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Synthetic Studies of Bacitracin. V.[1] Synthesis of Thiazoline Peptides from Cysteine Peptides by Dehydration Procedure

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Synthesis of thiazoline peptide from cysteine peptide by dehydration with hydrochloric acid in non-aqueous solvent was investigated. By this method, ethyl 2-benzyloxycarbonylaminomethyl-R-Δ^2-thiazoline-4-carboxylate and 2-benzyloxycarbonylaminomethyl-R-Δ^2-thiazoline-4-carbonyl-L-leucine ethyl ester were prepared and isolated in pure states. This synthetic method could give a promising route to a total synthesis of bacitracin A. However, the cysteine residue in the latter thiazoline peptide ester was found to be easily racemized under the basic condition.

In the previous paper,[1] the synthesis of thiazoline peptide by the coupling reaction of benzyloxycarbonylaminoalkylimino ethyl ether with ethyl L-cysteinate hydrochloride and the chemical behavior of the thiazoline ring in the peptide were investigated for the purpose of a total synthesis of an antibiotic bacitracin A. In the present investigation, an alternative method of the synthesis of the thiazoline peptide from cysteine peptide by dehydration with hydrogen chloride in non-aqueous solvent was studied. A formation of the thiazoline compound from glutathione or synthetic cysteine derivative in strong acid solution has been reported.[2-5] However, although the ultraviolet spectra characteristic for the thiazoline ring were observed in the solutions of the products of those experiments, any thiazoline derivative has not been isolated in a pure state so far.

A thiazoline derivative of a dipeptide containing cysteine residue was synthesized by the dehydration method according to the scheme as outlined in Fig. 1. Coupling of benzyloxycarbonylglycine with diethyl L-cystinate dihydrochloride[6] using *N*, *N'*-dicyclohexylcarbodiimide gave *N*, *N'*-bis-benzyloxycarbonylglycyl-L-cystine diethyl ester (I), which was then converted to benzyloxycarbonylglycyl-L-cysteine ethyl ester (II) by reduction with zinc and hydrochloric acid. A ring closure of the dipeptide (II) through dehydration by treatment with hydrogen chloride afforded ethyl 2-benzyloxycarbonylaminomethyl-R-Δ^2-thiazoline-4-carboxylate hydrochloride, from which the free thiazoline derivative (III) was obtained in crystalline state by removal of hydrogen chloride with an aqueous concentrated potassium carbonate solution. The thiazoline peptide derivative thus obtained was identical with the product of the coupling reaction of the iminoether with ethyl cysteinate hydrochloride.[1]

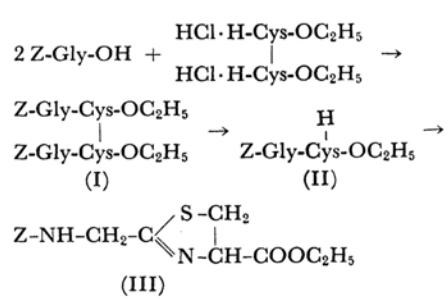

Fig. 1. Z=$C_6H_5CH_2OCO$-
Cysteine residue is of L-configuration.

A thiazoline derivative of a tripeptide was prepared as shown in Fig. 2. Condensation of *N*, *N'*-bisbenzyloxycarbonyl-L-cystine[7] with ethyl L-leucinate hydrochloride[8] by carbodiimide method gave *N*,*N'*-bisbenzyloxycarbonyl-L-cystinyl-di-L-leucine diethyl ester (IV), which was then treated with 30% hydrogen bromide in acetic acid to afford L-cystinyl-di-L-leucine diethyl ester dihydrobromide (V). Coupling of V with benzyloxycarbonylglycine by carbodiimide method gave *N*, *N'*-bisbenzyloxycarbonylglycyl-L-cystinyl-di-L-leucine diethyl ester (VI), which was then reduced

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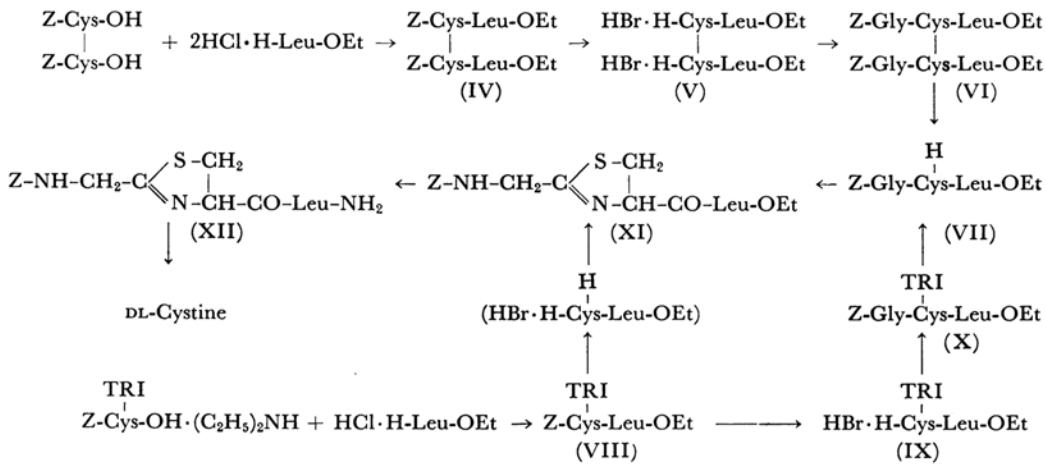

Fig. 2. $Z = C_6H_5CH_2OCO-$, $TRI = (C_6H_5)_3C-$
Cystine and leucine used are of L-configurations.

with zinc and hydrochloric acid to give benzyloxycarbonylglycyl-L-cysteinyl-L-leucine ethyl ester (VII).

The tripeptide (VII) was also prepared alternatively as follows. *N*-Benzyloxycarbonyl-*S*-trityl-L-cysteinyl-L-leucine ethyl ester (VIII) prepared by coupling of *N*-benzyloxycarbonyl-*S*-trityl-L-cysteine diethylamine salt[9] with ethyl L-leucinate hydrochloride[8] by carbodiimide method was treated with hydrogen bromide in acetic acid to give *S*-trityl-L-cysteinyl-L-leucine ethyl ester hydrobromide (IX). Although the removal of benzyloxycarbonyl group and trityl group could take place simultaneously in this reaction, both benzyl bromide and trityl bromide formed possibly attack the free thiol group in cysteine residue during concentration of the reaction mixture resulting in a formation of a mixture of *S*-benzyl and *S*-trityl derivative, from which *S*-trityl derivative (IX) could be isolated in crystalline state. Benzyloxycarbonylglycine was then coupled with IX by carbodiimide method to give benzyloxycarbonylglycyl-*S*-trityl-L-cysteinyl-L-leucine ethyl ester (X). According to the method of Zervas and Photaki,[10] X was converted to the silver mercaptide by the treatment with silver nitrate and pyridine. Removal of silver from the mercaptide by hydrochloric acid gave the tripeptide derivative (VII). The cysteine residue in VII was cyclized to the thiazoline ring by the action of hydrogen chloride in chloroform. A treatment of the product with potassium carbonate gave 2-benzyloxycarbonylaminomethyl-R-Δ^2-thiazoline-4-carbonyl-L-leucine ethyl ester (XI). This compound (XI) was found to be quite identical with the product of the coupling reaction of benzyloxycarbonylaminomethyl imino-ether[1] with L-cysteinyl-L-leucine ethyl ester hydrobromide which was prepared from *N*-benzyloxycarbonyl-*S*-trityl-L-cysteinyl-L-leucine ethyl ester by treatment with hydrogen bromide in acetic acid.

The thiazoline ester (XI) was treated with ammonia in ethanol to yield an acid amide (XII) as shown in Fig. 2. Acid hydrolysis followed by oxidation of XII gave optically inactive cystine. This finding indicates that the asymmetric carbon atom involved in the thiazoline ring is readily racemized under basic condition not only in the form of the thiazoline carboxylic acid as demonstrated in the previous paper[1] but also in the form of the thiazoline carbonyl derivative as shown above. For the racemization of the thiazoline involved in peptide chain, in other words, the cysteine residue in the original peptide needs not locate at the *C*-terminal position.

Experimental

All melting points are uncorrected. Ultraviolet spectra were obtained in 95% ethanol and 12 N hydrochloric acid - 95% ethanol (1 : 1) with a Hitachi EPS-2 spectrophotometer.

***N, N'*-Bisbenzyloxycarbonylglycyl-L-cystine Diethyl Ester (I).** To a suspension of 11.0 g (0.03 mol) of diethyl L-cystinate dihydrochloride[6] in 100 m*l* of methylene chloride was added 6.0 g (0.06 mol) of triethylamine. After stirring for 30 min, the mixture was washed with water, dried over magnesium sulfate and then evaporated *in vacuo*. To the solution of the residue obtained in 150 m*l* of tetrahydrofuran, 12.3 g (0.06 mol) of benzyloxycarbonylglycine and 13.6 g (0.066 mol) of *N, N'*-dicyclohexylcarbodiimide were added. After the reaction mixture had been stirred for several hours, 1 m*l* of acetic acid was added to it. *N, N'*-dicyclohexylurea formed was filtered off, and the filtrate was evaporated *in vacuo* to dryness. The residue was dissolved in ethyl acetate and the solution was washed successively with 0.5 N hydrochloric acid, water, a 5% aqueous sodium hydrogen carbonate solution,

9) R. G. Hiskey and W. P. Tucker, *J. Am. Chem. Soc.*, **84**, 4794 (1962).
10) L. Zervas and I. Photaki, *ibid.*, **84**, 3887 (1962).

and water. Upon evaporation *in vacuo* after drying with sodium sulfate and recrystallization of the residue from ethanol - water, 16.5 g (81%) of crystals of I was obtained; mp 92—93°C, $[\alpha]_D^{18}$ −53.3° (*c* 3.60, dimethylformamide).

Found: C, 53.11; H, 5.61; N, 8.25; S, 9.30%. Calcd for $C_{30}H_{38}O_{10}N_4S_2$: C, 53.08; H, 5.64; N, 8.25; S, 9.45%.

Benzyloxycarbonylglycyl-L-cysteine Ethyl Ester (II). To a mixture of 17.0 g (0.025 mol) of I and 7.5 g of zinc dust in 100 m*l* of ethanol was added 16.5 m*l* of concentrated hydrochloric acid with vigorous stirring on cooling at 0°C within a period of 20 min. The mixture was stirred for an additional 10 min, and then filtered. The filtrate was concentrated *in vacuo* at 30°C. An oily product separated out on addition of water was extracted with ethyl acetate. The extract was washed with water, dried and then evaporated *in vacuo*. Recrystallization of the residual solid from ethyl acetate - petroleum ether gave 14.0 g (82%) of crystals of II, mp 79—80°C, $[\alpha]_D^{17}$ −8.6° (*c* 5.31, dimethylformamide).

Found: C, 53.07; H, 5.91; N, 8.21; S, 9.30%. Calcd for $C_{15}H_{20}O_5N_2S$: C, 52.92; H, 5.92; N, 8.23 S, 9.42%.

Ethyl 2-Benzyloxycarbonylaminomethyl-R-Δ^2-thiazoline-4-carboxylate (III). A solution of 2.0 g (0.006 mol) of II in 20 m*l* of chloroform was saturated with dry hydrogen chloride gas on cooling in an ice bath. The mixture was allowed to stand at room temperature overnight, and then concentrated *in vacuo*. To the residue was added 100 m*l* of ether, and the mixture was treated with a concentrated aqueous solution of potassium carbonate. The ether layer was washed with water, dried over sodium sulfate and then evaporated to dryness to give crystals. Recrystallization from ether - petroleum ether gave 1.3 g (67%) of III; mp 75—76°C, $[\alpha]_D^{23}$ +75.9° (*c* 3.34, ethanol).

Found: C, 55.99; H, 5.80; N, 8.41; S, 9.69%. Calcd for $C_{15}H_{18}O_4N_2S$; C, 55.88; H, 5.63; N, 8.69; S, 9.95%.

***N, N'*-Bisbenzyloxycarbonyl-L-cystinyl-di-L-leucine Diethyl Ester (IV).** To a solution of 19.6 g (0.1 mol) of ethyl L-leucinate hydrochloride[8] in 100 m*l* of chloroform were added 10.1 g (0.1 mol) of triethylamine and then 200 m*l* of ether. The precipitate formed was filtered off, and the filtrate was evaporated *in vacuo*. To the solution of the residue obtained in 200 m*l* of tetrahydrofuran, 25.4 g (0.05 mol) of *N, N'*-bisbenzyloxycarbonyl-L-cystine[7] and 22.6 g (0.11 mol) of *N, N'*-dicyclohexylcarbodiimide were added. The mixture was stirred for a few hours and then 2 m*l* of acetic acid was added. *N, N'*-Dicyclohexylurea thus formed was filtered off, and the filtrate was evaporated *in vacuo*. The solution of the residue in ethyl acetate was washed as usual, and dried over sodium sulfate. Evaporation of the solvent *in vacuo* gave a crystalline residue. It was recrystallized from ethanol-water to give pure crystals of II; wt 29.0 g (84%), mp 139—140°C, $[\alpha]_D^{19}$ −107° (*c* 3.25, dimethylformamide).

Found: C, 57.80; H, 6.89; N, 7.15; S, 8.18%. Calcd for $C_{38}H_{54}O_{10}N_4S_2$: C, 57.70; H, 6.88; N, 7.08; S, 8.11%.

L-Cystinyl-di-L-leucine Diethyl Ester Dihydrobromide (V). To a solution of 23.7 g (0.03 mol) of IV in 150 m*l* of glacial acetic acid was added 60 m*l* of 30% hydrogen bromide in acetic acid, and the mixture was kept at room temperature for 3 hr with occasional swirling. To the reaction mixture there was added 100 m*l* of dry ether. After the mixture had been stood at 0°C for 5 hr, the precipitate formed was filtered off, and then recrystallized from anhydrous ethanol - anhydrous ether to give crystals of V; wt 19.0 g (93%), mp 230.0—231.5°C, $[\alpha]_D^{18}$ −86.4° (*c* 4.43, water).

Found: C, 38.47; H, 6.51; N, 8.12%. Calcd for $C_{22}H_{44}O_6N_4S_2Br_2$: C, 38.60; H, 6.48; N, 8.18%.

***N, N'*-Bisbenzyloxycarbonylglycyl-L-cystinyl-di-L-leucine Diethyl Ester (VI).** To a solution of 13.7 g (0.02 mol) of V in 150 m*l* of chloroform was added 4.0 g (0.04 mol) of triethylamine. The mixture was stirred for 30 min, and then 200 m*l* of ether was added. The precipitate formed was filtered off, and the filtrate was concentrated *in vacuo*. To the solution of the residue thus obtained in 150 m*l* of tetrahydrofuran, 8.4 g (0.04 mol) of benzyloxycarbonylglycine and 9.1 g (0.044 mol) of *N, N'*-dicyclohexylcarbodiimide were added. The reaction mixture was stirred for a few hours, and then 1 m*l* of acetic acid was added to it. The urea derivative formed was removed by filtration and the filtrate was concentrated *in vacuo*. The residue obtained was dissolved in ethyl acetate, and the solution was washed as usual. Evaporation of the solvent *in vacuo* gave the residue, which was then recrystallized from ethyl acetate - petroleum ether to yield crystals of VI; wt 16.5 g (87%), mp 133—134°C, $[\alpha]_D^{14}$ −72.3° (*c* 4.07, dimethylformamide).

Found: C, 55.83; H, 6.68; N, 9.13; S, 7.04%. Calcd for $C_{42}H_{60}O_{12}N_6S_2$: C, 55.73; H, 6.68; N, 9.29; S, 7.09%.

***N*-Benzyloxycarbonyl-*S*-trityl-L-cysteinyl-L-leucine Ethyl Ester (VIII).** To a solution of 22.8 g (0.04 mol) of *N*-benzyloxycarbonyl-*S*-trityl-L-cysteine diethylamine salt[9] and 7.8 g (0.04 mol) of ethyl L-leucinate hydrochloride[8] in 200 m*l* of chloroform there was added 10.3 g (0.05 mol) of *N, N'*-dicyclohexylcarbodiimide. After the reaction mixture had been stirred for several hours, 1 m*l* of acetic acid was added, and the urea derivative formed was filtered off. The filtrate was evaporated to dryness and the residue obtained was dissolved in ether. The ethereal solution was washed as usual. Upon concentration *in vacuo* and recrystallization of the residue from ethanol - water gave crystals of VIII; wt 20.3 g (79%), mp 95—96°C, $[\alpha]_D^{15}$ −9.8° (*c* 15.08, dimethylformamide).

Found: C, 71.26; H, 6.61; N, 4.38; S, 5.01%. Calcd for $C_{38}H_{42}O_5N_2S$: C, 71.44; H, 6.63; N, 4.39; S, 5.02%.

***S*-Trityl-L-cysteinyl-L-leucine Ethyl Ester Hydrobromide (IX).** To a solution of 19.2 g (0.03 mol) of VIII in 90 m*l* of glacial acetic acid was added 30 m*l* of 30% hydrogen bromide in acetic acid. The mixture was kept at room temperature for 30 min with occasional swirling. The reaction mixture was concentrated *in vacuo* to dryness at 50°C under nitrogen. The residue was dissolved in 100 m*l* of dry benzene, and the solution was again concentrated to dryness. The residue thus obtained was then triturated with 200 m*l* of anhydrous ether and kept at 0°C. The precipitate formed was filtered off, and washed with ether. Recrystallization from ethyl acetate - anhydrous ether gave crystals of IX; wt 11.7 g (67%), mp 143—144°C, $[\alpha]_D^{15}$ +12.9° (*c* 5.21, ethanol).

Found: C, 61.02; H, 6.39; N, 4.73%. Calcd for

$C_{30}H_{37}O_3N_2SBr$: C, 61.53; H, 6.37; N, 4.78%.

Benzyloxycarbonylglycyl-*S*-trityl-L-cysteinyl-L-leucine Ethyl Ester (X). To a solution of 5.9 g (0.01 mol) of IX in 20 m*l* of chloroform there was added 1.0 g (0.01 mol) of triethylamine. The precipitate formed on addition of 50 m*l* of ether was filtered off, and the filtrate was evaporated *in vacuo*. To the solution of the residue obtained in 50 m*l* of tetrahydrofuran, 2.1 g (0.01 mol) of benzyloxycarbonylglycine and 2.2 g (0.011 mol) of *N*, *N'*-dicyclohexylcarbodiimide were added. The mixture was stirred for several hours, and then 1 m*l* of acetic acid was added. The urea derivative precipitated was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was dissolved in ethyl acetate, and the solution was washed as usual. Evaporation of the solvent and then crystallization of the residue from ethanol-water gave 6.3 g (90%) of X; mp 126—127°C, $[\alpha]_D^{16}$ −11.7° (*c* 6.20, dimethylformamide).

Found: C, 68.89; H, 6.50; N, 6.03; S, 4.51%. Calcd for $C_{40}H_{45}O_6N_3S$: C, 69.04; H, 6.52; N, 6.04; S, 4.61%.

Benzyloxycarbonylglycyl-L-cysteinyl-L-leucine Ethyl Ester (VII). a) To a mixture of 4.5 g (0.005 mol) of VI and 1.5 g of zinc dust in 40 m*l* of ethanol, 3.3 m*l* of concentrated hydrochloric acid was added slowly with vigorous stirring at 0°C over 15 min. Stirring at this temperature was continued for an additional 3 min. Undissolved zinc dust was filtered off and washed with ethanol. The combined filtrate was concentrated *in vacuo* at 30°C. On addition of water to the residue, an oily product was separated out, which was then extracted with ethyl acetate. The organic layer was washed with water, dried over magnesium sulfate, and evaporated *in vacuo*. The residue was recrystallized from ethyl acetate - petroleum ether to give crystals of VII; wt 3.9 g (86%), mp 114.0—115.5°C, $[\alpha]_D^{15}$ −14.9° (*c* 5.76, dimethylformamide).

Found: C, 55.71; H, 6.91; N, 9.07; S, 7.06%. Calcd for $C_{21}H_{31}O_6N_3S$: C, 55.61; H, 6.89; N, 9.26; S, 7.07%.

b) Upon addition of a solution of 1.0 g (0.006 mol) of silver nitrate and 0.5 m*l* (0.006 mol) of pyridine in 30 m*l* of ethanol to a warm solution of 4.2 g (0.006 mol) of X in 20 m*l* of absolute ethanol, silver mercaptide of benzyloxycarbonylglycyl-L-cysteinyl-L-leucine ethyl ester was precipitated. After allowing to stand at room temperature under nitrogen for 2 hr, it was filtered off, washed with ethanol and then dried; wt 3.2 g (95%).

To a solution of 3.2 g of the silver mercaptide in 30 m*l* of dimethylformamide, 0.75 m*l* of concentrated hydrochloric acid was added. The mixture was shaken at room temperature for 2 hr, and then heated on a boiling water bath for 1 min. The precipitate of silver chloride formed was removed by filtration and washed with a little amount of dimethylformamide. After an addition of chloroform to the filtrate, it was washed with water several times, dried over magnesium sulfate, and then evaporated *in vacuo*. Upon addition of water to the residue, crystals was separated out. Recrystallization from ethyl acetate - petroleum ether gave 2.0 g (74%) of VII; mp 115—116°C, $[\alpha]_D^{17}$ −13.6° (*c* 3.08, dimethylformamide).

Found: C, 55.73; H, 6.98; N, 9.00; S, 7.07%.

2-Benzyloxycarbonylaminomethyl-R-Δ^2-thiazoline-4-carbonyl-L-leucine Ethyl Ester (XI). a) A solution of 1.2 g (0.0025 mol) of VII in 10 m*l* of chloroform was saturated with dry hydrogen chloride gas on cooling at 0°C. The mixture was allowed to stand at room temperature overnight, and then evaporated *in vacuo* to dryness. The residue obtained was triturated with ether, and the ethereal solution was treated with a concentrated potassium carbonate solution. The ether layer was separated, washed with water, dried over sodium sulfate and evaporated to dryness to give a crude oil; wt 0.9 g. This was dissolved in a little amount of ether and subjected to the silica gel column chromatography (Mallincrodt Chemical Worker, 100 mesh, 10 g). Elution with petroleum ether - anhydrous ether gave 0.75 g (68%) of XI as an oil; $[\alpha]_D^{24}$ +43.3° (*c* 2.09, ethanol), λ_{max}^{EtOH} 252 mμ (ε 2700), $\lambda_{max}^{EtOH\text{-}HCl}$ 268 mμ (ε 5000).

Found: C, 57.83; H, 6.90; N, 9.65; S, 7.13%. Calcd for $C_{21}H_{29}O_5N_3S$: C, 57.91; H, 6.71; N, 9.65; S, 7.36%.

b) A solution of 6.4 g (0.01 mol) of VIII and 2 g of anisole in 30 m*l* of glacial acetic acid there was added 10 m*l* of 30% hydrogen bromide in acetic acid and the mixture was allowed to stand at room temperature for 30 min with occasional swirling. The precipitate of trityl bromide formed was filtered off; wt 1.8 g. The filtrate was concentrated *in vacuo* under nitrogen to give L-cysteinyl-L-leucine ethyl ester hydrobromide as a residual oil. It was washed with three portions of petroleum ether, dried over sodium hydroxide *in vacuo* and then dissolved in 20 m*l* of tetrahydrofuran. To this solution, 2.4 g (0.01 mol) of benzyloxycarbonylaminomethyl iminoether[1] was added, and the reaction mixture was kept at room temperature for 1 hr. The precipitate of ammonium bromide formed was filtered off; wt 0.70 g. The filtrate was evaporated *in vacuo*, and the residue obtained was dissolved in ether. The ethereal solution was washed with water, dried over sodium sulfate and then evaporated *in vacuo* to yield a crude oil of XI; wt 4.0 g. The oil was purified by chromatography on silica gel column (40 g) as in a); wt 2.0 g (45%).

2-Benzyloxycarbonylaminomethyl-Δ^2-thiazoline-4-carbonyl-L-leucinamide (XII). A solution of 4.4 g (0.01 mol) of XI in 100 m*l* of absolute ethanol was saturated with ammonia gas at 0°C. The mixture was kept at room temperature for 3 days, and then evaporated *in vacuo* below 40°C. The residual solid was crystallized from ethanol-water to give crystals of the amide; wt 3.0 g (73%), mp 174—176°C (decomp.), $[\alpha]_D^{21}$ −45.0° (*c* 2.01, dimethylformamide), λ_{max}^{EtOH} 252 mμ (ε 2700), $\lambda_{max}^{EtOH\text{-}HCl}$ 268 mμ (ε 4900).

Found: C, 56.00; H, 6.61; N, 13.69; S, 7.86%. Calcd for $C_{19}H_{26}O_4N_4S$: C, 56.14; H, 6.45; N, 13.78; S, 7.89%.

Acid Hydrolysis of XII. A mixture of 4.1 g (0.01 mol) of XII, 4.1 g of anisole and 100 m*l* of 6 N hydrochloric acid was refluxed for 5 hr. After cooling, the mixture was extracted twice with ether. The aqueous layer was evaporated *in vacuo* under nitrogen. To the residue obtained, 50 m*l* of water was added and then the evaporation was repeated. A solution of the final residue in 50 m*l* of water was adjusted to pH about 8.5 by addition of concentrated aqueous ammonia, and then air was bubbled through the solution until the nitroprusside reaction by a spot test became negative.

After addition of a drop of acetic acid, the reaction mixture was stood at 0°C for 2 days. The precipitate thus formed was filtered off and then dissolved in 30 m*l* of N hydrochloric acid. Undissolved substance was removed by filtration and the filtrate was adjusted to pH 5.5—6.0 with addition of N sodium hydroxide and an aqueous sodium acetate solution. After standing at 0°C for 2 days, the precipitate formed was filtered off and dried at 80°C *in vacuo* for 4 hr to afford optically inactive cystine; wt 0.68 g (57%).

Found: N, 11.39; S, 26.46%. Calcd for $C_6H_{12}O_4N_2S_2$: N, 11.66; S, 26.69%.

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