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Synthetic Studies of Bacitracin. IV.[1] Synthesis of Thiazoline Peptides by Iminoether Coupling Method

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For the purpose of a total synthesis of bacitracin A, a synthetic method of the thiazoline peptide was investigated. A benzyloxycarbonylaminoalkylimino ethyl ether derived from glycine, L-alanine, L-valine, L-leucine or L-isoleucine was coupled with ethyl L-cysteinate hydrochloride to afford the corresponding thiazoline peptide, that is, a dehydrated form of cysteine peptide. While L-cystine was obtained from acid hydrolyzates of those thiazoline peptides, hydrolysis of them after treatment with alkali or ammonia gave DL-cystine. Furthermore, alkaline treatment caused a racemization of the amino acid residue adjacent to the thiazoline ring too.

Since it had been ascertained that an antibiotic bacitracin A contained a thiazoline ring in its peptide chain,[2] the thiazoline ring has been known to exist in other natural substances too, for example, firefly luciferin[3] or thiostrepton[4]. In the second paper of the series of this study,[5] the attempt has been made to synthesize bacitracin A by cyclization of the dodecapeptide involving a cysteine residue to the thiazoline peptide unsuccessfully. The present investigation was undertaken to study the synthetic methods of peptide containing the thiazoline ring for the purpose of applying them to the synthesis of *N*-terminal peptide part of bacitracin A. Concerning the formation of the thiazoline derivative, the ring closure reaction of

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2) I. M. Lockhart, G. G. F. Newton and E. P. Abraham, *Nature*, **173**, 536 (1954); I. M. Lockhart and E. P. Abraham, *Biochem. J.*, **58**, 633 (1954); W. Hausmann, J. R. Weisiger and L. C. Craig, *J. Am. Chem. Soc.*, **77**, 723 (1955); J. R. Weisiger, W. Hausmann and L. C. Craig, *ibid.*, **77**, 731 (1955).

3) E. H. White, F. McCapra, G. F. Field and W. D. McElroy, *J. Am. Chem. Soc.*, **83**, 2402 (1961); E. H. White, F. McCapra and G. F. Field, *ibid.*, **85**, 337 (1963).

4) D. F. W. Cross, G. W. Kenner, R. C. Sheppard and C. E. Stehr, *J. Chem. Soc.*, **1963**, 2143.

5) Y. Ariyoshi, T. Shiba and T. Kaneko, This Bulletin, **40**, 2648 (1967).

glutathione[6] or synthetic cysteine peptides[7,8] to the thiazoline peptide by dehydration in a strong acidic solution has been demonstrated. On the other hand, a coupling reaction of nitrile or iminoether derived from the amino acid with a cysteine derivative can also give the thiazoline derivative.[3,7,9,10] However, only a few thiazoline peptides with acetyl[7] or benzyl group[9] as an amino protecting group have been prepared by this coupling reaction, but little has been known on the reaction of the compound containing the thiazoline ring.

In the present paper, the formation reaction of the thiazoline peptides by the coupling of a number of benzyloxycarbonylaminoalkyl iminoethers derived from the corresponding amino acids with ethyl L-cysteinate hydrochloride was studied, and a racemization of the amino acid residue comprized in the thiazoline peptide during a hydrolysis was investigated. It appears of importance to choose an appropriate protecting group of *N*-terminal amino group for the following reason. The thiazoline ring is known to be only stable under a strong acidic condition.[11,12] In order to obtain a free amino thiazoline peptide, therefore,

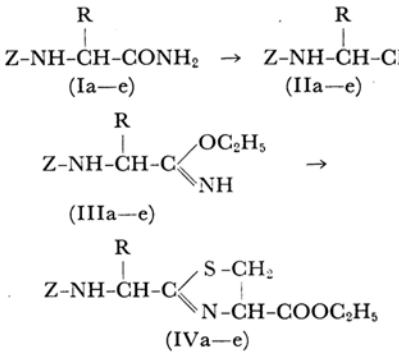

Fig. 1.

a) R=H, b) R=CH_3, c) R=$(CH_3)_2CH$,
d) R=$(CH_3)_2CHCH_2$, e) R=$(CH_3)(C_2H_5)CH$
Z=$C_6H_5CH_2OCO$-

it is required that the amino protecting group must be stable during a course of the formation of the thiazoline derivative and be able to be removed by an acidic medium where the thiazoline ring remains stable. The benzyloxycarbonyl group fulfills the above requirements.

Benzyloxycarbonylamino acid amide (Ia—e) of glycine, L-alanine, L-valine, L-leucine or L-isoleucine was converted to the corresponding nitrile (IIa—e) respectively by heating with *p*-toluenesulfonyl chloride in pyridine according to the method of Brookes *et al.*[13] as outlined in Fig. 1. The nitrile was treated with dry hydrogen chloride in a mixture of absolute ethanol and absolute ether to yield the corresponding iminoether hydrochloride by application of the method of Hill and Rabinowitz.[14,15] Free iminoethers (IIIa—e) were obtained from the hydrochlorides by removal of hydrogen chloride by treatment with aqueous concentrated potassium carbonate solution respectively. Among them, only the iminoether derivatives of glycine and L-alanine (IIIa,b) were obtained in crystalline states. Coupling of one of those iminoethers with ethyl L-cysteinate hydrochloride in anhydrous ethanol at room temperature gave ethyl 2-(1-benzyloxycarbonylaminoalkyl)-R-Δ^2-thiazoline-4-carboxylate (IVa—e). Except the thiazoline derivative derived from glycine (IVa) which was secured in a crystalline state, the reaction product (IVb—e) was purified by silica gel column chromatography using ether - petroleum ether as an eluting solvent. The ultraviolet spectra of those thiazoline derivatives showed the absorption maxima near 253 mμ in ethanol as well as near 268 mμ in ethanol - hydrochloric acid (1 : 1) in accordance with the data of Craig *et al.*[7]

The compound (IVa), one of the thiazoline derivatives, was hydrolyzed with hydrochloric acid and then oxidized to give L-cystine, while a similar treatment for the acid amide (Va) obtained from IVa by the action of ammonia in ethanol gave DL-cystine as shown in Fig. 2. On the other hand, the hydrolysis of IVa by sodium hydroxide gave sodium salt of thiazoline derivative (VI), an aqueous solution of which was then acidified to yield a mixture giving positive reactions both for ninhydrin and nitroprusside and showing the ultraviolet absorption maximum due to the S-CO group at about 230 mμ.[16] It seemed to be a mixture of *N*-benzyloxycarbonylglycylcysteine and glycyl-*S*-benzyloxycarbonylcysteine from the observation mentioned above. Hydrolysis of the

6) M. Calvin, "Glutathione," ed. by S. Colowick *et al.*, Academic Press, New York, N. Y. (1954), p. 3; G. Preaux and R. Lontie, *Biochem. J.*, **66**, 26p (1957); R. B. Martin and J. T. Edsall, *Bull. Soc. Chim. Biol.*, **40**, 1763 (1958); D. Garfinkel, *J. Am. Chem. Soc.*, **80**, 4833 (1958).
7) W. Stoffel and L. C. Craig, *J. Am. Chem. Soc.*, **83**, 145 (1961).
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12) W. Konigsberg, R. J. Hill and L. C. Craig, *J. Org. Chem.*, **26**, 3867 (1961).
13) P. Brookes, R. J. Clark, B. Majhofer, M. P. V, Mijovic and J. Walker, *J. Chem. Soc.*, **1960**, 925.
14) A. J. Hill and I. Rabinowitz, *J. Am. Chem. Soc.*, **48**, 732 (1926).
15) M. Mengelberg, *Chem. Ber.*, **89**, 1185 (1956).
16) M. Shimizu, G. Ohta, O. Nagase, S. Okada and Y. Hosokawa, *Chem. Pharm. Bull.*, **13**, 180 (1965).

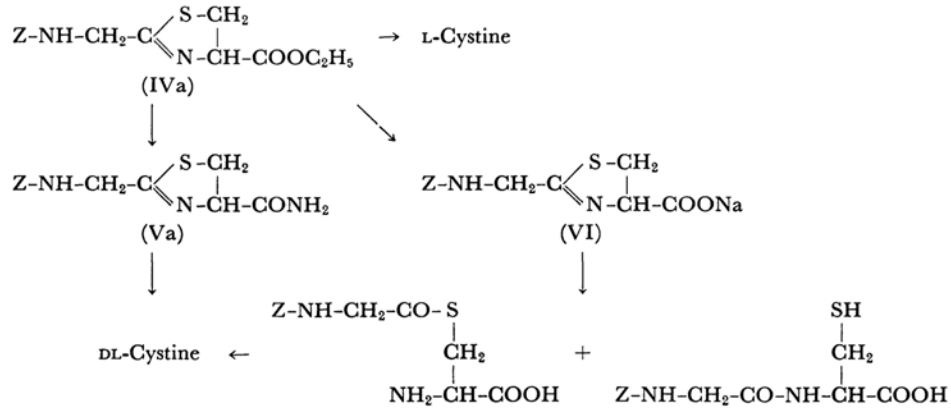

Fig. 2. $Z = C_6H_5CH_2OCO-$

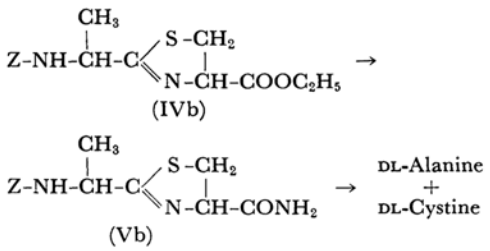

Fig. 3. $Z = C_6H_5CH_2OCO-$

mixture by hydrochloric acid followed by oxidation gave DL-cystine.

Furthermore, Vb, the other thiazoline acid amide, obtained from IVb by the same procedure as for Va was hydrolyzed by hydrochloric acid to afford DL-cystine as well as DL-alanine as shown in Fig. 3. The racemization of the alanine residue seems to be of particular interest in view of the easiness of the epimerization of L-isoleucine to D-alloisoleucine of *N*-terminal amino acid residue attached to the thiazoline ring in the molecule of bacitracin A.[12] From the results presented above, it is concluded that not only cysteine residue but also the amino acid adjacent to it in the molecule of thiazoline peptide has a tendency to be very easily racemized under the basic condition such as saponification by alkali or amidation by ammonia in ethanol.

Experimental

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

Benzyloxycarbonylaminoacetonitrile (IIa). To a solution of 20.8 g (0.1 mol) of benzyloxycarbonylglycinamide[17] (Ia) in 150 m*l* of pyridine there was added 22.9 g (0.12 mol) of *p*-toluenesulfonyl chloride, and the mixture was refluxed for 1 hr. It was concentrated *in vacuo*, and then diluted with ether. The ethereal solution was washed successively with 2 N hydrochloric acid and water, dried with sodium sulfate and then evaporated. The residue obtained was crystallized from ether - petroleum ether to give the nitrile (IIa); wt 15.9 g (78%), mp 61—62°C, lit.[15]; IIa prepared from aminoacetonitrile hydrochloride, mp 62°C.

Found: C, 63.27; H, 5.32; N, 14.35%. Calcd for $C_{10}H_{10}O_2N_2$: C, 63.15; H, 5.30; N, 14.73%.

L-2-Benzyloxycarbonylaminopropionitrile (IIb). A mixture of 22.2 g (0.1 mol) of benzyloxycarbonyl-L-alaninamide (Ib) in 150 m*l* of pyridine, and 22.9 g (0.12 mol) of *p*-toluenesulfonyl chloride was heated at 100°C for 1 hr, and then worked up as in the preceding experiment. Recrystallization of the product from ether - petroleum ether gave IIb; wt 16.8 g (82%), mp 84.0—85.5°C, $[\alpha]_D^{15}$ −69.1° (*c* 5.62, ethanol).

Found: C, 64.83; H, 5.90; N, 13.38%. Calcd for $C_{11}H_{12}O_2N_2$: C, 64.69; H, 5.92; N, 13.72%.

L - 2 - Benzyloxycarbonylaminoisovaleronitrile (IIc). A mixture of 12.5 g (0.05 mol) of benzyloxycarbonyl-L-valinamide (Ic), 75 m*l* of pyridine and 12.0 g (0.075 mol) of *p*-toluenesulfonyl chloride was refluxed for 10 min, and then worked up in a similar way to give the nitrile (IIc) in a crystalline state; wt 9.7 g (83%), mp 55—56°C, $[\alpha]_D^{12}$ −55.2° (*c* 4.48, ethanol).

Found: C, 67.56; H, 6.98; N, 12.08%. Calcd for $C_{13}H_{16}O_2N_2$: C, 67.22; H, 6.94; N, 12.06%.

L-2-Benzyloxycarbonylamino-4-methylvaleronitrile (IId). A mixture of 10.6 g (0.04 mol) of benzyloxycarbonyl-L-leucinamide[18] (Id), 60 m*l* of pyridine and 9.5 g (0.05 mol) of *p* - toluenesulfonyl chloride was refluxed for 1.5 hr. The nitrile obtained was purified by distillation *in vacuo*; bp 133—146°C/0.005 mmHg, wt 8.9 g (90%), mp 29.5—32.0°C, $[\alpha]_D^{24}$ −51.0° (*c* 5.86, ethanol).

Found: C, 68.47; H, 7.38; N, 11.51%. Calcd for $C_{14}H_{18}O_2N_2$: C, 68.27; H, 7.37; N, 11.37%.

L-2-Benzyloxycarbonylamino-3-methylvaleronitrile (IIe). In a similar way, a mixture of 5.3 g (0.02 mol) of benzyloxycarbonyl-L-isoleucinamide (Ie), 30 m*l* of pyridine and 4.1 g (0.022 mol) of *p*-toluenesulfonyl chloride was refluxed for 3 hr. The nitrile obtained was distilled *in vacuo;* bp 137—145°C/0.005 mmHg. Wt 4.1 g (83%), mp 28—29°C, $[\alpha]_D^{19}$ −48.3°

17) S. W. Fox and H. Wax, *J. Am. Chem. Soc.*, **73**, 2936 (1951).

18) O. K. Behrens and M. Bergmann, *J. Biol. Chem.*, **129**, 587 (1939).

(*c* 4.037, ethanol).

Found: C, 68.13; H, 7.29; N, 11.70%. Calcd for $C_{14}H_{18}O_2N_2$: C, 68.27; H, 7.37; N, 11.37%.

Benzyloxycarbonylaminoacetimino Ethyl Ether (IIIa). The hydrochloride of IIIa was prepared according to the method of Mengelberg[15]; yield 93%, mp 117—118°C (decomp.), lit.,[15] mp 118—119°C (decomp.).

To a suspension of 8.2 g (0.03 mol) of the hydrochloride in 100 m*l* of ether there was slowly added a concentrated potassium carbonate solution with continuous shaking, until the salt was completely decomposed. The ethereal solution was separated, dried with sodium sulfate and then evaporated *in vacuo*. The residue obtained was crystallized from ether - petroleum ether to give a free iminoether (IIIa); wt 6.7 g (95%), mp 58—59°C, ν_{max} 1680 cm^{-1} (–C=N–).

Found: C, 60.90; H, 6.90; N, 11.62%. Calcd for $C_{12}H_{16}O_3N_2$: C, 61.00; H, 6.83; N, 11.86%.

L-2-Benzyloxycarbonylaminopropioimino Ethyl Ether (IIIb). A solution of 4.1 g (0.02 mol) of IIb in a mixture of 1.1 g of anhydrous ethanol and 20 m*l* of anhydrous ether was saturated with dry hydrogen chloride on cooling in an ice bath, and allowed to stand at room temperature for 1 hr. The solvent was evaporated *in vacuo* to give an oily material which was then suspended in 50 m*l* of ether. To the suspension, a concentrated potassium carbonate solution was slowly added with continuous shaking. The ethereal solution was separated, dried with magnesium sulfate, and then evaporated *in vacuo*. Recrystallization of the residue thus obtained from ether - petroleum ether gave crystals of IIIb; wt 3.4 g (68%), mp 71.5—73.0°C, $[\alpha]_D^{24}$ −1.4° (*c* 12.65, anhydrous ethanol), ν_{max} 1660 cm^{-1} (–C=N–).

Found: C, 62.35; H, 7.18; N, 10.96%. Calcd for $C_{13}H_{18}O_3N_2$: C, 62.38; H, 7.25; N, 11.19%.

L - 2 - Benzyloxycarbonylaminoisovaleroimino Ethyl Ether (IIIc). In a similar manner described above, the iminoether (IIIc) was obtained as a crude oil from 7.0 g (0.03 mol) of IIc, 1.7 g of anhydrous ethanol, and 30 m*l* of anhydrous ether; wt 3.7 g (45%), ν_{max} 1660 cm^{-1} (–C=N–).

L - 2 - Benzyloxycarbonylamino - 4 - methylvaleroimino Ethyl Ether (IIId). The iminoether (IIId) was also prepared as a crude oil from 8.4 g (0.034 mol) of IId, 1.9 g of anhydrous ethanol, and 30 m*l* of anhydrous ether by the similar procedure; wt 4.8 g (49%), ν_{max} 1660 cm^{-1} (–C=N–).

L - 2 - Benzyloxycarbonylamino - 3 - methylvaleroimino Ethyl Ether (IIIe). In a similar manner, the iminoether (IIIe) was obtained as a crude oil from 7.4 g (0.03 mol) of IIe, 1.7 g of anhydrous ethanol, and 30 m*l* of anhydrous ether; wt 3.7 g (42%), ν_{max} 1660 cm^{-1} (–C=N–).

Ethyl 2-Benzyloxycarbonylaminomethyl-R-Δ^2-thiazoline-4-carboxylate (IVa). To a solution of 6.4 g (0.036 mol) of ethyl L-cysteinate hydrochloride[19] in 40 m*l* of anhydrous ethanol there was added 7.1 g (0.03 mol) of IIIa. After the reaction mixture had been kept at room temperature for 1 hr, ammonium chloride deposited was filtered off; wt 1.2 g. The filtrate was concentrated *in vacuo*, and the residue obtained was dissolved in ether. The solution was washed with water, dried with sodium sulfate, and then evaporated to give crystals. Recrystallization from ether - petroleum ether gave IVa; wt 6.4 g (66%), mp 75—76°C, $[\alpha]_D^{25}$ +75.2° (*c* 4.31, ethanol), λ_{max}^{EtOH} 253 mμ (ε 3300), $\lambda_{max}^{EtOH\text{-}HCl}$ 267 mμ (ε 7300).

Found: C, 55.99; H, 5.68; N, 8.62; S, 9.79%. Calcd for $C_{15}H_{18}O_4N_2S$: C, 55.88; H, 5.63; N, 8.69; S, 9.95%.

Ethyl 2-(L-1-Benzyloxycarbonylaminoethyl)-R-Δ^2-thiazoline-4-carboxylate (IVb). To a solution of 4.5 g (0.024 mol) of ethyl L-cysteinate hydrochloride[19] in 30 m*l* of anhydrous ethanol was added 5.0 g (0.02 mol) of IIIb. The mixture was worked up as described above. Evaporation of the solvent gave an oily material; wt 6.1 g. This was dissolved in 10 m*l* of anhydrous ether, and the solution was placed on the top of a column which was prepared from silica gel (Mallinckrodt Chemical Works; 100 mesh, 30 g) suspended in petroleum ether (bp 40—50°C). The column was eluted with anhydrous ether - petroleum ether (1 : 1) to give pure oily IVb; wt 3.8 g (63%), $[\alpha]_D^{24}$ +60.9° (*c* 4.01, ethanol), λ_{max}^{EtOH} 252 mμ (ε 2600), $\lambda_{max}^{EtOH\text{-}HCl}$ 268 mμ (ε 6600).

Found: C, 56.86; H, 6.04; N, 8.21; S, 9.42%. Calcd for $C_{16}H_{20}O_4N_2S$: C, 57.12; H, 5.99; N, 8.33; S, 9.53%.

Ethyl 2-(L-1-Benzyloxycarbonylamino-2-methylpropyl)-R-Δ^2-thiazoline-4-carboxylate (IVc). By the same procedure to that described above, the thiazoline derivative (IVc) was prepared from 2.0 g (0.011 mol) of ethyl L-cysteinate hydrochloride[19] and 2.5 g (0.009 mol) of IIIc in 25 m*l* of anhydrous ethanol. Purification of 2.2 g of an oily crude product by silica gel column chromatography gave 0.9 g (32%) of pure IVc; $[\alpha]_D^{27}$ +35.1° (*c* 3.26, ethanol), λ_{max}^{EtOH} 253 mμ (ε 2500), $\lambda_{max}^{EtOH\text{-}HCl}$ 269 mμ (ε 5500).

Found: C, 59.26; H, 6.81; N, 7.69; S, 8.51%. Calcd for $C_{18}H_{24}O_4N_2S$: C, 59.32; H, 6.64; N, 7.69; S, 8.80%.

Ethyl 2-(L-1-Benzyloxycarbonylamino-3-methylbutyl)-R-Δ^2-thiazoline-4-carboxylate (IVd). Condensation of 3.7 g (0.02 mol) of ethyl L-cysteinate hydrochloride[19] with 4.8 g (0.016 mol) of IIId carried out in a similar way yielded 5.1 g of an oily product. A solution of it in ether was run through a column of silica gel to give 3.4 g (68%) of pure IVd; $[\alpha]_D^{27}$ +42.1° (*c* 3.81, ethanol), λ_{max}^{EtOH} 253 mμ (ε 2800), $\lambda_{max}^{EtOH\text{-}HCl}$ 268 mμ (ε 6800).

Found: C, 60.29; H, 7.00; N, 7.34; S, 8.33%. Calcd for $C_{19}H_{26}O_4N_2S$: C, 60.29; H, 6.92; N, 7.40; S, 8.47%.

Ethyl 2-(L-1-Benzyloxycarbonylamino-2-methylbutyl)-R-Δ^2-thiazoline-4-carboxylate (IVe). Similarly, the condensation of 2.8 g (0.015 mol) of ethyl L-cysteinate hydrochloride[19] with 3.6 g (0.0125 mol) of IIIe gave 2.9 g of an oily product. It was dissolved in ether and applied to the silica gel column chromatography to yield 1.2 g (43%) of pure IVe; $[\alpha]_D^{27}$ +25.8° (*c* 3.64, ethanol), λ_{max}^{EtOH} 252 mμ (ε 2300), $\lambda_{max}^{EtOH\text{-}HCl}$ 270 mμ (ε 4900).

Found: C, 59.92; H, 6.98; N, 7.46; S, 8.29%. Cacld for $C_{19}H_{26}O_4N_2S$: C, 60.29; H, 6.92; N, 7.40; S, 8.47%.

Isolation of L-Cystine from Acid Hydrolyzate of IVa. A solution of 3.2 g (0.01 mol) of IVa and 1.1

19) V. du Vigneaud, R. Dorfmann and H. S. Loring, *J. Biol. Chem.*, **98**, 577 (1932).

g (0.01 mol) of anisole in 100 m*l* of 3 N hydrochloric acid was refluxed for 4 hr. The reaction mixture was cooled and extracted with ether. The aqueous layer was concentrated *in vacuo* to a half volume in a stream of nitrogen, and then titrated with 0.1 N iodine solution at 0°C; about 100 m*l* of the solution was required. The slight excess of iodine was removed with a sodium thiosulfate solution. The reaction mixture was adjusted to pH 3.5—4.0 with a sodium acetate solution, and kept at 0°C for 24 hr. The precipitate formed was filtered off, and dissolved in 20 m*l* of N hydrochloric acid. After removal of undissolved material by filtration, the filtrate was adjusted to pH 3.5—4.0 with an aqueous sodium acetate solution. After the solution had been kept at 0°C for 24 hr, crystals of L-cystine formed was filtered off, and dried at 80°C *in vacuo* for 3 hr; wt 0.70 g (58%), $[\alpha]_D^5$ −238°C (*c* 1.41, N HCl).

Found: C, 30.29; H, 5.13; N, 11.80; S, 26.44%. Calcd for $C_6H_{12}O_4N_2S_2$: C, 29.99; H, 5.04; N, 11.66; S, 26.69%.

2-Benzyloxycarbonylaminomethyl-Δ^2-thiazoline-4-carboxamide (Va). A solution of 6.4 g (0.02 mol) of IVa in 60 m*l* of anhydrous ethanol was saturated with ammonia at 0°C, and the mixture was kept at room temperature for 2 days. The solution was concentrated *in vacuo* below 40°C. The residue thus obtained was recrystallized from ethanol - water to give 5.2 g (90%) of Va; mp 120—121°C, λ_{max}^{EtOH} 252 mμ (ε 2600), $\lambda_{max}^{EtOH\text{-}HCl}$ 268 mμ (ε 5200); it showed no optical rotation in D line.

Found: C, 53.19; H, 5.28; N, 14.44; S, 10.85%. Calcd for $C_{13}H_{15}O_3N_3S$: C, 53.23; H, 5.15; N, 14.32; S, 10.93%.

Isolation of DL-Cystine from Acid Hydrolyzate of Va. A solution of 2.9 g (0.01 mol) of Va and 1.1 g (0.01 mol) of anisole in 50 m*l* of 12 N hydrochloric acid was refluxed for 1 hr. The solution was worked up as in the isolation of L-cystine to give DL-cystine; wt 0.75 g (63%).

Found: C, 30.02; H, 5.08; N, 11.47; S, 26.35%. Calcd for $C_6H_{12}O_4N_2S_2$: C, 29.99; H, 5.04; N, 11.66; S, 26.69%.

Sodium 2-Benzyloxycarbonylaminomethyl-Δ^2-thiazoline-4-carboxylate (VI). To a solution of 3.2 g (0.01 mol) of IVa in 50 m*l* of acetone was added 11 m*l* (0.011 mol) of N sodium hydroxide. The solution was stirred at room temperature for 1 hr, diluted with 200 m*l* of acetone, and then kept at 0°C for 5 hr. A sodium salt thus formed was filtered off, washed with acetone and dried at 50—60°C *in vacuo* over phosphorus pentoxide for 2 days to give hygroscopic crystals of optically inactive VI; wt 2.9 g (91%), $\lambda_{max}^{H_2O}$ 253 mμ (ε 2900).

Found: C, 48.52; H, 4.24; N, 8.62; Na, 7.03%. Calcd for $C_{13}H_{13}O_4N_2SNa$: C, 49.36; H, 4.14; N, 8.86; Na, 7.27%.

Isolation of DL-Cystine from Acid Hydrolyzate of VI. A solution of 1.6 g (0.005 mol) of the sodium salt (VI) in 50 m*l* of water was adjusted to pH 3.0 with N hydrochloric acid, and then kept at 0°C for 1 hr. The precipitate formed was filtered off and dried at 80°C for 5 hr to give a solid which seemed to be a mixture of *N*-benzyloxycarbonylglycyl-DL-cysteine and glycyl-*S*-benzyloxycarbonyl-DL-cysteine; wt 1.2 g (77%), mp 126—128°C (decomp.). It gave positive reactions both for nitroprusside and ninhydrin. $\lambda_{max}^{N\ NaOH}$ 235 mμ (ε 5500), $\lambda_{max}^{N\ HCl}$ 232 mμ (ε 3900) (characteristic for the –S–CO– group).[16]

A solution of 0.62 g (0.002 mol) of the above mixture in 25 m*l* of 3 N hydrochloric acid was refluxed for 2 hr, and then worked up in the usual manner for oxidation to afford 0.14 g (58%) of DL-cystine.

2-(1-Benzyloxycarbonylaminoethyl)-Δ^2-thiazoline-4-carboxamide (Vb). A solution of 1.0 g (0.003 mol) of IVb in 20 m*l* of anhydrous ethanol was saturated with ammonia at 0°C. The reaction mixture was kept at room temperature for 2 days. The solution was evaporated *in vacuo* below 40°C. The residue thus obtained was crystallized from ethanol - water to yield optically inactive Vb; wt 0.8 g (87%), mp 116—118°C, λ_{max}^{EtOH} 252 mμ (λ 2900), $\lambda_{max}^{EtOH\text{-}HCl}$ 268 mμ (ε 6200).

Found: C, 54.60; H, 5.65; N, 13.82; S, 10.36%. Calcd for $C_{14}H_{17}O_3N_3S$: C, 54.70; H, 5.58; N, 13.67; S, 10.43%.

Isolation of DL-Cystine and DL-Alanine from Acid Hydrolyzate of Vb. To a mixture of 3.1 g (0.01 mol) of Vb and 1.1 g (0.01 mol) of anisole was added 100 m*l* of 6 N hydrochloric acid. The mixture was refluxed for 3 hr. After cooling, it was extracted twice with ether. An aqueous layer was evaporated to dryness *in vacuo* in a stream of nitrogen. The residue obtained was dissolved in 50 m*l* of water, and the evaporation *in vacuo* was repeated. After the residue thus obtained had been dried *in vacuo* at 50°C over sodium hydroxide for 5 hr to remove hydrochloric acid completely, it was dissolved again in 50 m*l* of water, and the solution was adjusted to pH about 8.5 with 28% aqueous ammonia. Through the solution, air was bubbled until no more positive nitroprusside reaction was given on a spot test. After an addition of a drop of acetic acid, the mixture was allowed to stand at 0°C for 24 hr. A crude cystine precipitated was filtered off, and dissolved in 30 m*l* of N hydrochloric acid. The solution was adjusted to pH 5.5—6.0 with N sodium hydroxide, and kept at 0°C for 2 days. The precipitate of DL-cystine thus formed was filtered off and dried; wt 0.8 g (67%).

The filtrate from the crude cystine was evaporated *in vacuo*. The residue obtained was triturated with 300 m*l* of methanol, and the mixture was allowed to stand at room temperature for 24 hr. The precipitate was filtered off, and dissolved in 50 m*l* of water. After filtration of undissolved material, the filtrate was added to a column of Dowex 50 H^+ form (50 m*l*). The column was washed with 200 m*l* of water until a neutral washing was obtained, and then eluted with 100 m*l* of aqueous N ammonia. The eluate was evaporated *in vacuo*. Recrystallization of the residue from water - ethanol gave 0.62 g (35%) of optically inactive alanine.

Found: C, 40.29; H, 7.85; N, 15.64%. Calcd for $C_3H_7O_2N$: C, 40.44; H, 7.92; N, 15.72%.

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