was placed on an IRC-50 (XE-64) column (1.9 × 43.3 cm.). The chromatogram was developed with the same buffer at room temperature with a flow rate of 5.5 ml. per hour. The tubes were analyzed by determination of the absorption at 275 mμ and the Folin–Lowry color reaction.[22] The contents of the tubes of the main peak were pooled and the solution was lyophilized three times to remove the ammonium acetate. From this peak, 38 mg. of 9-sarcosine lysine-vasopressin was obtained (35% based on the weight of material placed on the column). In another chromatogram the recovery in terms of weight was approximately 40%. The pressor activity of the purified 9-sarcosine lysine-vasopressin was 0.4–0.5 unit per mg.

Paper electrophoresis was performed with 0.1 *M* pyridine acetate buffer of *p*H 4.0 at 400 V. on Whatman No. 3 MM paper.[23] 9-Sarcosine lysine-vasopressin traveled as a single spot in the cathode direction at the same rate as lysine-vasopressin. Analytical ion exchange chromatography (5.99 mg., 0.9 × 12.7 cm. column of XE-64, 0.5 *M* ammonium acetate buffer of *p*H 6.38) gave one symmetrical peak. Paper chromatography with the solvent system butanol–acetic acid–water (4:1:5) showed the compound to travel as a single spot with the same R_F value (0.2) as lysine-vasopressin. Amino acid analysis of a hydrolysate on the starch column[24] gave the following amino acid content expressed in molar ratios (with the ratio of phenylalanine arbitrarily taken as 1): phenylalanine 1.0, tyrosine 1.0, proline 0.7, glutamic acid 1.0, aspartic acid + sarcosine 1.9,[25] lysine 0.7, cystine 0.8, ammonia 3.2.

A sample for analysis was dried at 100° for 8 hr. over P_2O_5.

Anal. Calcd. for $C_{47}H_{67}O_{13}N_{12}S_2 \cdot C_2H_4O_2$: C, 52.1; H, 6.33; N, 16.1. Found: C, 52.4; H, 6.32; N, 16.0.

(22) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).

(23) S. P. Taylor, Jr., V. du Vigneaud and H. G. Kunkel, *ibid.*, **205**, 45 (1953).

(24) S. Moore and W. H. Stein, *ibid.*, **178**, 53 (1949).

(25) Aspartic acid and sarcosine emerge from a starch column as one single peak. The color yields of both amino acids are approximately the same.

[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE, NEW YORK 21, N. Y.]

Synthesis of Structures Related to Bacitracin A

BY W. STOFFEL AND L. C. CRAIG

RECEIVED AUGUST 12, 1960

When all the available evidence for the structure of bacitracin A is considered, the most probable one is that shown in Fig. 1.[1–4] It would seem most likely that a linear dodecapeptide is first synthesized by the organism and that two rings are then formed as shown in Fig. 2. The thiazoline ring is now well established,[3–6] but direct evidence for the linkage connecting the aspartyl-β-carboxyl group and the ε-amino-nitrogen of the lysine has not been obtained. However, if our earlier interpretation[7] of peptide 17 is the correct one, it is a tripeptide, His·Asp·Lys. Since a pentapeptide (Asp_2, Lys, Orn, Ileu) also was isolated and since the Lys·Orn and Ileu-α-amino-Lys linkages have been well established, there seems little doubt but that the second aspartic acid is attached to the first. It is the one found by Lockhart and Abraham[8] to be more easily split off on acid hydrolysis and to be dextrorotatory. The reduction experiments of Swallow and Abraham[9] further show that this aspartic acid carries the single amide group of bacitracin on its α-carboxyl.

Recently in this Laboratory[10] partial hydrazinolysis experiments to be reported soon have shown that the larger ring is easily split to liberate one of the amino groups of lysine. Since all of the amino acids originally present in bacitracin A still appear to be connected, the linkage split must be the ε-amino group of lysine known to be attached[8] to an aspartic acid. The β-carboxyl of an aspartic acid might be expected to be more easily split by hydrazine than the other amide linkages in bacitracin.

Interesting as these unusual linkages are they are no more so than the ones directly concerned with the thiazoline ring. Here it seemed wise to confirm certain of the proposed structures by synthesis. This paper will report the synthesis of a number of thiazoline derivatives and the required intermediates for these and still other thiazoline peptides. Since the thiazoline ring system has been postulated to be present in proteins[11] but not proved to be in any protein as yet, it seemed of considerable interest apart from the bacitracin problem to learn more of the requirements as regards substitution which would permit cysteine peptides to cyclize to the thiazoline ring and yet have sufficient stability to remain in this form.

The compounds synthesized are (I) 2-methylthiazoline and its methyl iodide derivative, (II) 2-(1-acetamino-2-methyl-propyl)-thiazoline, (III) ethyl-2-(1-acetamino-2-methyl-propyl)-4-carboxythiazoline, the dipeptides and their derivatives given in Table I and the tripeptides, pentapeptides and their derivatives given in Table II.

In the tables and the Experimental part which follow, the standard amino acid abbreviations are used. Other abbreviations used are: Z = carbobenzoxy, Bz = benzyl, Me = methyl, DCC = dicyclohexylcarbodiimide, Ac = acetyl.

The typical absorption spectrum of the thiazoline ring is well known[1,3] and is shown by bacitracin A in Fig. 3a. The latter has a similar extinction co-

(1) L. C. Craig, Wm. Konigsberg and R. J. Hill, in Ciba Foundation Symposium on Amino Acids and Peptides with Antimetabolic Activity, J. and A. Churchill, Ltd., London, 1958, p. 226.

(2) E. P. Abraham, "Biochemistry of Some Peptides and Steroid Antbiotics," John Wiley and Sons, Inc., New York, N. Y., 1957.

(3) Wm. Konigsberg and L. C. Craig, THIS JOURNAL, **81**, 3452 (1959).

(4) E. P. Abraham and G. G. F. Newton, ref. 1, p. 205.

(5) J. R. Weisiger, W. Hausmann and L. C. Craig, THIS JOURNAL, **77**, 3123 (1955).

(6) I. M. Lockhart, E. P. Abraham and G. G. F. Newton, *Biochem. J.*, **61**, 534 (1955).

(7) W. Hausmann, J. R. Weisiger and L. C. Craig, THIS JOURNAL, **77**, 723 (1955).

(8) I. M. Lockhart and E. P. Abraham, *Biochem. J.*, **62**, 645 (1956).

(9) D. L. Swallow and E. P. Abraham *ibid.*, **72**, 326 (1959).

(10) R. J. Hill, unpublished experiments.

(11) K. Linderstrøm-Lang and C. F. Jacobsen, *J. Biol. Chem.*, **137**, 443 (1941).

TABLE I

DIPEPTIDES AND/OR DERIVATIVES

	Compound	Starting compound	Method	M.p., °C.	Yield, %
IV	Z-ileu-S-Bz-cysOEt(L,L)	Z-ileu(L) + S-Bz-cysOEt(L)	Mix. anhyd.	...	78
V	Z-ileu-S-Bz-cysNHNH$_2$(L,L)	Cmpd. IV	NH$_2$NH$_2$	180	85
VI	Z-ileu-S-Bz-cys(L,L)	Cmpd. IV	Sapon.	189	72
VII	Z-ileu-S-Bz-cysCH$_2$CN(L,L)	Cmpd. VI	ClCH$_2$CN	144	85
VIII	Z-ileu-cys(L,L)	Z-ileu-SC$_6$H$_5$(L) + cys(L)	C$_6$H$_5$SH	158	89
IX	Ileu-cys(L,L)	Cmpd. VI	Na/NH$_3$ (liq.)	164–165	55
X	Z-S-Bz-cys-leuOMe(L,L)	Z-S-Bz-cys(L) + leuOMe(L)	Mix. anhyd.	...	64
XI	S-Bz-cys-leuOMe·HBr	Cmpd. X	HBr–ACOH	...	92
XII	Z-S-Bz-cys-leuNH$_2$(L,L)	Z-S-Bz-cys(L) + leuNH$_2$(L)	Mix. anhyd.	174	66
XIII	S-Bz-cys-leuNH$_2$(L,L)	Cmpd. XII	HBr–ACOH	110–112	70
XIV	Z-(γ-Bz-glu)-ileuOMe(D,L)	Z-γ-Bz-glu(D) + ileuOMe(L)	DCC	123	80
XV	γ-Bz-glu-ileuOMe(D,L)·HBr	Cmpd. XIV	HBr–ACOH	...	90
XVI	Z-ileu-pheOMe(L,D)	Z-ileu(L) + pheOMeHCl(D)	DCC	...	90
XVII	Z-ileu-phe(L,D)	Cmpd. XVI	Sapon.	164–165	95
XVIII	Z-ileu-pheNHNH$_2$(L,D)	Cmpd. XVI	NH$_2$NH$_2$	225	87
XIX	Z-(β-Bz-asp)-β-Bz-aspNH$_2$(L,D)	Z-β-Bz-asp(L) + β-aspNH$_2$(D)·HBr	DCC	131–132	96
XX	β-Bz-asp-β-Bz-aspNH$_2$·HBr(L,D)	Cmpd. XIX	HBr–ACOH	...	86

efficient at 253 mμ in ethanol as the simpler derivatives with the least substituted derivative showing the highest absorption. In strong HCl the absorption band shifts to a longer wave length[8] as is shown in Fig. 3b. In order to show that this shift is due to the quaternization of the ring nitrogen, the N-methyl derivative of 2-methyl-thiazoline was prepared. In ethanol this derivative gave a band at the longer wave length as did the other derivatives in strong HCl.

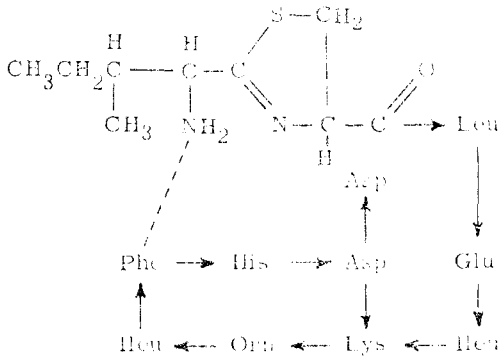


Fig. 1.—Cyclic structure of bacitracin.

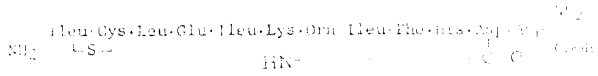


Fig. 2.—Linear formula of bacitracin.

It might be expected that isoleucylcysteine and other peptides containing this N-terminal sequence would cyclize readily to give thiazolines. However, this did not prove to be the case. Reduced glutathione[12] forms the ring to a considerable extent in concentrated HCl, but the ring does not stay closed except in strong acid. None of the cysteine peptides prepared in Tables I and II with the N-terminal amino group free showed any tendency to form the thiazoline ring in concentrated hydrochloric acid. Nor could the ring be induced to form with a variety of reagents such as methanolic HCl, BF_3 and dicyclohexylcarbodiimide.

(12) M. Calvin, "Symposium on Glutathione," Academic Press, Inc., New York, N. Y., 1954, p. 21.

On the other hand, the characteristic thiazoline absorption was readily obtained when the N-terminal amino group was substituted by the acetyl group and the basicity of this nitrogen thus removed.

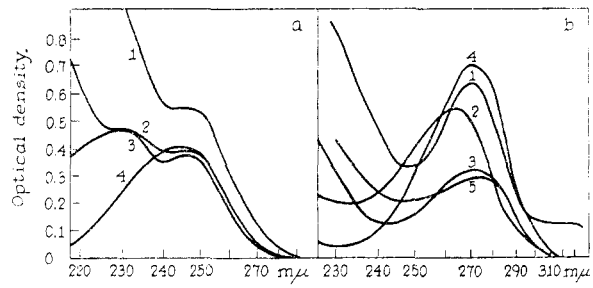
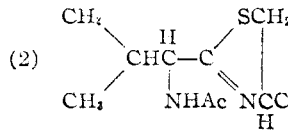
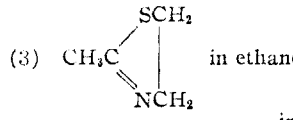


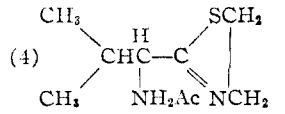
Fig. 3.—Absorption spectrum curves of: (1) Bacitracin A in ethanol, 1a (ϵ 1.45 × 10^{-3}); in concd. HCl, 1b (ϵ 4.9 × 10^3).



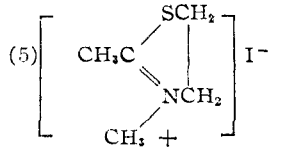
(2) in ethanol, 2a (ϵ 1.5 × 10^3); in concd. HCl, 2b (ϵ 2.4 × 10^3)



(3) in ethanol, 3a (ϵ 4.0 × 10^3); in concd. HCl, 3b (ϵ 6.2 × 10^3).



(4) in ethanol, 4a (ϵ 2.0 × 10^3); in concd. HCl, 4b (ϵ 6.6 × 10^3).



(5) in ethanol, 5b (ϵ 6 × 10^3).

This observation would seem to lend some support to the theory expressed in formula 1 that the terminal isoleucine nitrogen of bacitracin is not

TABLE II

TRIPEPTIDES AND/OR DERIVATIVES

	Compound	Starting compound	Method	M.p., °C.	Yield, %
XXI	Z-ileu-S-Bz-cys-leuOMe(L,L,L)	Cmpd. VI + leuOMe(L)	Mix. anhyd.	...	90
	Z-ileu-S-Bz-cys-leuOMe(L,L,L)	Cmpd. VII + leuOMe(L)	$ClCH_2CN$	...	79
	Z-ileu-S-Bz-cys-leuOMe(L,L,L)	Cmpd. XI + Z-ileu(L)	Mix. anhyd.	...	67
XXII	Z-ileu-S-Bz-cys-leu(L,L,L)	Cmpd. XXI	Sapon.	165	70
XXIII	Z-ileu-S-Bz-cys-leuNH_2(L,L,L)	Cmpd. VI + leuNH_2(L)	DCC	252–253	82
XXIV	Ileu-S-Bz-cys-leuNH_2(L,L,L)	Cmpd. XXIII	HBr–AcOH	176	80
XXV	N-Ac-ileu-S-Bz-cys-leu(L,L,L)	Cmpd. XXII	1 HBr–AcOH	270	80
XXVI	N-Ac-ileu-S-Bz-cys-leuNH_2(L,L,L)	Cmpd. XXIII	2 AcCl–pyr.	278	84
XXVII	Ileu-cys-leu(L,L,L)	Cmpd. XXII	Na/NH_3 (liq.)	145	67
XXVIII	Z-ileu-S-Bz-cys-leu$NHNH_2$(L,L,L)	Cmpd. XXI	NH_2NH_2	228	90
XXIX	Z-ileu-phe-N(im)-Bz-his-OET(L,D,L,)	Cmpd. XVIII and N(im)-Bz-his-OET(L)·2HCl	Azide	136	80
XXX	Z-ileu-phe-N(im)-Bz-his-$NHNH_2$(L,D,L)	Cmpd. XXIX	NH_2NH_2	194	98
	Pentapeptides				
XXXI	Z-ileu-S-Bz-cys-leu-γ-Bz-glu-ileuOMe (L,L,L,L,D)	Cmpd. XXVIII + XV	Azide	152–154	50
XXXII	Z-ileu-S-Bz-cys-leu-β-Bz-asp β-Bz-aspNH_2 (L,L,L,L,D)	Cmpd. XXII + XX	DCC	158	57
XXXIII	N-Ac-ileu-S-Bz-cys-leu-β-Bz-asp-β-aspNH_2 (L,L,L,L,D)	Cmpd. XXV + XX	DCC	...	75

entirely free. Such a possibility seems to be indicated by dinitrophenylation together with subsequent hydrolysis experiments and other data previously presented.[5] Further discussion of this problem will be postponed until it can be supported by the behavior of other synthetic derivatives closely related to bacitracin.

Experimental

The amino acid starting materials were purchased from Mann Research Laboratories Inc., New York 6. In the case of crystalline derivatives melting points (uncorrected) were determined with a micro-hot-stage apparatus. The elementary analyses were made by Mr. D. Rigakos. Ultraviolet spectra were taken with the Cary recording spectrophotometer model 14 PM or the Spectracord UV spectrophotometer model 4000. Optical rotatory dispersions were measured with the Rudolph photoelectric polarimeter model 200.

The purities of the free peptides were checked where possible by paper chromatography and paper electrophoresis. The systems used for the paper chromatography were (1) 2-butanol–3% aqueous ammonia (100:40) and (2) 2-butanol–88% formic acid–water (75:15:10). The paper electrophoresis was performed at a *p*H of 5.6 with a solution containing 0.8% pyridine and 0.2% acetic acid.

2-Methyl thiazoline was synthesized by the method of Kuhn and Drawert.[13] A sample of this base was converted to the quaternary N-methyl iodide by reaction with methyl iodide. After recrystallization from ethanol, it melted at 235° and gave satisfactory analytical figures.

2-(1-Acetamino-2-methyl-propyl)-thiazoline.—The intermediate, α-aminoisovaleronitrile, was prepared by a modified Strecker synthesis.[14] It was acetylated with acetic anhydride in pyridine; 2.6 g. of the oil so obtained was treated with 1.5 g. of cysteamine and 5 ml. of absolute ethanol. The mixture was refluxed until the liberation of ammonia ceased. After evaporation of the ethanol, the solid residue was recrystallized from water; yield 3.1 g.

Anal. Calcd. for $C_9H_{16}N_2OS$: C, 54.0; H, 8.05; N, 14.0. Found: C, 54.0; H, 7.93; N, 13.9.

Ethyl-2-(1-acetamino-2-methyl-propyl)-4-carboxythiazoline.—A solution of 4.0 g. of N-acetyl-α-aminoisovaleronitrile, 1.8 ml. of absolute ethanol and 25 ml. of dry ether was saturated at −5° with dry gaseous HCl. The hygroscopic imino ether (5.7 g.) precipitated immediately. It was suspended with 5.0 g. of L-cysteine ethyl ester hydrochloride in 40 ml. of dry methylene chloride and 3.8 ml. of dry triethylamine added. After stirring at room temperature in an atmosphere of nitrogen for 48 hours the precipitate of triethylammonium chloride was filtered off and washed with ether. The combined filtrates were evaporated *in vacuo* and the residue was taken up in benzene. The solution was refluxed 20 minutes with Norite, filtered and the benzene evaporated. The yield approximated 96% of the theory. Final purification was accomplished by short-path distillation to a cold finger (140° bath temp., 0.1 mm.); $n^{25}D$ 1.4681.

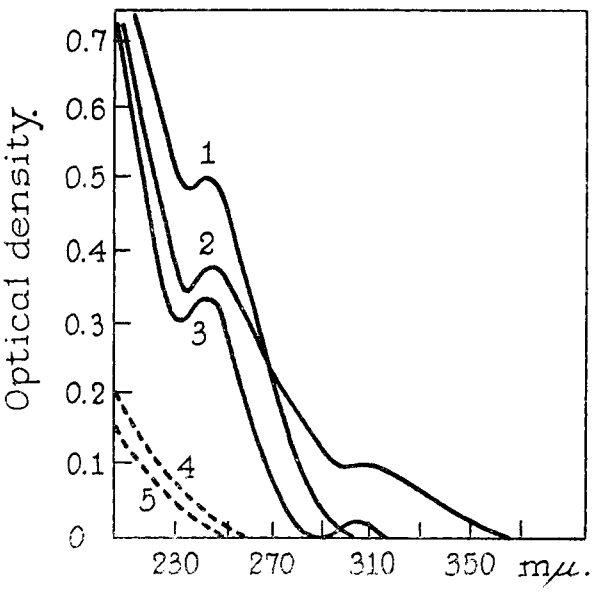


Fig. 4.—Absorption spectrum curves in ethanol of: 1, bacitracin (ϵ 1.45 × 10^3); 2, N-Ac-ileu.cys.leu (after treatment with HCl–MeOH), ϵ 1.2 × 10^3; 3, N-Ac-ileu.cys.leu.-asp.aspNH_2 (after treatment with HCl–MeOH), ϵ 1.5 × 10^3; 4, N-Ac-ileu.cys.leu.asp.aspNH_2; 5, N-Ac-ileu.cys.leu.

Anal. Calcd. for $C_{12}H_{20}N_2O_3S$: C, 52.9; H, 7.35; N, 10.3. Found: C, 52.8; H, 7.31; N, 10.1.

Z-Isoleucyl-S-Bz-cysteine Ethyl Ester (L,L).—Z-L-Isoleucine was prepared according to Bergmann and Zervas.[15]

(13) R. Kuhn and F. Drawert, *Ann.*, **590**, 55 (1954).

(14) R. E. Steiger, *Org. Syntheses*, **22**, 13 (1942).

(15) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

S-Benzyl-L-cysteine was prepared according to Gortner and Hoffmann.[16] The ethyl ester of this was then prepared.[17]

Ethyl chlorocarbonate (2.95 g., 9.5 μmoles) was added dropwise to a well-stirred solution of 2.55 g. (9.5 μmoles) of Z-L-isoleucine and 1.3 ml. of triethylamine in 40 ml. of dry tetrahydrofuran cooled to −15°. After 15 minutes the precipitated triethylamine hydrochloride was filtered off in a precooled flask. To the filtrate was added a solution containing 2.25 g. (9.5 moles) of S-Bz-cysteine ethyl ester hydrochloride in 25 ml. of dry chloroform and 1.3 ml. of triethylamine. After stirring for 15 minutes at 0° and 1 hour at room temperature the solvent was evaporated. The residue was taken up in ethyl acetate and successively washed with 2 *N* HCl, 5% $NaHCO_3$ and water. It was dried over Na_2SO_4. The oil remaining after evaporation of the solvent was not characterized but was used directly to prepare compounds V and VI in Table I.

The hydrazide, compound V of Table I, was prepared by treatment of 2.2 g. of the oil with 3 ml. of hydrazine hydrate in 24 ml. of 95% ethanol at room temperature overnight. It melted at 181°.

Z-Isoleucyl-S-Bz-cysteine (L,L) was prepared from 3.6 g. of the above oil in 30 ml. of dioxane by adding 8 ml. of 1 *N* NaOH with stirring. After an hour water was added and the unsaponified material was extracted with ether. The crystalline precipitate obtained on acidification with 2 *N* HCl at 0° was recrystallized from methanol; yield 3.2 g., m.p. 189°.

Anal. Calcd. for $C_{24}H_{30}N_2O_5S$: C, 62.9; H, 6.58; N, 6.12. Found: C, 62.8; H, 6.35; N, 6.22.

The cyanomethyl ester (VII of Table I) was prepared by treatment of 0.92 g. of the above peptide derivative with a solution containing 0.28 g. of triethylamine and 2.10 g. of chloroacetonitrile for 24 hours at room temperature.

Z-Ileucylcysteine (L,L).—Z-L-Isoleucyl thiophenol ester was prepared according to the general method of Wieland.[18] It melted at 86–87°; 1.3 g. of this ester was dissolved in 10 ml. of pyridine and 0.55 g. of L-cysteine added. After standing overnight the dipeptide was precipitated with 2 *N* HCl and the precipitate taken up in ethyl acetate. It was washed with 2 *N* HCl and water. The solvent was evaporated and the residue dried at 90° and 0.01 mm. pressure. The residue was recrystallized from a mixture of ethanol and water; m.p. 158°.

Anal. Calcd. for $C_{17}H_{24}N_2O_5S$: C, 55.4; H, 6.56; N, 7.60. Found: C, 55.6; H, 6.71; N, 7.65.

Isoleucylcysteine (L,L).—Carbobenzoxyisoleucyl-S-benzylcysteine (3.0 g.) was dissolved in 50 ml. of liquid ammonia. Sodium in small pieces was added, taking care to exclude moisture. The temperature was that of the boiling point of the ammonia. After the blue color had persisted for 20 minutes, sufficient ammonium chloride was added to neutralize the excess of sodium. The ammonia was evaporated in a stream of dry nitrogen. The gray residue was dissolved in 3 *N* acetic acid and the solution was extracted with ether. The dipeptide was precipitated from the aqueous layer with mercuric acetate (20%). The white voluminous precipitate was washed with oxygen-free water three times with centrifuging. It was suspended in 8 ml. of 1 *N* HCl and a rapid flow of H_2S introduced. The precipitate was filtered off with the aid of Celite. The filtrate was lyophilized. The residue, 0.95 g., melted at 165°.

Anal. Calcd. for $C_9H_{19}N_2O_3SCl$: C, 39.9; H, 7.08; N, 10.4. Found: C, 39.7; H, 6.95; N, 10.3.

An alternative way of isolating the peptide following the reduction was by countercurrent distribution. In the system 2-butanol–0.01 *N* HCl at 200 transfers, a well-separated band with a *K* of 0.60 was obtained. Recovery gave material with the correct analysis and melting at 164–165°.

Z-γ-Bz-Glutamyl Isoleucyl Methyl Ester (D,L).—D-Glutamic acid was prepared by enzymatic resolution[19] of D,L-carbobenzoxyglutamic acid with papain. D-Z-γ-Bz-glutamic acid was obtained by partial hydrolysis of the dibenzyl derivative according to the directions of Hanby, Waley and Watson.[19] L-Isoleucine methyl ester hydrochloride was synthesized by the method of Boissonnas, *et al.*[20]

A mixture of 1.8 g. of Z-γ-Bz-D-glutamic acid, 0.90 g. of L-isoleucine methyl ester hydrochloride and 0.70 of triethylamine was dissolved in 25 ml. of dry tetrahydrofuran. Dicyclohexylcarbodiimide, 1.10 g., was added and the mixture stirred for 6 hours at room temperature. Glacial acetic acid, 0.1 ml., was added. After filtering off the precipitated dicyclohexyl urea, the filtrate was evaporated to dryness. The residue, taken up in ethyl acetate, was washed twice with 2 *N* HCl, 5% $NaHCO_3$, then water and dried over $MgSO_4$. The solvent was evaporated and the residue recrystallized from ethanol; yield 2.00 g., m.p. 123°.

Anal. Calcd. for $C_{27}H_{34}N_2O_7$: C, 65.0; H, 6.81; N, 5.62. Found: C, 65.0; H, 6.87; N, 5.58.

Z-(β-Bz-Aspartyl)-β-Bz-asparagine (L,D).—Z-β-Bz-aspartic acid was synthesized by partial saponification of Z-L-aspartic acid dibenzyl ester according to Berger and Katchalski.[21] The benzyl ester of D-asparagine was prepared by hydrolysis of Z-D-asparagine benzyl ester with 2.5 *N* HBr in glacial acetic acid. The carbobenzoxy derivative could be prepared according to the method of Bergmann, *et al.*,[22] or somewhat easier by treatment of the silver salt of Z-D-asparagine with benzyl chloride in tetrahydrofuran.

A mixture of 1.8 g. of Z-β-L-aspartic acid, 1.04 g. of dicyclohexylcarbodiimide and 1.5 g. of D-asparagine benzyl ester hydrobromide was suspended in 50 ml. of dry methylene chloride with 0.7 ml. of triethylamine. After stirring for 6 hours, 0.3 ml. of glacial acetic acid was added and the dicyclohexylurea filtered off. The solvent was evaporated and the residue was taken up in ethyl acetate, washed successively with 2 *N* HCl, 5% $NaHCO_3$ and water. It was dried over Na_2SO_4 and evaporated to dryness. The residue, 2.2 g., was recrystallized from ethanol; m.p. 131–132°.

Anal. Calcd. for $C_{30}H_{31}N_3O_8$: C, 64.2; H, 5.57; N, 7.48. Found: C, 64.4; H, 5.84; N, 7.75.

Z-Isoleucyl-S-Bz-cysteinylleucine (L,L,L).—Ethyl chlorocarbonate, 50 ml., was added to a mixture of 2.5 g. of Z-isoleucyl-S-Bz-cysteine (L,L), and 0.76 ml. of dry triethylamine dissolved in 25 ml. of dry tetrahydrofuran with vigorous stirring. It was cooled to −10° and after 30 minutes the precipitated triethylamine hydrochloride was filtered off. A solution of 0.80 g. of L-leucine methyl ester in 25 ml. of tetrahydrofuran was added to the filtrate and stirring continued for 2 hours at room temperature. The solvent was evaporated *in vacuo* and the residue was taken up in ethyl acetate. The solution was washed successively with 2 *N* HCl, 5% $NaHCO_3$ and water. After drying over Na_2SO_4 the solvent was evaporated. The oily residue was saponified at room temperature in dioxane with 4.0 ml. of 2 *N* NaOH. After 1 hour 25 ml. of water was added and the dioxane evaporated *in vacuo*. The unsaponified material was extracted with ether. Acidification of the aqueous layer with 2 *N* HCl gave 2.0 g. of the substituted tripeptide. It was recrystallized from 95% ethanol; m.p. 165°, $[\alpha]^{25}D$ −94° (*c* 1.628% in pyridine). The rotatory dispersion was normal.

Anal. Calcd. for $C_{30}H_{41}N_3O_5S$: C, 63.0; H, 7.23; N, 7.35. Found: C, 63.0; H, 6.85; N, 7.25.

Isoleucylcysteinylleucine hydrochloride (L,L,L) was prepared by reduction of the above derivative with sodium and liquid ammonia and isolation of the product through the mercaptide as described under the preparation of isoleucylcysteine. It gave a strong nitroprusside reaction and melted at 145°.

Anal. Calcd. for $C_{15}H_{30}N_3O_4SCl$: C, 46.9; H, 7.82; N, 10.8. Found: C, 46.8; H, 7.84; N, 10.8.

N-Acetylisoleucyl-S-Bz-cysteinylleucine Amide (L,L,L).—Z-Isoleucyl-S-Bz-cysteinylleucine amide was prepared by condensing Z-isoleucyl-S-Bz-cysteine (L,L) and L-leucine amide with the dicyclohexylcarbodiimide reagent. The carbobenzoxy protecting group was removed with HBr–acetic acid at room temperature (2 *N* HBr in 32% aqueous acetic acid). The product was acetylated with acetyl chloride in pyridine and precipitated from the pyridine solution with 4 *N* HCl. The precipitate was recrystallized from a mixture of ethanol and ethyl acetate; m.p. 278°.

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Anal. Calcd. for $C_{24}H_{38}N_4O_4S$: N, 11.7. Found: N, 11.9.

Reduction and Cyclization of N-Acetyl-isoleucyl-S-Bz-cysteinylleucine.—This compound was prepared from the corresponding carbobenzoxy derivative in the same way as the amide above. A sample, 380 mg., was reduced with sodium and liquid ammonia as described for the isoleucyl-cysteine derivative. The residue was dissolved in acetate buffer at *p*H 4.6, the toluene extracted with ethyl ether and the tripeptide precipitated with *p*-chloromercuribenzoate. The precipitate was collected by centrifuge and washed four times with oxygen-free water. It was suspended in 0.1 *N* HCl and treated with H_2S. The sulfide precipitate was filtered and the filtrate lyophilized. The crystalline residue was dissolved in dry methanol and dry HCl introduced at −20°. The product showed the characteristic absorption of the thiazoline ring as shown in Fig. 4.

Z-Isoleucyl-S-Bz-cysteinylleucyl-β-Bz-aspartyl-β-Bz-asparagine (L,L,L,L,D).—A sample of Z-isoleucyl-S-Bz-cysteinylleucine weighing 0.900 g. was dissolved in 50 ml. of dry methylene chloride, treated with 0.33 g. of dicyclohexylcarbodiimide and 0.80 g. of β-Bz-aspartyl-β-Bz-asparagine hydrobromide. The mixture was cooled to −5° and 0.25 ml. of triethylamine added slowly with stirring. Stirring was continued 48 hr. at +5°.

After adding 0.1 ml. of glacial acetic acid and stirring for 1 hour, the dicyclohexylurea was filtered off and the filtrate evaporated to dryness. The residue was taken up in ethyl acetate and washed successively with 2 *N* HCl, 5% $NaHCO_3$ and water. The solvent was evaporated and the residue purified by a 50-transfer countercurrent distribution in a system made from chloroform, benzene, methanol and 0.1 *N* aqueous acetic acid (20:10:28:7 volume proportions). A central cut of the main band, tubes 7–16, was taken for recovery. This gave 0.89 g. of the pentapeptide with a melting point of 158–160°.

Anal. Calcd. for $C_{52}H_{64}N_6O_{11}S$: C, 63.7; H, 6.58; N, 8.59. Found: C, 64.1; H, 6.74; N, 8.35.

The acetyl derivative was synthesized by the same procedure except that the acetyl tripeptide was used as starting material in place of the carbobenzoxy derivative. The pentapeptide derivative was reduced with sodium and liquid ammonia in the same way as was the isoleucylcysteinyl-leucine derivative and converted to the thiazoline with methanolic HCl. The product gave the characteristic absorption spectra shown in Fig. 4.

Z-Isoleucylphenylalanyl-N(im)-Bz-histidine Hydrazide (L,D,L).—Z-Isoleucylphenylalanine methyl ester (L,D) was prepared from Z-isoleucine and phenylalanine by the dicyclohexylcarbodiimide method. The hydrazide crystallized from a solution containing 3.5 g. of the ester and 4.0 ml. of hydrazine hydrate in 50 ml. of ethanol after refluxing for 1 hour. The crystals weighed 2.95 g. and melted at 225° after recrystallization from ethanol.

Anal. Calcd. for $C_{28}H_{30}N_4O_4$: C, 64.8; H, 7.09; N, 13.1. Found: C, 65.0; H, 7.22; N, 12.8.

N(im)-Bz-histidine was prepared according to the method of du Vigneaud and Behrens.[23] The yield was 85% of the theoretical. It melted at 247°. The ethyl ester hydrochloride was prepared by treating the carbobenzoxy derivative, 10.0 g., suspended in 50 ml. of dry ethanol with dry gaseous HCl. The flask was cooled in ice. After 4 hours the ethanol was evaporated and again treated with ethanol and dry HCl. The solution was concentrated to 10 ml. and the hydrochloride precipitated with dry ethyl ether; 12.2 g. of material melting at 128° was obtained after drying over P_2O_5 and recrystallizing from ethanol–ether.

Anal. Calcd. for $C_{15}H_{21}N_3O_2Cl_2$: C, 52.0; H, 6.11; N, 12.1. Found: C, 52.2; H, 6.26; N, 12.0.

The isoleucylphenylalanyl hydrazide derivative (1.41 g.) above was converted to the azide by treatment with 0.55 g. of sodium nitrite in 4 ml. of water, 30 ml. of glacial acetic acid and 5 ml. of 2 *N* HCl at 0–5°. This gave 1.3 g. of the azide which was condensed with 1.10 g. of the histidine derivative as follows. A mixture of 25 ml. of dimethylformamide and 0.70 ml. of triethylamine was stirred over a period of 36 hours at 5°. The tripeptide ester was dissolved in ethyl acetate, washed with 2 *N* HCl, 5% $NaHCO_3$ and water. It was dried over Na_2SO_4. After recrystallization from ethanol, 1.58 g. was obtained which melted at 136°.

The ester was converted to the hydrazide by dissolving it in 25 ml. of ethanol containing 2 ml. of hydrazine hydrate and allowing the solution to stand at 25° overnight. The product weighed 1.3 g. and melted at 194° after recrystallizing from ethanol.

Anal. Calcd. for $C_{36}H_{43}N_7O_5$: C, 66.1; H, 6.64; N, 15.0. Found: C, 65.9; H, 6.70; N, 14.9.

Summary

A number of cysteine peptide derivatives similar to the N-terminal part of bacitracin A have been synthesized. It was found that the thiazoline ring would not form by loss of water unless the basic nature of the terminal amino group was repressed by acetylation.

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