarbamoylglycine (1 g.) in ethanol (75 c.c.) at 0° was saturated with dry hydrogen chloride for 1 hr. The solution was shaken at room temperature for 12 hr., by which time the solution was homogeneous. Ethanol was removed under reduced pressure and the residual oil was dissolved in ethyl acetate (30 c.c.). After being washed with 5% sodium hydrogen carbonate, followed by water, the solution was evaporated under reduced pressure. The residue (0·97 g.), crystallised from water, had m. p. 79° (Found : C, 37·0; H, 6·1; N, 16·7; S, 19·5. $C_5H_{10}O_2N_2S$ requires C, 37·0; H, 6·2; N, 17·3; S, 19·8%). Light absorption in 95% EtOH : $\lambda_{max.}$ 241 mμ. The infrared spectrum had the following bands : 3414 (s), 3314 (s), 3200 (s), 3105 (w), 2984 (w), 2677 (vw), 1732 (vs), 1634 (vs), 1573 (s), 1446 (s), 1378 (s), 1356 (s), 1304 (m), 1256 (m), 1232—1217 (vs, unresolved), 1191 (sh), 1174 (sh), 1095 (w), 1045 (m), 1019 (m), 959 (s), 918 (vw), 863 (w), 758 (w), 739 (m), 712 (m) $cm.^{-1}$. The foregoing ester (0·8 g.) was dissolved in warm 2N-hydrochloric acid and kept at room temperature overnight. White prisms, m. p. and mixed m. p. 228—229° (decomp.), of 2-thiohydantoin (0·5 g.) were deposited. *N*-Thiocarbamoylglycine ethyl ester (50 mg.) was dissolved in saturated ethanolic ammonia (2 c.c.); after 3 hr. at room temperature, paper chromatography in butan-1-ol–water revealed

that all the ester had disappeared and that 2-thiohydantoin (R_F 0·54) and a trace of ultraviolet-absorbing material (R_F 0·28) were present. The solution was evaporated and the residue was crystallised from water (charcoal), affording 2-thiohydantoin (13 mg.), m. p. 222—224° (decomp.).

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STAVELEY RESEARCH LABORATORIES,
THE UNIVERSITY, SHEFFIELD, 10.

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