phorus pentachloride (2.9 g.) in small portions with shaking. The mixture was shaken until a clear solution resulted and the ether evaporated *in vacuo* at a bath temperature of 10–15°. The resulting crystalline acid chloride was washed with petroleum ether and dried *in vacuo;* yield 2.2 g. (81%).

A solution of the above acid chloride in ether (40 ml.) was added to an ice-cold ethyl acetate solution (210 ml.) of L-tyrosine methyl ester (prepared from 5 g. of the hydrochloride) and the mixture kept at room temperature for 24 hours. The suspension was filtered, the filtrate evaporated to dryness *in vacuo* and the resulting oily compound dissolved in methanol (30 ml.). Hydrazine hydrate (800 mg.) was added, the mixture refluxed for five minutes and then kept at room temperature for 24 hours. The hydrazide which had crystallized was collected and recrystallized from aqueous methanol; yield 3.5 g. (97%); m.p. 204–207° with decomposition.

Anal. Calcd. for $C_{18}H_{22}O_5N_4S$: C, 53.2; H, 5.5; N, 13.8; S, 7.9. Found: C, 53.4; H, 5.4; N, 13.8; S, 7.6.

Tosylglycyl-L-tyrosylglycine Carbobenzoxyhydrazide.—Tosylglycyl-L-tyrosine hydrazide (2.5 g.) was dissolved in 2 *N* hydrochloric acid (13.4 ml.) and the solution cooled at 0°. A solution of sodium nitrite (460 mg.) in water (2 ml.) was added, and the azide which precipitated was extracted with three 100-ml. portions of ice-cold ethyl acetate. The ethyl acetate extracts were combined, washed with water and dried over sodium sulfate. The solution was then added to a methanol solution (40 ml.) of glycine carbobenzoxyhydrazide (prepared from 1.73 g. of the hydrochloride) and the mixture kept at room temperature for 20 hours. The suspension was filtered, the solvents removed *in vacuo* and the residue triturated with 1 *N* hydrochloric acid (20 ml.) when crystallization occurred. The compound was collected, washed with water, dried and recrystallized from aqueous methanol; yield 2.35 g. (64%); m.p. 185–187°; $\alpha^{26}D$ −3 ± 2°(*c* 1, in methanol).

Anal. Calcd. for $C_{28}H_{31}O_8N_5S$: C, 56.3; H, 5.2; N, 11.7; S, 5.4. Found: C, 56.3; H, 5.2; N, 12.0; S, 5.5.

Carbobenzoxyglycylglycyl-DL-leucine Carbobenzoxyhydrazide.[17]—An ice-cold ethyl acetate solution (120 ml.) of carbobenzoxydiglycine azide (prepared from 1.57 g. of the hydrazide) was added to an ethanol solution (10 ml.) of DL-leucine carbobenzoxyhydrazide (prepared from 1.39 g. of the hydrochloride) and the mixture kept at room temperature for 19 hours. The suspension was filtered, and the filtrate evaporated to dryness *in vacuo* and the residue dissolved in ethyl acetate (25 ml.). The solution was washed with 1 *N* hydrochloric acid (30 ml.), 10% sodium bicarbonate (30 ml.) and water (30 ml.), dried over sodium sulfate, and evaporated to dryness *in vacuo*. The residue was recrystallized from ethanol; yield 1.4 g. (47%); m.p. 196–199°; $\alpha^{26}D$ 0 ± 1° (*c* 10, in glacial acetic acid).

Anal. Calcd. for $C_{26}H_{33}O_7N_5$: C, 59.2; H, 6.3; N, 13.3. Found: C, 59.1; H, 6.2; N, 13.4.

Triglycine Hydrazide Dihydrochloride. a. From Carbobenzoxytriglycine Hydrazide.—A suspension of carbobenzoxytriglycine hydrazide (1.0 g.) in water (5 ml.) and 1 *N* hydrochloric acid (6.3 ml.) was hydrogenated over spongy palladium until the evolution of carbon dioxide ceased. The catalyst was removed by filtration, the filtrate concentrated to a small volume and placed in a refrigerator. The resulting crystals were collected and dried; yield 0.7 g. (84%); m.p. 195–205° with decomposition.

Anal. Calcd. for $C_6H_{15}O_3N_5Cl_2$: N, 25.4; Cl, 25.7. Found: N, 25.7; Cl, 25.4.

b. **From Carbobenzoxytriglycine Carbobenzoxyhydrazide.**—A suspension of carbobenzoxytriglycine carbobenzoxyhydrazide (0.3 g.) in 50% aqueous methanol (14 ml.) and 3 *N* hydrochloric acid (4.4 ml.) was hydrogenated over spongy palladium until the evolution of carbon dioxide ceased. The catalyst was removed by filtration, the filtrate evaporated to dryness *in vacuo* and the resulting oil placed in a refrigerator where crystallization occurred. The crystals were washed with absolute ethanol and dried; yield 0.16 g. (91%); m.p. 195–205° with decomposition. A sample for analysis was recrystallized from water.

Anal. Calcd. for $C_6H_{15}O_3N_5Cl_2$: N, 25.4. Found: N, 25.8.

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Studies on Polypeptides. IV.[1] Remarks Regarding the Use of the Phenylthiocarbonyl Protecting-group in Peptide Synthesis

BY ADOLF LINDENMANN,[2] NOORUL HAQ KHAN AND KLAUS HOFMANN[3]

A number of phenylthiocarbonyl dipeptide esters were prepared and their reaction with lead acetate in 70% ethanol was investigated. Contrary to previous claims this reaction led to the formation of hydantoin derivatives and not to dipeptide esters. This observation limits the practical applicability of the phenylthiocarbonyl group as a tool in peptide synthesis. The method may provide the basis for a generally applicable procedure for the preparation of hydantoins. The reaction of phenylthiocarbonylglycine carbobenzoxyhydrazide with lead acetate in ethanol afforded 2-carbobenzoxy-3,6-dioxohexahydro-1,2,4-triazine and not glycine carbobenzoxyhydrazide.

Phenylthiocarbonyl chloride (I) has been suggested as a group-protecting reagent in peptide synthesis.[4] This acid chloride reacts with α-amino acid esters (II) to form phenylthiocarbonyl-α-amino acid esters (III) which are readily converted into phenylthiocarbonyl-α-amino acids (IV) by acid hydrolysis. These latter compounds in the form of their chlorides (V) may be coupled with other α-amino acid esters (VI) to form phenylthiocarbonyldipeptide esters (VII). Short heating with lead acetate in 70% ethanol, it is claimed, removes the phenylthiocarbonyl blocking-group from such dipeptide derivatives with the formation of lead phenylmercaptide and dipeptide esters. Since this method seemed to offer considerable promise as a new, generally applicable procedure in peptide chemistry, we have undertaken the present investigation.

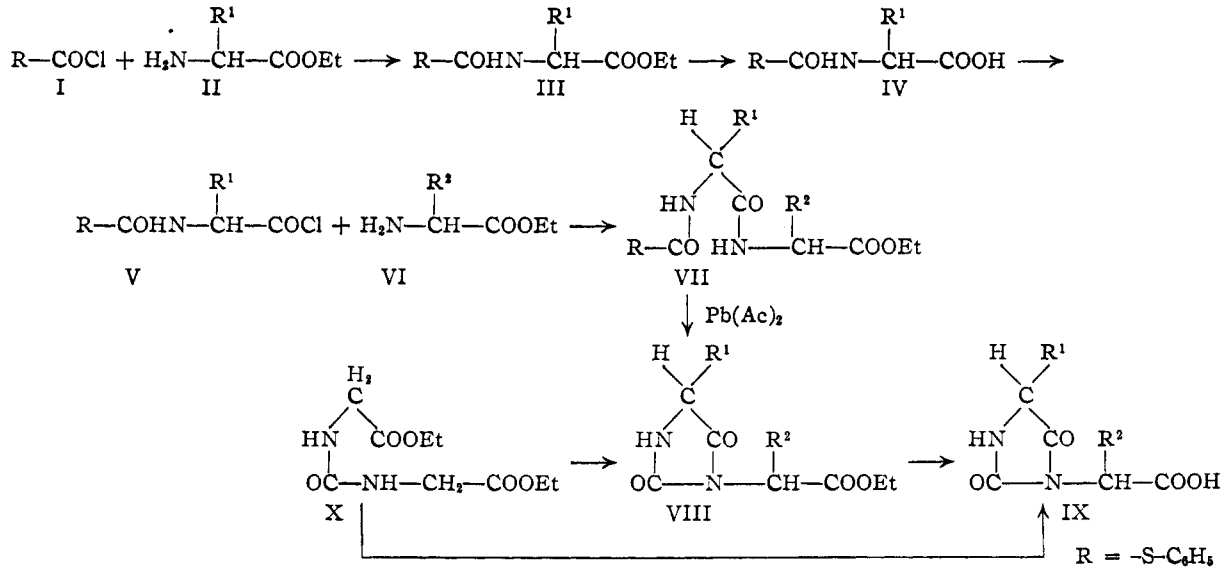
Phenylthiocarbonylglycine ethyl ester (III, R^1 = H) and phenylthiocarbonyl-DL-alanine methyl ester were prepared and were converted into the respective phenylthiocarbonyl-α-amino acids by acid hydrolysis. By coupling of the appropriate phenylthiocarbonyl-α-amino acid chloride with the desired α-amino acid ester, phenylthiocarbonyldiglycine ethyl ester (VII, R^1 and R^2 = H), phenylthiocarbonylglycyl-DL-alanine methyl ester and phenylthiocarbonyl-DL-alanyl-DL-alanine ethyl ester (VII)

(1) For paper III see K. Hofmann, A. Lindenmann, M. Z. Magee and N. H. Khan, THIS JOURNAL, **74**, 470 (1952).

(2) Postdoctorate Research Fellow from the University of Basel, Switzerland. The participation of Dr. Lindenmann in this study was made possible by a fellowship from the American-Swiss Foundation for Scientific Exchange, Inc.

(3) This investigation was supported by grants from the U. S. Public Health Service, The Rockefeller Foundation in New York, and Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

(4) G. C. H. Ehrensvärd, *Nature*, **159**, 500 (1947).



R¹ and R² = Me) were prepared. The latter dipeptide ester was separated into a diastereomer A of melting point 124–126° and a diastereomer B melting at 130–132° by fractional crystallization. In addition to its preparation from phenylthiocarbonylglycyl chloride and glycine ethyl ester, phenylthiocarbonyldiglycine ethyl ester also was obtained in high yield from phenylthiocarbonyl chloride and diglycine ethyl ester.

The reaction of diethyl L-glutamate with phenylthiocarbonyl chloride afforded diethyl phenylthiocarbonyl-L-glutamate in the form of an oil which was readily converted into phenylthiocarbonyl-L-glutamic acid by acid hydrolysis. Heating with acetic anhydride converted this compound into its anhydride from which phenylthiocarbonyl-L-glutamic acid was regenerated upon exposure to dilute hydrochloric acid. The optical rotation of the regenerated acid was identical with that of the starting material, demonstrating that anhydride formation was not accompanied by racemization. Phenylthiocarbonyl-L-glutamic acid anhydride may thus serve as a starting material for the preparation of phenylthiocarbonyl-L-glutamyl peptides.

Although a number of the above-mentioned compounds have been recorded previously, their synthesis and elementary composition are presented in this study since this information is not available in the original paper.[4] The phenylthiocarbonyl derivatives obtained during this investigation represent well crystallized compounds, exhibiting sharp melting points. They may offer certain advantages for the characterization of peptides.

The removal of the phenylthiocarbonyl group from four phenylthiocarbonyl dipeptide esters by treatment with lead acetate in 70% ethanol was then investigated. Short heating of phenylthiocarbonyldiglycine ethyl ester (VII, R¹ and R² = H) with an equimolar quantity of lead acetate in 70% ethanol resulted in the formation of a copious precipitate of lead phenylmercaptide. The evolution of only traces of carbon dioxide was noticed during the reaction. Evaporation of the colorless filtrate from the lead phenylmercaptide afforded a crystalline ester melting at 118–120°. The substance was soluble in ether, did not form a hydrochloride upon treatment with ethanolic hydrogen chloride and failed to liberate nitrogen under the conditions of the Van Slyke amino-nitrogen determination. This behavior of the compound excluded the presence of the expected diglycine ethyl ester acetate. Acid hydrolysis converted the ester into an acid melting at 196–198°. The ester and the acid were identified as ethyl hydantoin-3-acetate (VIII, R¹ and R² = H) and hydantoin-3-acetic acid (IX, R¹ and R² = H), respectively, by comparison with authentic samples prepared from N,N′-bis-(carbethoxymethyl)-urea (X).[5]

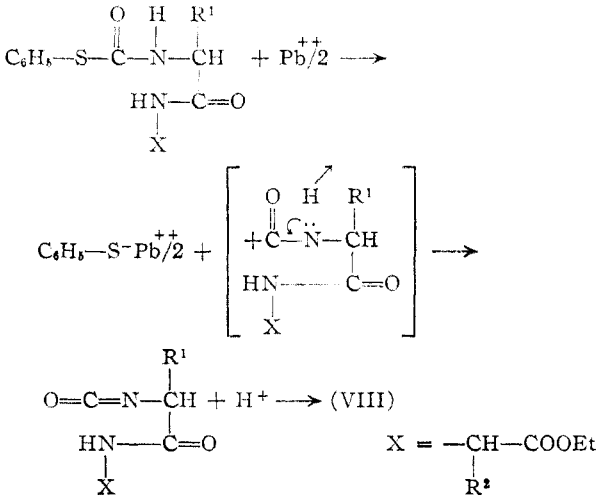
The treatment of phenylthiocarbonylglycyl-DL-alanine methyl ester with lead acetate afforded an oily ester not containing a free amino group. Hydrolysis converted this substance into a crystalline acid $C_6H_8O_4N_2$ which must be regarded as *dl*-α-methylhydantoin-3-acetic acid (IX, R¹ = H; R² = Me). Similar results were obtained when the two diasteromeric phenylthiocarbonyl-DL-alanyl-DL-alanines were treated with lead acetate. Both compounds afforded oily esters which again failed to liberate nitrogen under the conditions of the Van Slyke amino-nitrogen determination. Acid hydrolysis converted these esters into two diastereomeric acids which were identified by mixed melting point determinations with authentic samples as the two racemic α,5-dimethylhydantoin-3-acetic acids (IX, R¹ and R² = Me). The necessary reference samples for this comparison were prepared according to the procedures of Gränacher and collaborators.[6,7]

The finding that short exposure to lead acetate in ethanol converted four phenylthiocarbonyldipeptide esters into derivatives of hydantoin-3-acetic acid and not into dipeptide esters suggests that other phenylthiocarbonyl peptide derivatives will most likely exhibit a similar behavior. Therefore, this method has little practical significance as a tool in peptide chemistry but may provide the

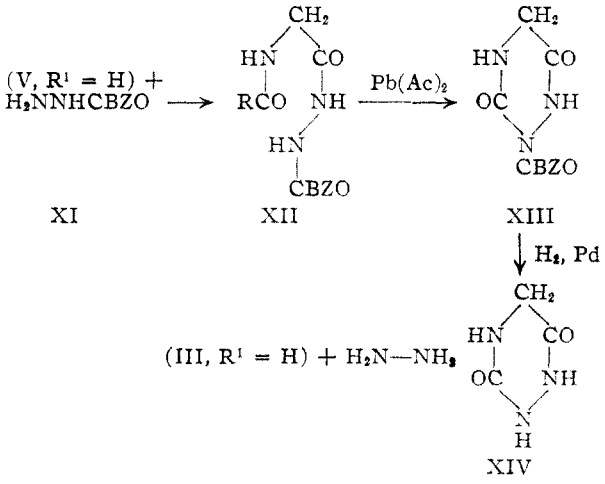
(5) R. Locquin and V. Cerchez, *Bull. soc. chim.*, **49**, 309 (1939).
(6) Ch. Gränacher and H. Landolt, *Helv. Chim. Acta*, **10**, 799 (1927).
(7) Ch. Gränacher and C. Wolf, *ibid.*, **11**, 172 (1928).

basis for a generally applicable procedure for the preparation of hydantoin derivatives.

The reaction of phenylthiocarbonyldipeptide esters with plumbous ion may involve the initial formation of an isocyanate through elimination of a phenylmercaptide ion and expulsion of a proton. This isocyanate in turn may react with the neighboring peptide nitrogen to form the final product.



In a recent communication[1] we have presented a number of procedures for the preparation of α-amino acid carbobenzoxyhydrazides. In an attempt to find additional routes to these interesting compounds, we have prepared phenylthiocarbonylglycine carbobenzoxyhydrazide (XII) by the interaction of phenylthiocarbonylglycyl chloride (V, R^1 = H) and carbobenzoxyhydrazine (XI) and have investigated its behavior toward lead acetate.



Short refluxing of (XII) in the presence of an equimolar quantity of lead acetate in ethanol afforded, in addition to lead phenylmercaptide, a substance of the composition $C_{11}H_{11}O_4N_3$. This compound contained no amino-nitrogen and failed to give the known hydrochloride of glycine carbobenzoxyhydrazide when it was subjected to ethanolic hydrogen chloride. Hydrogenation removed the carbobenzoxy group with the formation of a new material of the composition $C_3H_5O_2N_3$. This compound is formulated as 3,6-dioxohexahydro-1,2,4-triazine (XIV) since it also resulted from the interaction of phenylthiocarbonylglycine ethyl ester (III, R^1 = H) with hydrazine hydrate. The reaction of phenylthiocarbonylglycine carbobenzoxyhydrazide with lead acetate therefore led to the formation of 2-carbobenzoxy-3,6-dioxohexahydro-1,2,4-triazine (XIII) and did not afford glycine carbobenzoxyhydrazide.

Experimental[8]

Phenylthiocarbonylglycine Ethyl Ester.—To a cooled suspension of glycine ethyl ester hydrochloride (15 g.) in chloroform (50 ml.) was added diethylamine (11 ml.) and the mixture shaken until a clear solution had resulted. Ether (150 ml.) was added and the precipitate of diethylamine hydrochloride removed by filtration. The filtrate was evaporated to dryness *in vacuo* and the oily residue dissolved in chloroform (30 ml.). The solution was cooled in an ice-bath and a solution of phenylthiocarbonyl chloride[9] (9 g.) in chloroform (15 ml.) added. The mixture was kept at room temperature for four hours and was then washed with three 40-ml. portions of 2 *N* hydrochloric acid, 40 ml. of water, and dried over sodium sulfate. The chloroform was removed and the residue recrystallized from ethanol; yield 11.2 g. (89%); m.p. 104–106°; literature m.p. 104°.[4]

Anal. Calcd. for $C_{11}H_{13}O_3NS$: N, 5.9. Found: N, 5.7.

Phenylthiocarbonyl-DL-alanine Methyl Ester.—To a chloroform solution (70 ml.) of DL-alanine methyl ester, prepared from DL-alanine methyl ester hydrochloride (11.0 g.) and diethylamine as described above, was added a solution of phenylthiocarbonyl chloride (7.1 g.) in chloroform (20 ml.) and the mixture kept at room temperature for 14 hours. The solution was washed with three 40-ml. portions of water, dried over sodium sulfate and evaporated to give an oil which soon solidified. The material was recrystallized from a mixture of methanol and petroleum ether; yield 7.3 g. (74%); m.p. 55–56°.

Anal. Calcd. for $C_{11}H_{13}O_3NS$: C, 55.2; H, 5.5; N, 5.9; S, 13.4. Found: C, 55.0; H, 5.5; N, 5.6; S, 13.4.

Phenylthiocarbonylglycine.—A suspension of phenylthiocarbonylglycine ethyl ester (10 g.), in a 1:1 mixture of glacial acetic and concentrated hydrochloric acids (35 ml.), was refluxed for ten minutes. The resulting clear solution was cooled in an ice-bath and water (100 ml.) was added slowly. The resulting crystals were collected, washed with water and dried *in vacuo* at room temperature; yield 7.9 g. (89%); m.p. 153–154°; literature m.p. 155°.[4] A sample for analysis was recrystallized from a mixture of ethyl acetate and petroleum ether.

Anal. Calcd. for $C_9H_9O_3NS$: N, 6.6. Found: N, 6.5.

Phenylthiocarbonyl-DL-alanine.—A suspension of phenylthiocarbonyl-DL-alanine methyl ester (7.3 g.), in a 1:1 mixture of glacial acetic and concentrated hydrochloric acids (20 ml.), was refluxed for 15 minutes, when a clear solution resulted. The mixture was cooled in an ice-bath and water (100 ml.) was added. The resulting crystals were recrystallized from a mixture of methanol and water; yield 5.5 g. (80%); m.p. 136–138°.

Anal. Calcd. for $C_{10}H_{11}O_3NS$: C, 53.3; H, 5.0; N, 6.2; S, 14.2. Found: C, 53.1; H, 5.1; N, 6.0; S, 14.2.

Phenylthiocarbonyl-L-glutamic Acid.—To an ice-cold chloroform solution (140 ml.) of diethyl L-glutamate, prepared from 20 g. of the hydrochloride,[10] was added a solution of phenylthiocarbonyl chloride (9.3 g.) in chloroform (20 ml.) and the mixture kept at room temperature for three hours. The solution was then extracted with three 50-ml. portions of 1 *N* hydrochloric acid and 50 ml. of water, dried over sodium sulfate and the chloroform removed *in vacuo*. The oily residue was dissolved in a mixture of glacial acetic and hydrochloric acids 4:1 (50 ml.) and the solution refluxed for 30 minutes. The solvents were removed *in vacuo* at 50°

(8) The melting points were determined with short-stem Anschütz thermometers and are uncorrected. The microanalyses were performed in our microanalytical laboratory by Mr. George L. Stragand. The Van Slyke determinations were performed by Miss Anna Bridgwater. Petroleum ether (b.p. 30–60°) was employed.

(9) M. Rivier, *Bull. soc. chim.*, [4] **1**, 733 (1907).

(10) F. A. King and D. A. A. Kidd, *J. Chem. Soc.*, 3315 (1949).

bath temperature and the residue washed by decantation with petroleum ether. Trituration of the residue with water resulted in crystallization. The crystals were washed with water, dried at room temperature and recrystallized from a mixture of ethyl acetate and petroleum ether; yield 7.2 g. (47%); m.p. 111–113°; $\alpha^{26}D$ −22 ± 2° (*c* 1, in methanol).

Anal. Calcd. for $C_{12}H_{13}O_5NS$: C, 50.9; H, 4.6; N, 4.9; S, 11.3. Found: C, 50.7; H, 4.6; N, 4.9; S, 11.6.

Phenylthiocarbonyl-L-glutamic Acid Anhydride.—Phenylthiocarbonyl-L-glutamic acid (1 g.) was heated in acetic anhydride (10 ml.) at 100–110° for five minutes and the solution evaporated to dryness *in vacuo* at 50° bath temperature. Chloroform (5 ml.) was added to the residue and the solution again evaporated to dryness. The resulting crystalline mass was recrystallized from chloroform; yield 0.79 g. (84%); m.p. 166–168°.

Anal. Calcd. for $C_{12}H_{11}O_4NS$: C, 54.3; H, 4.2; N, 5.3; S, 12.1. Found: C, 54.1; H, 4.0; N, 5.2; S, 11.9.

Treatment of a sample of the anhydride (0.15 g.) with 1 *N* hydrochloric acid (5 ml.) for 30 minutes at 80° regenerated phenylthiocarbonyl-L-glutamic acid: $\alpha^{27}D$ −22 ± 2° (*c* 1, in methanol).

Phenylthiocarbonylglycyl Chloride.—To an ice-cold suspension of phenylthiocarbonylglycine (2 g.) in dry ether (40 ml.) was added, in small portions, powdered phosphorus pentachloride (2 g.) and the mixture shaken in an ice-bath until a clear solution resulted (20–30 minutes). The solution was filtered, the clear filtrate evaporated to dryness *in vacuo*, and the crystalline residue washed with petroleum ether and recrystallized from ether; yield 2.0 g. (92%); m.p. 83–85°; literature m.p. 87°.[4]

Phenylthiocarbonyl-DL-alanyl Chloride.—To an ice-cold suspension of phenylthiocarbonyl-DL-alanine (5.5 g.) in ether (100 ml.) was added, with cooling and shaking, powdered phosphorus pentachloride (5.2 g.) and the mixture shaken until a clear solution was obtained (25 minutes). The solution was filtered, the ether evaporated *in vacuo*, and the resulting crystals washed with petroleum ether and dried *in vacuo;* yield 5.3 g. (88%). A sample for analysis was recrystallized from a mixture of ether and petroleum ether; m.p. 81–85°.

Anal. Calcd. for $C_{10}H_{10}O_2NSCl$: C, 49.3; H, 4.1; N, 5.7; S, 13.2; Cl, 14.5. Found: C, 49.5; H, 4.0; N, 5.6; S, 13.6; Cl, 14.6.

Phenylthiocarbonyldiglycine Ethyl Ester. Method A.—To a chloroform solution (130 ml.) of diglycine ethyl ester (12.5 g.) was added a solution of phenylthiocarbonyl chloride (6.7 g.) in chloroform (20 ml.) and the mixture kept at room temperature for 15 hours. The resulting precipitate, containing a mixture of the desired ester and diglycine ethyl ester hydrochloride, was collected, the filtrate evaporated to dryness *in vacuo* and the crystalline residue washed with water and dried. The precipitate mentioned above was ground with water in a mortar, filtered, washed with water and dried. This material was combined with the material from the filtrate and recrystallized from ethanol; yield 10.8 g. (94%); m.p. 131–133°; literature m.p. 127°.[4]

Anal. Calcd. for $C_{13}H_{16}O_4N_2S$: C, 52.7; H, 5.4; N, 9.5; S, 10.8. Found: C, 52.7; H, 5.2; N, 9.6; S, 11.1.

Method B.—To a cooled solution of glycine ethyl ester (prepared from 1.8 g. of the hydrochloride) in chloroform (20 ml.) was added a solution of phenylthiocarbonylglycyl chloride (1.5 g.) in chloroform (10 ml.) and the mixture kept at room temperature for seven hours. The mixture was washed with 1 *N* hydrochloric acid (40 ml.) and water (20 ml.), dried over sodium sulfate and the chloroform removed. The residue was recrystallized from ethanol; yield 1.5 g. (77%); m.p. 128–129°. A mixed melting point determination with the material prepared according to method A gave no depression.

Phenylthiocarbonylglycyl-DL-alanine Methyl Ester.—To a chloroform solution (100 ml.) of DL-alanine methyl ester (prepared from 6.5 g. of hydrochloride) was added a solution of phenylthiocarbonylglycyl chloride (5.0 g.) in chloroform (100 ml.) and the mixture kept at room temperature for 20 hours. The solvent was removed *in vacuo* and the residue twice washed by decantation with petroleum ether. The resulting crystalline mass was triturated with water, filtered, dried *in vacuo* at room temperature and recrystallized from a mixture of benzene and petroleum ether; yield 4.7 g. (72%); m.p. 121–123°; literature m.p. 116°.[4]

Anal. Calcd. for $C_{15}H_{16}O_4N_2S$: N, 9.5. Found: N, 9.3.

Phenylthiocarbonyl-DL-alanyl-DL-alanine Ethyl Ester.—To an ice-cold chloroform solution (50 ml.) of DL-alanine ethyl ester (prepared from 6.6 g. of the hydrochloride) was added a solution of phenylthiocarbonyl-DL-alanyl chloride (5.2 g.) in chloroform (40 ml.) and the mixture kept at room temperature for 20 hours. The solution was washed with 1 *N* hydrochloric acid (50 ml.) and water (80 ml.), dried over sodium sulfate and evaporated to dryness *in vacuo*. The oily residue was washed by decantation with petroleum ether and triturated with ethyl acetate when crystallization occurred. The crystals were collected, washed with a 1:1 mixture of ethyl acetate and ether and dried *in vacuo*. Two recrystallizations from a mixture of ethyl acetate and petroleum ether gave the diastereomer B; yield 2 g. (29%); m.p. 130–132°.

Anal. Calcd. for $C_{15}H_{20}O_4N_2S$: C, 55.6; H, 6.2; N, 8.6; S, 9.9. Found: C, 55.7; H, 6.0; N, 8.7; S, 9.9.

The combined mother liquors and washings from the preparation of the diastereomer B were triturated with ether and the resulting crystals collected and washed with ether. Recrystallization from a mixture of ethyl acetate and petroleum ether gave the diastereomer A; yield 0.77 g. (11%); m.p. 124–126°.

Anal. Calcd. for $C_{15}H_{20}O_4N_2S$: C, 55.6; H, 6.2; N, 8.6; S, 9.9. Found: C, 55.6; H, 6.0; N, 8.5; S, 10.0.

Ethyl Hydantoin-3-acetate.—To a warm solution of lead acetate (384 mg.) in 70% ethanol (35 ml.) was added phenylthiocarbonyldiglycine ethyl ester (600 mg.), and the mixture heated for five minutes at 80–85° and cooled to room temperature. The copious precipitate of lead phenylmercaptide was removed by filtration and the filtrate evaporated to dryness *in vacuo*. The residue was dried and recrystallized from ether; yield 310 mg. (82%); m.p. 118–120°. The compound failed to liberate nitrogen in the Van Slyke amino-nitrogen determination. A mixed melting point determination with an authentic sample of ethyl hydantoin-3-acetate[5] gave no depression.

Anal. Calcd. for $C_7H_{10}O_4N_2$: C, 45.2; H, 5.4; N, 15.0. Found: C, 45.1; H, 5.3; N, 15.1.

Hydantoin-3-acetic Acid.—A sample of the above ester (650 mg.) was saponified with concentrated hydrochloric acid (4 ml.) and ether (10 ml.),[6] and the acid recrystallized from water; yield 415 mg. (75%); m.p. 196–198°. Admixture of this acid with an authentic sample of hydantoin-3-acetic acid[5,6] gave no depression of the melting point.

Anal. Calcd. for $C_5H_6O_4N_2$: N, 17.7. Found: N, 17.9.

***dl*-α-Methylhydantoin-3-acetic Acid.**—To a warm solution of lead acetate (2.2 g.) in 70% ethanol (30 ml.) was added phenylthiocarbonylglycyl-DL-alanine methyl ester (3.3 g.) and the mixture refluxed for 10 minutes. The ensuing copious precipitate of lead phenylmercaptide was removed by filtration and the filtrate concentrated to dryness *in vacuo* to give an oil which failed to crystallize; yield 1.8 g. (91%). The compound failed to liberate nitrogen in the Van Slyke amino-nitrogen determination. The above ester (1.6 g.) was dissolved in a mixture of concentrated hydrochloric acid (10 ml.) and ether (20 ml.) and the solution kept at room temperature for 24 hours. The insoluble portions were removed by filtration and the clear filtrate was evaporated to dryness *in vacuo*. The residue was crystallized from a small quantity of methanol; yield 0.73 g. (40%); m.p. 175–179°.

Anal. Calcd. for $C_6H_8O_4N_2$: C, 41.9; H, 4.7; N, 16.3. Found: C, 41.9; H, 4.4; N, 16.1.

DL-α,5-Dimethylhydantoin-3-acetic Acid. Diastereomer A.—Treatment of phenylthiocarbonyl-DL-alanyl-DL-alanine ethyl ester (diastereomer A) (0.73 g.) with lead acetate (0.43 g.) in 70% ethanol (40 ml.) in the manner described above gave ethyl DL-α,5-dimethylhydantoin-3-acetate as an oil; yield 0.47 g. (98%). Only traces of carbon dioxide were liberated during the lead acetate treatment, and the ester failed to liberate nitrogen under the conditions of the Van Slyke amino-nitrogen determination. A sample (0.40 g.) was hydrolyzed with concentrated hydrochloric acid (4 ml.) and ether (10 ml.) in the usual manner, and the resulting acid recrystallized from a mixture of ethyl acetate and petroleum ether; yield 0.29 g. (83%); m.p. 182–185°. This material gave no melting point depression when mixed with an authentic sample of the high melting diastereomer

of *dl*-α,5-dimethylhydantoin-3-acetic acid. The reference compound which was prepared according to Gränacher and Landolt[6] melted at 181–184°; literature m.p. 187–189°.

Anal. Calcd. for $C_7H_{10}O_4N_2$: N, 15.0. Found: N, 14.8.

Diastereomer B.—Treatment of phenylthiocarbonyl-DL-alanyl-DL-alanine ethyl ester (diastereomer B) (1.2 g.) with lead acetate (0.7 g.) in 70% ethanol (40 ml.) afforded an oily ester; yield 0.74 g. (93%). The compound failed to liberate nitrogen in the Van Slyke determination. Hydrolysis of this ester (0.57 g.)[6] gave a crystalline acid; yield 0.26 g. (52%); m.p. 152–155°. This substance gave no depression when admixed with an authentic sample of the low melting diastereomer of *dl*-α,5-dimethylhydantoin-3-acetic acid. The reference compound which was prepared according to Gränacher and Wolf[7] melted at 153–156°; literature m.p. 158–160°.

Anal. Calcd. for $C_7H_{10}O_4N_2$: N, 15.0. Found: N, 15.1.

Phenylthiocarbonylglycine Carbobenzoxyhydrazide.—To a solution of phenylthiocarbonylglycine chloride (2.2 g.) in chloroform (50 ml.) was added with cooling a solution of carbobenzoxyhydrazine (3.2 g.) in chloroform (60 ml.) and the mixture kept at room temperature for 16 hours. The compound was isolated in the usual manner and recrystallized from a mixture of ethyl acetate and petroleum ether; yield 2.9 g. (84%); m.p. 132–134°.

Anal. Calcd. for $C_{17}H_{17}O_4N_3S$: C, 56.8; H, 4.8; N, 11.7; S, 8.9. Found: C, 56.6; H, 4.6; N, 11.4; S, 8.6.

2-Carbobenzoxy-3,6-dioxohexahydro-1,2,4-triazine.—To a warm solution of lead acetate (790 mg.) in 70 per cent. ethanol (60 ml.) was added phenylthiocarbonylglycine carbobenzoxyhydrazide (1.5 g.) and the mixture kept at 80–85° for 6 minutes, when it was cooled to room temperature. The lead phenylmercaptide was removed by filtration, the filtrate evaporated to dryness *in vacuo* and the residue recrystallized from ethanol; yield 820 mg. (78%); m.p. 168–169°.

Anal. Calcd. for $C_{11}H_{11}O_4N_3$: C, 53.0; H, 4.4; N, 16.9; Found: C, 53.5; H, 4.4; N, 16.7.

3,6-Dioxohexahydro-1,2,4-triazine. A. By Hydrogenation of 2-Carbobenzoxy-3,6-dioxohexahydro-1,2,4-triazine.—A sample of the triazine (500 mg.) was hydrogenated in the usual manner over spongy palladium in methanol (20 ml.) containing three drops of ethanolic hydrogen chloride. The reaction product was recrystallized from dioxane; yield 210 mg. (91%); m.p. 194–195°. The compound failed to liberate nitrogen in the Van Slyke amino-nitrogen determination.

Anal. Calcd. for $C_3H_5O_2N_3$: C, 31.3; H, 4.4; N, 36.5. Found: C, 31.2; H, 4.2; N, 36.7.

B. By Treatment of Phenylthiocarbonylglycine Ethyl Ester with Hydrazine.—A solution of phenylthiocarbonylglycine ethyl ester (1 g.) and hydrazine hydrate (0.2 ml.) in 70% ethanol (10 ml.) was refluxed for three hours. The mixture was cooled when the reaction product began to crystallize. Crystallization was completed by keeping the mixture at room temperature for 20 hours. The crystals were collected and recrystallized from dioxane; yield 0.2 g. (41%); m.p. 195–196°. No depression of the melting point was observed when this material was admixed with a sample of the same compound prepared according to method A above.

Anal. Calcd. for $C_3H_5O_2N_3$: N, 36.5. Found: N, 36.4.

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The Preparation of Active, Non-antidiuretic Hydrolyzates of ACTH

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Acid hydrolysis of concentrates of ACTH has given products which are active in patients with rheumatoid arthritis. Selected conditions for the hydrolysis retain the hydrolyzate's full adrenocorticotropic activity, but destroy the substance responsible for excessive sodium and water retention in the patient. Such acid hydrolyzates may be purified to yield fractions which are still free of the side effects mentioned above, but which are active clinically at lower dosage levels. The clinical results have been in agreement with the animal assays for both adrenocorticotropic and antidiuretic activity. Pepsin digestion does not destroy the antidiuretic activity of the ACTH preparations studied, but this effect is lost upon subsequent acid treatment.

Acid hydrolyzates of concentrates of the adrenocorticotropic hormone (ACTH) have been found active in rheumatoid arthritis, and appear to possess some therapeutic advantages over unhydrolyzed ACTH, or pepsin digests of ACTH.

ACTH has been described as a protein of molecular weight about 20,000.[1,2] The ability possessed by the hormone to stimulate the adrenal cortex in animals was not destroyed, according to Li,[3] by pepsin digestion or acid hydrolysis under relatively mild conditions. The announcement[4] that ACTH was active in rheumatoid arthritis was followed shortly by reports that fractions of pepsin digests of ACTH freed of materials of high molecular weight by trichloroacetic acid treatment[5] or by dialysis[6] were also active in treating this disease. Other workers have confirmed the activity of pepsin digests of ACTH in rheumatoid arthritis, and have shown that the digests can be fractionated to yield products with many times the activity of the original hormone.[7]

The administration of ACTH preparations to human subjects results in a number of metabolic changes, which include the retention by the subject of sodium and of water.[8] The protein-free peptide mixtures of Li produced metabolic changes including sodium and water retention indistinguishable from those effected by administration of unhydrolyzed ACTH.[5,9] Most recently it has been re-

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