512. *Amination and Acylation Reactions by Homocysteine Thiolactone and N-Benzyloxycarbonylhomocysteine Thiolactone.*

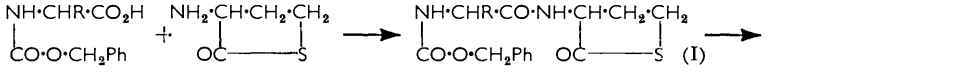
By R. Laliberté, Y. Knobler, and M. Frankel.

The α-amino-group of homocysteine thiolactone shows a reactivity analogue to that of homoserine lactone; in α-benzyloxycarbonylamino-γ-thiobutyrolactone the reactivity of the carbonyl group to nucleophilic attacks is much less than that of the analogous *O*-lactone.

By using homocysteine thiolactone as aminating agent, peptide γ-thiolactones have been synthesised from *N*-protected amino-acids. By using α-benzyloxycarbonylamino-γ-thiobutyrolactone, amides and mixed dioxopiperazines of homocystine have been obtained.

Homoserine lactone (α-amino-γ-butyrolactone) can be coupled with *N*-protected amino-acids to give homoserine-peptidolactones, and α-benzyloxycarbonylamino-γ-butyrolactone is an effective acylating agent for sodium salts of amino-acid and for amines.[1] The present paper concerns reactions of the analogous homocysteine thiolactone (α-amino-γ-thiobutyrolactone), studied in respect of peptide coupling between protected amino-acids and homocysteine thiolactone as well as the use of α-benzyloxycarbonylamino-γ-thiobutyrolactone as acylating agent. It was of interest to compare these thiolactones in relation to the corresponding *O*-lactones and to synthesise of peptides and aminolytic products of homocysteine and of homocystine.

Coupling of homocysteine thiolactone with *N*-benzyloxycarbonylamino-acids by the mixed anhydride method proceeded smoothly without affecting the thiolactone structure. *N*-(α-*N*-Benzyloxycarbonylaminoacyl)homocysteine thiolactones (I) were thus prepared with glycine (a), alanine (b), valine (c), methionine (d), phenylalanine (e), and glycylphenylalanine. Removal of the benzyloxycarbonyl group with hydrogen bromide in acetic acid then led to the peptide thiolactone hydrobromides (II) of homocysteine.

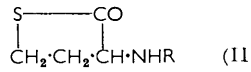


(I) a, R = H; b, R = Me; c, R = Pr^{i}; d, R = $CH_2 \cdot CH_2 \cdot SMe$; e, R = CH_2Ph. (II) a, R = H; b, R = $Ph \cdot CH_2$

[1] Sheradsky, Knobler, and Frankel, *J. Org. Chem.*, 1961, **26**, 2710.

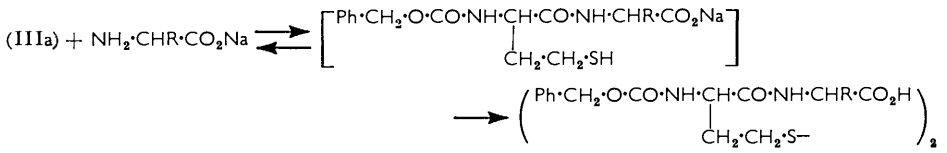
The α-amino-group of homocysteine thiolactone also reacted with phenyl isocyanate without effecting the thiolactone structure, giving *N*-phenylcarbamoylhomocysteine thiolactone.

N-Benzyloxycarbonylhomocysteine lactone (IIIa) was synthesised in good yield by treating homocysteine thiolactone hydrochloride with benzyl chloroformate in the presence of a weak base; the known *N*-benzoyl (IIIb) and *N*-acetyl (IIIc) derivatives were prepared by a simplified technique.

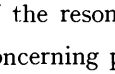


(III) a, R = CO·O·CH_2Ph; b, R = Bz; c, R = Ac.

For acylation, amino-acids were heated in ethanolic sodium ethoxide for several hours with an equivalent of *N*-benzyloxycarbonylhomocysteine thiolactone (IIIa), and treated as in the case of the *O*-lactone.[1] Contrary to the reaction of *N*-benzyloxycarbonylhomoserine lactone, which gave good yields of homoserylpeptides, the thiolactone (IIIa) gave only low yields of impure *N*-protected homocystine peptides (IV) when oxygen was passed through the solution. In general, the disulphide side of the equilibrium is favoured, as seen also in other aminolytic reactions of the thiolactone.



(IV) a, R = CH_2Ph; b, R = Me; c, R = Bu^i.

The weaker reactivity of the thiolactone (IIIa) than of its *O*-analogue is considered to be due to its sulphur atom. The thiolactone (IIIa) shows the carbonyl stretching absorption in the infrared spectrum at 5·9 μ (1680 ± 10 cm.$^{-1}$). The low frequency, compared with that for the normal lactone, probably indicates an appreciable contribution of the resonance form (),[2a] and thus deactivation. The available information concerning parallel aminolytic experiments on esters and on thiol-esters is insufficient to permit an estimation of their reactivity orders (S > O or S < O).[2b]

Studies of the effect of structure on the rate of aminolysis of linear thiol esters [2c] showed the reactivity orders expected on the basis of electronic effects only. Nucleophilic substitution of the thiol-ester carbonyl group is favoured by such SR groups which increase the inductive effect of the C–S bond (). Were the $-I_s$ effect dominant, lower reactivity is to be expected of the thiolactones (III) than of the corresponding *O*-lactones ($-I_s$ of SR < $-I_s$ of OR).[3] However, the polarisability $-I_d$, an important factor of this S_N2 mechanism,[2c] is relatively less effective in the corresponding OR groups.[4a] Further, kinetic data for the hydrolysis of ethyl acetate and thiolacetate [4b] suggest greater reactivity of the *O*-lactones, in accordance with the solvation of the more negative and smaller *O*-alkyl oxygen atom.

The red colour in the sensitive nitroprusside test in sodium hydroxide solution appears immediately with the three α-acylamino-γ-thiobutyrolactones (IIIa, b, c), indicating rapid opening to the γ-mercapto-acids. On using the more weakly basic aqueous sodium hydrogen carbonate, this colour appears after a short time with α-acetamido- (IIIc), and

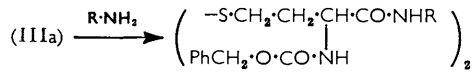
[2] Kharasch, "Organic Sulfur Compounds," Pergamon Press, Oxford, 1961, pp. (*a*) 54, (*b*) 431—432; (*c*) Schwyzer, *Helv. Chim. Acta*, 1953, **36**, 414.

[3] Gould, "Mechanism and Structure in Organic Chemistry," Holt, Rinehart, and Winston, New York, 1959, p. 207.

[4] (*a*) Gould, ref. 3, pp. 259—261; (*b*) Schaefgen, *J. Amer. Chem. Soc.*, 1948, **70**, 1308.

later or not at all, with α-benzamido- (IIIb) and with α-benzyloxycarbonylamino-γ-thiobutyrolactone (IIIa). It is thus possible to distinguish between the α-acetamido-γ-thiolactone (IIIc) and the other two, more hindered thiolactones.

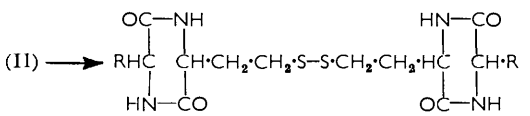
Aminolysis of the *N*-benzyloxycarbonyl derivative (IIIa) could be carried out with nucleophilic agents such as ammonia, benzylamine, or hydrazine. Oxidation in the presence of an excess of the amine accelerated the reaction and gave better yields and purer products. In this way, the diamide (Va), the dibenzylamide (Vb), and the dihydrazide (Vc) of *NN'*-dibenzyloxycarbonylhomocystine were prepared. The dihydrazide (Vc) might be useful for further peptide syntheses of homocystine by Curtius's method.



(V) a, R = H; b, R = CH_2Ph; c, R = NH_2.

Aminolysis of *N*-benzyloxycarbonylhomoserine *O*-lactone and thiolactone (IIIa) was slower with hydrazine than with the more basic benzylamine or ammonia and here again the greater reactivity of the *O*-lactone becomes apparent.* With the weakly basic hydroxylamine, reaction failed for thiolactone (IIIa), but occurred with the *O*-lactone, affording the hydroxamic acid.[7]

The acylating reactivity of the homocysteine thiolactonic system is increased by intramolecular aminolysis. Peptide thiolactone hydrobromides (II) were cyclised in slightly alkaline solution in the presence of oxygen, giving good yields of the mixed dioxopiperazines (VI) of homocystine and other amino-acids. For the *N*-protected peptide



(VI) a, R = Me; b, R = Pr^i; c, R = CH_2Ph.

thiolactone (Ib), lower activity of the α-acylamino-thiolactonic system than of the *O*-analogue can again be seen: the peptide thiolactone (Ib) did not react with salts of amino-acids or with α-amino-γ-butyrolactone to achieve elongation of a peptide chain.

Experimental

Infrared absorption spectra were determined on a Perkin-Elmer model 137 spectrophotometer.

Preparation of N-*Benzyloxycarbonylpeptide γ-Thiobutyrolactones* (I).—*N-Benzyloxycarbonylglycylhomocysteine thiolactone* (Ia). *N*-Benzyloxycarbonylglycine (10·5 g., 0·051 mole) and triethylamine (5·1 g., 0·051 mole) in toluene (100 ml.) and chloroform (50 ml.) were cooled to 0°, ethyl chloroformate (5·4 g., 0·050 mole) was added, and the mixture was stirred in the cold for 20 min. A cooled solution of homocysteine thiolactone hydrochloride (7·4 g., 0·048 mole) and triethylamine (10·2 g., 0·10 mole) in chloroform (130 ml.) was added. Stirring in the cold was continued for 2 hr. and the mixture left overnight at room temperature. Solvents were evaporated under a vacuum, the product was dissolved in ethyl acetate (200 ml.), and triethylamine hydrochloride was filtered off. Ethyl acetate was removed under a vacuum and the residue was dissolved in an excess of toluene and crystallised very slowly on storage at −10°. N-(N-*Benzyloxycarbonylglycyl*)*homocysteine thiolactone* (Ia) (7·2 g., 50%) melted at 87° (Found: C, 54·6; H, 5·3; N, 9·1; Equiv., 308·0. $C_{14}H_{16}N_2O_4S$ requires C, 54·5; H, 5·2; N, 9·1%; Equiv., 308·3).

* A similar difference has been observed between α-benzamido-γ-butyrolactone and α-benzamido-γ-thiobutyrolactone (IIIb).[5] With the latter, which is the less hindered, experiments on peptide-bond formation (in aqueous alkali) have been reported,[6] and some *S*-phenylmercuri-*N*-benzoyl-peptides have been obtained.

[5] Unpublished observation with Miss E. Boni.
[6] Benesh and Benesh, *J. Amer. Chem. Soc.*, 1956, **78**, 1597.
[7] Unpublished observation with S. Bittner.

Equivalent weights of *N*-benzyloxycarbonylpeptides (I) and other *N*-acyl derivatives of homocysteine thiolactone were determined by alkali-hydrolysis to the γ-mercapto-acids and iodometric titration of the thiol group (see below).

Materials.—*N*-Benzyloxycarbonylamino-acids were prepared by the simplified version of the Schotten–Baumann procedure suggested by Greenstein and Winitz.[8] Homocysteine thiolactone hydrochloride was from Aldrich Chemical Co., U.S.A.

N-(N-*Benzyloxycarbonylalanyl*)*homocysteine thiolactone* (Ib). This was prepared as above from *N*-benzyloxycarbonylalanine (11·5 g., 0·051 mole). After being kept overnight the reaction mixture was cooled and the *compound* was filtered off and crystallised from methanol. Recrystallised from ethanol or chloroform, it (12 g., 77%) melted at 170° (Found: C, 55·5; H, 5·8; N, 8·8%; Equiv., 322·0. $C_{15}H_{18}N_2O_4S$ requires C, 55·7; H, 5·6; N, 8·7%; Equiv., 322·4).

N-(N-*Benzoyloxycarbonylvalyl*)*homocysteine thiolactone* (Ic). Preparation, as above, was from *N*-benzyloxycarbonylvaline (2·5 g., 0·010 mole). After being kept overnight, the mixture was cooled and the product was filtered off and crystallised from methanol. After evaporation, the residue was dissolved in methanol and another portion was obtained by adding water. The *thiolactone* (Ic) (2·9 g., 83%), crystallised from propan-2-ol, melted at 220° (Found: C, 58·1; H, 6·8; N, 8·0%; Equiv., 349·0. $C_{17}H_{22}N_2O_4S$ requires C, 58·3; H, 6·3; N, 8·0%; Equiv., 350·5).

N-(N-*Benzyloxycarbonylmethionyl*)*homocysteine thiolactone* (Id). This preparation was from *N*-benzyloxycarbonylmethionine (2·8 g., 0·010 mole). The solvent was evaporated and the residue crystallised from methanol by addition of water. Recrystallised from toluene the *thiolactone* (3·2 g., 84%) melted at 140° (Found: C, 54·3; H, 5·8; N, 7·2%; Equiv., 382·0. $C_{17}H_{22}N_2O_4S_2$ requires C, 54·3; H, 5·8; N, 7·3%; Equiv., 382·5).

N-(N-*Benzyloxycarbonylphenylalanyl*)*homocysteine thiolactone* (Ie). A similar reaction mixture, including *N*-benzyloxycarbonylphenylalanine (15 g., 0·050 mole), was kept overnight at 10°. A product (A) was obtained which, crystallised from methanol, melted at 179° (7·5 g., 39%) (Found: C, 63·2; H, 5·8; N, 6·9%). Evaporation of the mother-liquor gave a solid that was dissolved in the methanolic solution used for the former crystallisation, and on addition of water a second product (B), melting at 149°, was obtained (7·5 g., 39%) (Found: C, 63·1; H, 5·8; N, 6·9%). This m. p. remained unchanged on repeated crystallisation of this material from propan-2-ol or ethyl acetate.

Equivalent weights of 395·0 (required, 398·5) for both products were obtained by thiol titration with iodine after opening of the thiolactone: 350 mg. of the product were dissolved in methanol (50 ml.) and aliquot parts (10 ml.) were used. 1·75N-Sodium hydroxide (5 ml.) was added. After 5 min. the solution was acidified with acetic acid (3 ml.), and a known excess of iodine in methanol was added (0·1N; 10 ml.). The excess of iodine was titrated back with 0·1N-sodium thiosulphate (8·26 ml.).

The products were optically inactive. Infrared spectra were almost identical. Debenzyloxycarbonylation of both products (A) and (B) gave the same peptide thiolactone hydrobromide (IIb), identified by mixed m. p. and infrared spectra. These two products may be racemic pairs. In some cases only one *thiolactone* (13·2 g., 68%) was obtained, melting at 178° after crystallisation from methanol (Found: N, 7·0. $C_{21}H_{22}N_2O_4S$ requires C, 63·3; H, 5·6; N, 7·0%).

N-[N-(N-*Benzyloxycarbonylglycyl*)*phenylalanyl*]*homocysteine thiolactone.* In this preparation *N*-(*N*-benzyloxycarbonylglycyl)phenylalanine [9] (3·56 g., 0·010 mole) was used. After addition of homocysteine thiolactone, the mixture was stirred for 30 min. at 0° and for 2 hr. at room temperature. The solvent was evaporated under a vacuum and the residue was dissolved in methanol. Water was added, and the *thiolactone* (2·3 g., 53%) filtered off; when recrystallised from ethanol and then from butan-1-ol, it melted at 163° (Found: C, 60·4; H, 5·8; N, 9·1%; Equiv., 454·5. $C_{23}H_{25}N_3O_5S$ requires C, 60·6; H, 5·5; N, 9·2%; Equiv., 455·5). The biuret reaction was positive.

Glycylhomocysteine Thiolactone Hydrobromide (IIa).—The thiolactone (Ia) (500 mg., 0·0016 mole) was dissolved at 30° in acetic acid containing 25% of hydrogen bromide (10 ml.). The *hydrobromide* began to crystallise within 10 min. After 30 min., dry ether (100 ml.) was added,

[8] Greenstein and Winitz, " Chemistry of the Amino Acids," John Wiley & Sons, New York, 1961, Vol. II, p. 891.

[9] Vaughan and Osato, *J. Amer. Chem. Soc.*, 1952, **74**, 676.

the mixture was chilled, and the solvent was decanted. The solid was dissolved in propan-2-ol (20 ml.), and ether (50 ml.) was added again. The *hydrobromide* (IIa) (410 mg., 98%), when collected and then washed with ethyl acetate and crystallised from ethanol, melted at 240° (decomp.) [Found: N(Van Slyke), 5·9, (Kjeldahl), 10·9; Br, 31·9. $C_6H_{11}BrN_2O_2S$ requires N, 5·5, 11·0; Br, 31·3%].

Phenylalanylhomocysteine Thiolactone Hydrobromide (IIb).—The Product A (Ie) (1 g., 0·0025 mole) was dissolved at 30° in a 25% solution (10 ml.) of hydrogen bromide in acetic acid. After 30 min. dry ether (150 ml.) was added and the mixture was left overnight in the cold. The solvent was decanted, the solid was dissolved in dry ethanol, and ether was added. The *hydrobromide* (IIb) (680 mg., 80%) melted at 173° (from nitromethane) [Found: N(Van Slyke), 4·0, (Kjeldahl), 7·9; Br, 23·0. $C_{13}H_{17}BrN_2O_2S$ requires N, 4·0, 8·1; Br, 23·2%].

N-*Phenylcarbamoylhomocysteine Thiolactone*.—To homocysteine thiolactone hydrochloride (1·54 g., 0·01 mole) in cooled aqueous sodium hydrogen carbonate (1 g. in 20 ml.), phenyl isocyanate (1·08 g., 0·009 mole) in ether (20 ml.) was added. The mixture was stirred for 1 hr., and the *derivative* (1·65 g., 70%) was filtered off and crystallised from ethanol; it melted at 192° (Found: N, 11·9%; Equiv., 235·0. $C_{11}H_{12}N_2O_2S$ requires N, 11·9%; Equiv., 236·3). The infrared spectrum showed the typical shift of the thiolactone carbonyl band from 5·65 to 5·95 μ, when compared with that of *N*-phenylcarbamoylhomoserine lactone,[10] other bands resembling those of the latter.

N-*Benzyloxycarbonylhomocysteine Thiolactone* (IIIa).—To homocysteine thiolactone hydrochloride (10 g., 0·065 mole) in aqueous sodium hydrogen carbonate (11 g., 0·13 mole, in 200 ml.), benzyl chloroformate (12 g., 0·07 mole) in ether (100 ml.) was added at room temperature with stirring. After 1 hr. the product was filtered off and crystallised from 90% propan-2-ol. The *benzyloxycarbonyl derivative* (IIIa) (16 g., 98%) melted at 106° (Found: C, 57·6; H, 5·4; N, 5·6%; Equiv., 250·0. $C_{12}H_{13}NO_3S$ requires C, 57·4; H, 5·2; N, 5·6%; Equiv., 251·3); it had a thiolactone carbonyl band at 5·9 μ, as a shoulder on the first amide band at 6·0 μ. There is another sharp band at 11·8 μ (Nujol).

N-*Benzoylhomocysteine Thiolactone* (IIIb).—To homocysteine thiolactone hydrochloride (5 g., 0·0325 mole) in aqueous sodium hydrogen carbonate (6 g., 0·07 mole, in 200 ml.), benzoyl chloride (6 g., 0·043 mole) in ether (100 ml.) was added and the mixture was stirred at room temperature for 1 hr. The benzamido-thiolactone (IIIb) (6·9 g., 95%), crystallised from toluene or water, melted at 142—143° (cf. du Vigneaud *et al.*[11]) (Found: N, 6·3%; Equiv., 221·0. Calc. for $C_{11}H_{11}NO_2S$: N, 6·3%; Equiv., 221·3), $\lambda_{max.}$ (in Nujol) 5·95s and 11·85m μ.

N-*Acetylhomocysteine Thiolactone* (IIIc).—Homocysteine thiolactone hydrochloride (5 g., 0·0325 mole), anhydrous sodium acetate (3 g., 0·036 mole), and acetic anhydride (8 ml.) were suspended in dioxan (100 ml.). After 3 hours' refluxing, the solid was filtered off and the solvent was evaporated. Crystallised from toluene, the acetamido-thiolactone (IIIc) (4·2 g., 82%) melted at 110° (cf. Benesh *et al.*[6]) (Found: N, 8·6%; Equiv., 158·0. Calc. for $C_6H_9NO_2S$: N, 8·8%; Equiv., 159·2), $\lambda_{max.}$ (in Nujol) 5·95s and 11·75ms μ.

NN′-*Dibenzyloxycarbonylhomocystinyldiphenylalanine* (IVa).—To sodium (115 mg., 0·005 mole) in ethanol (100 ml.), phenylalanine (825 mg., 0·005 mole) was added. When this had dissolved, the thiolactone (IIIa) (1·25 g., 0·005 mole) was added and the solution was refluxed for 6 hr. with oxygen passing through it. The mixture was evaporated, the residue was dissolved in water, and the solution filtered, acidified with hydrochloric acid, and again evaporated to dryness. Salts were removed by addition of acetone, and the crude NN′-*dibenzyloxycarbonylpeptide* (IVa) (250 mg., 30%) was obtained after evaporation under a vacuum at 40°. It melted at 117—118° (Found: N, 6·4. $C_{42}H_{46}N_4O_{10}S_2$ requires N, 6·8%). The ninhydrin reaction was negative. The infrared spectrum showed characteristic bands for CO_2^- and the first and second amide bands. The same spectrum was observed for compounds (IVb) and (IVc).

NN′-*Dibenzyloxycarbonylhomocystinyldialanine* (IVb), prepared as above (yield 25%), melted at 65° (Found: N, 7·9. $C_{30}H_{38}N_4O_{10}S_2$ requires N, 8·3%).

NN′-*Dibenzyloxycarbonylhomocystinyldi-L-leucine* (IVc) (yield 30%) melted at 74° (Found: N, 7·0. $C_{36}H_{50}N_4O_{10}S_2$ requires N, 7·3%).

Analogous reactions were attempted in the absence of oxygen with amino-acids in acetic

[10] Frankel, Knobler, and Ammar, *Bull. Res. Council Israel*, 1962, **11**, *A*, 6.
[11] Du Vigneaud, Patterson, and Hunt, *J. Biol. Chem.*, 1938, **126**, 217.

acid, and with their sodium salts or their ethyl esters in methanol or dimethylformamide. Under these conditions starting materials were recovered.

NN′-*Dibenzyloxycarbonylhomocystine Diamide* (Va).—The thiolactone (IIIa) (1 g., 0·004 mole) in methanol (10 ml.) and concentrated aqueous ammonia (40 ml.) was left at room temperature for 4 days in a large flask filled with oxygen. The *diamide* (Va) (1·06 g., 99%) was filtered off and crystallised from nitromethane or propan-2-ol; it melted at 196°. The nitroprusside test (NaOH) was negative (Found: N, 10·5. $C_{24}H_{30}N_4O_6S_2$ requires N, 10·5%). It had $\lambda_{max.}$ (in Nujol) 2·95s μ for a primary amide (NH), in addition to a band for a secondary amide at 3·1 μ.

NN′-*Dibenzyloxycarbonylhomocystine Dibenzylamide* (Vb).—The thiolactone (IIIa) (800 mg., 0·0032 mole) and benzylamine (2 ml.) were dissolved in methanol (60 ml.) in a large flask filled with oxygen. After a week at room temperature, the *dibenzylamide* (Vb) (1·05 g., 92%) was filtered off and crystallised from toluene; it melted at 155° (Found: N, 7·9. $C_{38}H_{42}N_4O_6S_2$ requires N, 7·8%).

NN′-*Dibenzyloxycarbonylhomocystine Dihydrazide* (Vc).—The thiolactone (IIIa) (2·5 g., 0·01 mole) and hydrazine (4 ml.) in propan-2-ol (30 ml.) were left for 20 hr. at room temperature in presence of oxygen. After filtration and crystallisation from nitromethane, the *dihydrazide* (Vc) (2·54 g., 90%) melted at 176° (Found: C, 51·2; H, 5·8. $C_{24}H_{32}N_6O_6S_2$ requires C, 51·2; H, 5·7%). The infrared spectrum resembled that of the following homoserine hydrazide.

N-*Benzyloxycarbonylhomoserine Hydrazide*.—α-Benzyloxycarbonylamino-γ-butyrolactone [1] (2·35 g., 0·01 mole) was dissolved in ethanol (50 ml.) with gentle heating, hydrazine hydrate (2 g., 0·04 mole) was added, and the solution was left at room temperature. The *hydrazide* was precipitated within 2 hr. quantitatively. It melted at 155° (Found: C, 54·4; H, 6·3. $C_{12}H_{17}N_3O_4$ requires C, 54·0; H, 6·3%).

Attempted Reaction of N-*Benzyloxycarbonylhomocysteine Thiolactone* (IIIa) *with Hydroxylamine*.—The thiolactone (IIIa) (2·5 g., 0·01 mole) was dissolved in absolute ethanol (50 ml.) and hydroxylamine (0·66 g., 0·02 mole), from hydroxylamine hydrochloride (1·4 g., 0·02 mole) and sodium ethoxide (0·46 g. of Na) in ethanol (35 ml.) was added. The solution was left for 20 hr. at room temperature under nitrogen, acidified with ethanolic hydrogen chloride to stop the reaction, and evaporated under a vacuum at 40°. The residue was washed several times with aqueous sodium hydrogen carbonate, and the undissolved material was filtered off and identified as the starting thiolactone (IIIa) (2·2 g., 88%) by mixed m. p. and infrared spectrum. Only a small quantity of the hydroxamic acid could have been formed but the solution gave a weak ferric chloride test and a slight precipitate was obtained with copper acetate solution from the acidified sodium hydrogen carbonate filtrate.

The equivalent reaction with the analogous *O*-lactone yielded, under the same conditions, 70—75% of α-benzyloxycarbonylamino-γ-hydroxybutyrohydroxamic acid.[7]

Di-[2-(5-*methyl*-3,6-*dioxopiperazin*-2-*yl*)*ethyl*] *Disulphide* (VIa).—*N*-(*N*-Benzyloxycarbonylalanyl)homocysteine thiolactone (Ib) (500 mg., 0·0016 mole) was added to hydrogen bromide (18%) in acetic acid (10 ml.), and after 1 hr. the salt was precipitated with ether (150 ml.). The hygroscopic material was washed with ether, then dissolved in aqueous sodium hydrogen carbonate (50 ml.; pH 8·5), and air was bubbled through the solution for 3 days. After filtration, the *product* (VIa) (200 mg., 72%) crystallised from dimethylformamide, then melting at 303° (Found: C, 44·5; H, 6·2; N, 14·5. $C_{14}H_{22}N_4O_4S_2$ requires C, 44·9; H, 5·9; N, 14·9%).

The infrared spectrum of the three homocystinyldi(dioxopiperazines) (VIa, b, c) showed only one carbonyl amidic band at 6·0 μ characteristic of cyclic amides (there was no carbonyl II band at 6·6 μ).

Di-[2-(5-*isopropyl*-3,6-*dioxopiperazin*-2-*yl*)*ethyl*] *Disulphide* (VIb).—*N*-(*N*-Benzyloxycarbonylvalyl)homocysteine thiolactone (Ic) (500 mg., 0·0014 mole) gave, as above, a hygroscopic solid that was dissolved in water (50 ml.); sodium hydrogen carbonate was added (to give pH 8·5) and air was bubbled through the solution for 3 days. The *disulphide* (VIb) (220 mg., 72%) was filtered off and, crystallised from dimethylformamide, melted at 316° (decomp.). (Found: C, 50·0; H, 7·2; N, 13·2. $C_{18}H_{30}N_4O_4S_2$ requires C, 50·2; H, 7·0; N, 13·0%).

Di-[2-(5-*benzyl*-3,6-*dioxopiperazin*-2-*yl*)*ethyl*] *Disulphide* (VIc).—(*a*) Phenylalanylhomocysteine thiolactone hydrobromide (IIb) (500 mg., 0·0145 mole) in aqueous sodium hydrogen carbonate (30 ml.; pH ~8·5) was left for 1 hr. at room temperature while air was passed through the solution. The *disulphide* (VIc) (350 mg., 92%) was filtered off and, crystallised from

dioxan, melted at 237° (Found: C, 59·1; H, 6·0; N, 10·5. $C_{26}H_{30}N_4O_4S_2$ requires C, 59·3; H, 5·8; N, 10·7%).

The product was precipitated very rapidly from water; it was also obtained from an aqueous solution of phenylalanylhomocysteine thiolactone hydrobromide (IIb) (pH ~5). The product (VIc) crystallised slowly even if the acidity was increased (until pH ~1). When the thiolactone hydrobromide (IIb) was dissolved in an excess (5 equivalents) of hydrobromic acid (pH < 1), no precipitation of the disulphide (VIc) occurred. Precipitation was not accelerated by passing oxygen through the solution or by cooling.

(*b*) Thiolactone hydrobromide (IIb) (500 mg., 0·0145 mole) was dissolved in water and left for three weeks at room temperature. The product (VIc) crystallised slowly (460 mg., 60%); when washed with water until free from bromide and dried (P_2O_5), it melted at 238° (Found: C, 59·2; H, 6·2; N, 10·5%). By a mixed m. p. determination and infrared spectrum it was shown to be identical with that prepared as in (*a*).

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Department of Organic Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel. [*Received, October 29th,* 1962.]