their generosity in supplying us with the material this study was based on, and for making available to us serological data which were of great value in selecting the particular patient. We also wish to thank Drs. W. Yount and R. Carey of Boston, and Dr. R. D. Levere, New York City, for their kind cooperation in following other patients and supplying us with material. We are grateful to Mrs. Penny Ryan, Mr. Connie Mahoney, and Mr. Gerhard Bitterlich for their expert technical assistance.

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The Chemistry of Tyrocidine. VI. The Amino Acid Sequence of Tyrocidine C*

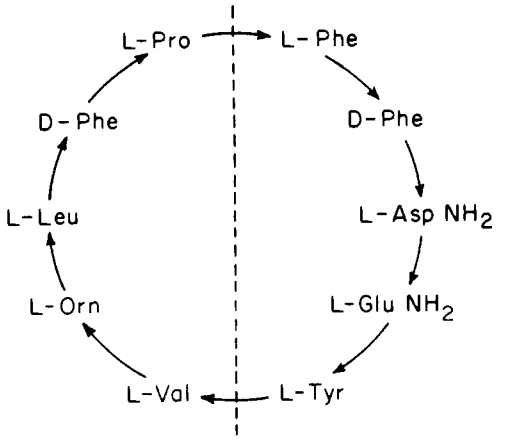
Michael A. Ruttenberg, Te Piao King, and Lyman C. Craig

ABSTRACT: A third major component of the antibiotic cyclic decapeptide series of tyrocidine, designated tyrocidine C, has been isolated by countercurrent distribution and its amino acid composition has been determined. A novel peptide cleavage reaction employing $LiAlH_4$ resulted in a single split at the acyl-proline linkage and thereby converted the peptide from a cyclic to a linear structure. This linear structure, in contrast to its cyclic precursor, could be digested by pepsin, and it was subsequently degraded in a manner which permitted the following unique sequence to be assigned:

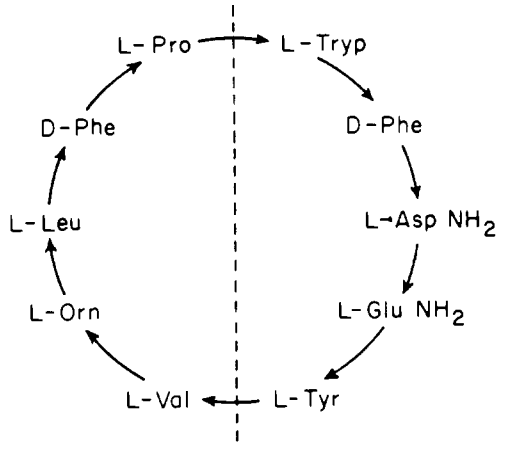
┌L-prolyl-L-tryptophanyl-D-tryptophanyl-L-
| asparaginyl-L-glutaminyl-L-tyrosyl-L-valyl-
| L-ornithyl-L-leucyl-D-phenylalanyl-┐
└──────────────────────────────────┘

The discovery more than 25 years ago of the antibiotic polypeptides produced by *Bacillus brevis* (Dubos, 1939; Hotchkiss and Dubos, 1940) was an event of considerable interest not only because of the therapeutic potential of such substances but because at the time it was thought they would serve as readily available "pure" models to develop methods for the study of proteins. However, with the introduction of countercurrent distribution it was found that the two polypeptide fractions, gramicidin (Craig *et al.*, 1950) and tyrocidine (Battersby and Craig, 1952a), were each mixtures of three or more closely related polypeptides differing in their amino acid composition.

* From the Laboratories of the Rockefeller Institute, New York City. *Received September 30, 1964.* This study was made in partial fulfillment for the Ph.D. degree by Michael A. Ruttenberg. It was partially supported by a research grant (AM 02493-06) from the U.S. Public Health Service.



Amino acid sequence of Tyrocidine A



Amino acid sequence of Tyrocidine B

FIGURE 1: (A) Amino acid sequence of tyrocidine A. The arrows indicate a C-to-N linkage. The dotted line indicates the pentapeptide Val-Orn-Leu-Phe-Pro, which is also present in gramicidin S (see Discussion). (B) Amino acid sequence of tyrocidine B.

Other classes of these polypeptides from different organisms studied by countercurrent distribution were also found to be mixtures (Craig, 1956); the individual members in each class usually contained a characteristic number of amino acid residues but differed by specific amino acid replacements. This experience suggested that such heterogeneity might be expected in the larger proteins provided sufficiently selective analytical and separation methods were developed. Since that time greatly refined methods have been developed but have failed to reveal the expected heterogeneity. From this experience a more selective biosynthetic mechanism for the proteins, perhaps a different one, seems to be implied. More recent study (Mach, 1963) by incorporation experiments has indicated that this is correct.

Considerable insight into the mechanism of the biosynthesis of proteins has been gained in the past few years. With this as a background the question of the way the antibiotic polypeptides are synthesized becomes of greater scientific interest. Obviously for this type of investigation it will be helpful to know the precise structures and differences between each member of a class.

The composition and amino acid sequences of tyrocidine A (Battersby and Craig, 1952a,b; Paladini and Craig, 1954) and tyrocidine B (King and Craig, 1955a,b) have previously been established in this laboratory (Figure 1) but a third component designated tyrocidine C has not been so well characterized. It is the purpose of this paper to report the purification and characterization of tyrocidine C. For the structural study, use has been made of the previously observed specific reductive cleavage of acyl-proline bonds by $LiAlH_4$ (Ruttenberg *et al.*, 1964).

Experimental

Materials. The tyrocidine C was separated by countercurrent distribution from crude tyrocidine, Wallerstein no. ON 13554. We wish to thank the Wallerstein Company for a gift of the sample. All solvents used were redistilled. The $LiAlH_4$ was prepared as described previously (Ruttenberg *et al.*, 1964). Pepsin and carboxypeptidase A were obtained from Worthington Biochemical Corp.

Methods. The procedure for acid hydrolysis was that described previously (Ruttenberg *et al.*, 1964). Amino acid analyses were performed on a Spinco Model 120 amino acid analyzer by the method of Spackman *et al.* (1958), using loads of approximately 0.3 μmole of each constituent amino acid. Tryptophan was determined both by amino acid analysis and spectrophotometric assay using the experimentally determined absorbance for tyrocidine C in H_2O at 280 mμ of 1.21×10^4. Phenylalaninol was measured on the amino acid analyzer as described by Ruttenberg *et al.* (1964). Edman degradation was performed according to the modified procedure of Konigsberg and Hill (1962). Carboxypeptidase digestion was performed as described by Guidotti (1960). The synthesis and chromatography of the dinitrophenyl (DNP) derivatives was according to the method of Fraenkel-Conrat *et al.* (1955). The rotations of the DNP-amino acids were measured on a Schmidt and Haensch polarimeter using the D line of sodium and their concentrations were determined spectrophotometrically at 350 mμ. The values used as standards for the specific rotations and extinctions were those reported by Rao and Sober (1954).

The isolation of the free amino acids for measurement of optical rotation was performed on columns of Aminex Q-15 and Q-50 using volatile buffers of pyridine formate as described by Eaker (1962) with *p*H and ionic strength the same as the citrate buffers used with the Spinco amino acid analyzer. The rotations were measured at 435 mμ on a Rudolph Model 80 spectropolarimeter using a mercury arc source; the concentrations were determined by chromatography

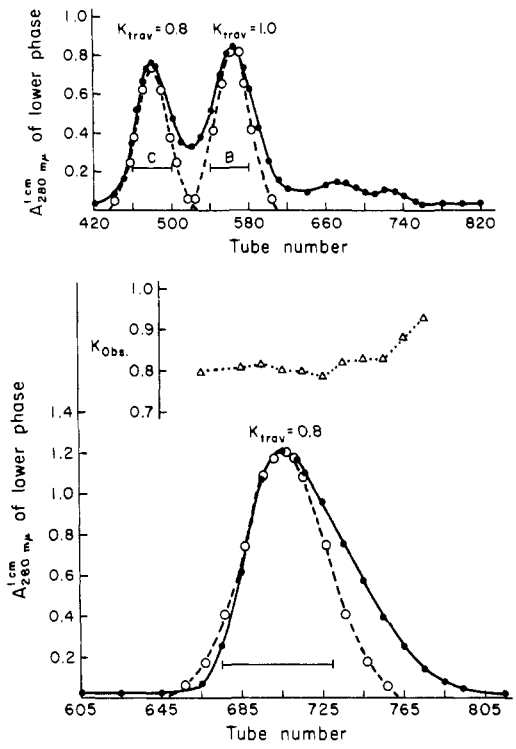


FIGURE 2: (A) Countercurrent distribution of 5 g of crude tyrocidine in the system acetic acid–water–2-butanol–cyclohexane–pyridine (3:7:6:4:0.2). The analysis was made after recycling to 1124 transfers in a 420-tube automatic machine employing 10 ml of each phase. (B) Redistribution of the tyrocidine C from (A). The analysis is after 1600 transfers in a 1000-tube automatic machine employing 3 ml of each phase. The same system as in (A) was used. O-------O, theoretical Gaussian distribution; ●——●, experimental points.

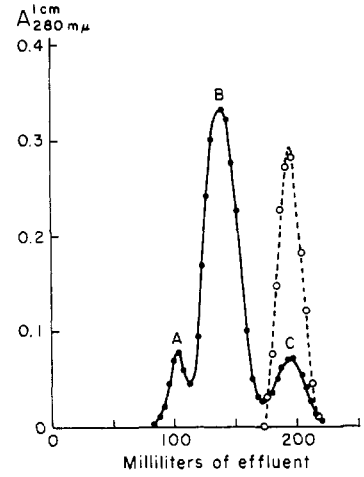


FIGURE 3: Sephadex chromatography of 4 mg of a mixture of tyrocidines A, B, and C, ●——●, and of 1 mg of tyrocidine C isolated from the countercurrent distribution of Figure 2B, O-------O. A 0.9 × 150-cm column of Sephadex G-25 was employed. The tyrocidine was eluted with 10% acetic acid (v/v) at a flow rate of 10 ml/hour; 2-ml fractions were collected.

of samples on the amino acid analyzer. The values for specific rotations used as standards were those listed by Greenstein and Winitz (1961).

Purification of Tyrocidine C. Five g of crude tyrocidine was purified by countercurrent distribution in a system consisting of acetic acid–water–2-butanol–cyclohexane–pyridine (3:7:6:4:0.2). This system has an advantage over the chloroform–methanol–0.1 N HCl system previously employed (Battersby and Craig, 1952a) in that no esterification of the peptide can take place. The disadvantage of this system is its slow settling time (3 minutes, as compared to 40 seconds for the chloroform system). Analysis by absorbance at 280 mμ after recycling to 1124 transfers in a 420-tube machine employing 10 ml of each phase gave the pattern in Figure 2A. The positions of tyrocidines B and C are clear but the position of tyrocidine A is not discernible because of its low absorbance at this wavelength.

The tyrocidine C was further purified by redistribution in the same solvent system. Analysis after 1600 transfers gave the pattern in Figure 2B. The purity of this redistributed tyrocidine C was checked by chromatography on Sephadex G-25 in 10% acetic acid (Figure 3). This technique may be used also to fractionate the tyrocidines on an analytical scale as shown by the pattern in Figure 3 given by a 4-mg mixture of the three tyrocidines. The fact that Sephadex chromatography gave a single peak indicated that the tyrocidine C sample was probably pure. A portion was submitted to amino acid analysis, the results of which are given in Table I. The amino acids listed accounted for 98% of the total nitrogen and 99% of the weight of the sample. This gave further support to the thesis of purity.

A sample of the tyrocidine C was treated with an excess of fluorodinitrobenzene (FDNB) and subsequently hydrolyzed for 24 hours with 6 N HCl in an evacuated sealed tube which had been flushed with nitrogen. The hydrolysate was diluted with water and extracted with ether. The ether extract was subjected to paper chromatography in the toluene system as described by Fraenkel-Conrat *et al.* (1955). The only DNP-amino acids detected were δ-DNP-ornithine and *O*-DNP-tyrosine. This indicated the absence of an amino-terminal residue.

Cleavage of Tyrocidine C by $LiAlH_4$. The cleavage of tyrocidine C with $LiAlH_4$ was performed on 100 mg of peptide using a 10-fold molar excess of 2.3 M $LiAlH_4$ in tetrahydrofuran. The details of the cleavage reaction have been described previously (Ruttenberg *et al.*, 1964). Tetrahydrofuran (200 ml) was used as solvent. The reaction was allowed to proceed for 1 hour at 0°. At the end of this time the reduction was stopped with 3 ml of 6 N HCl. The solution was brought to dryness in a rotary evaporator, and the residue was dissolved in 10 ml of 50% acetic acid and desalted on a 2.5 × 200-cm column of Sephadex G-25. The desalted material was lyophilized and the last traces of excess

TABLE I: Amino Acid Analysis of Tyrocidine C Isolated by Countercurrent Distribution.

Amino Acid	Mole Ratio in Tyrocidine C before $LiAlH_4$ Reduction[a]	Nearest Whole Number	Mole Ratio in Tyrocidine C after $LiAlH_4$ Reduction[b]	Nearest Whole Number
Aspartic acid	0.98	1	0.99	1
Glutamic acid	1.05	1	1.01	1
Proline	1.12	1	1.03	1
Valine	1.02	1	1.00	1
Leucine	1.00	1	1.00	1
Tyrosine	1.00	1	1.02	1
Phenylalanine	1.03	1	0.06	0
Tryptophan[c]	1.96	2	1.89	2
Ornithine	1.05	1	1.01	1
Ammonia	2.29	2	2.05	2
Phenylalaninol	0.00	0	0.91	1

[a] Material obtained from countercurrent distribution (Figure 2B). The sample contained 15.50% nitrogen. [b] Material obtained after treatment with $NaBH_4$ and chromatography on phosphocellulose (Figure 4B). [c] Also determined spectrophotometrically.

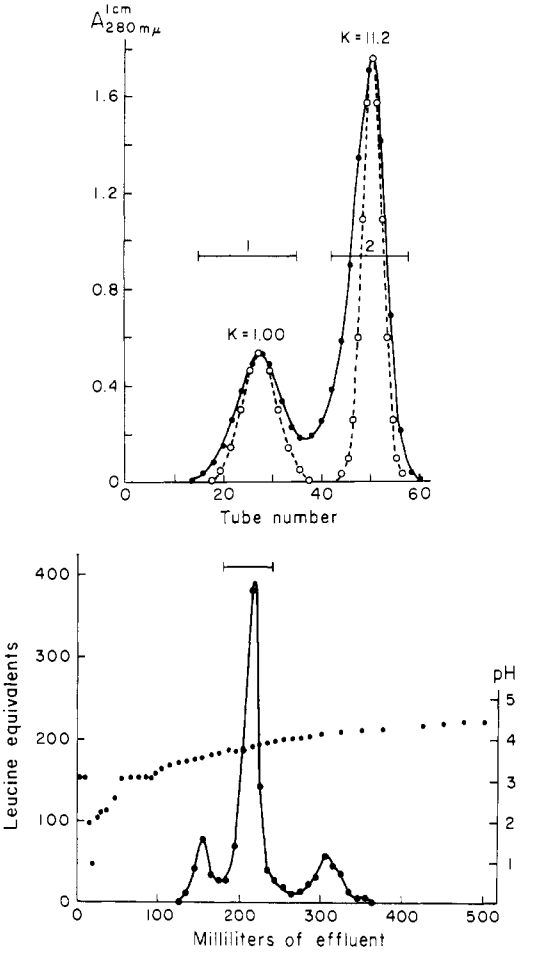


FIGURE 4: (A) Countercurrent distribution of the $LiAlH_4$ reduction product of 100 mg of tyrocidine C after subsequent treatment with $NaBH_4$. The system consisted of chloroform–methanol–0.1 N HCl (2:2:1). Analysis after 55 transfers in a 60-tube machine employing 3 ml of each phase. O-------O, theoretical Gaussian distribution; ●——●, experimental points. (B) Phosphocellulose chromatography of the material from peak 2 of the countercurrent distribution of reduced tyrocidine C (4A). The material was eluted with a linear gradient of pyridine formate buffer: First buffer, 400 ml of 0.1 M pyridine formate, *p*H 3.00, second buffer, 400 ml of 2 M pyridine formate, *p*H, 4.50. The sample was applied in 10 ml of 50% formic acid.

acetic acid were removed by relyophilization from water. The peptide was then dissolved in 50% ethanol and the solution was added to a 10-fold excess of $NaBH_4$. The reduction was allowed to proceed for 2 hours at 25°. At the end of this time the reaction was stopped with 50% acetic acid and the material was brought to dryness in a rotary evaporator. The dry residue was dissolved in 50% acetic acid and rechromatographed on Sephadex G-25 to remove the salts derived from the $NaBH_4$. The desalted peptide was then subjected to countercurrent distribution in the system chloroform–methanol–0.1 N HCl. Analysis after fifty-five transfers gave the pattern in Figure 4A. The first peak contained intact tyrocidine C which when treated with $LiAlH_4$ behaved identically with the original starting material. Peak 2 contained the crude cleaved peptide in approximately 50% yield. This material was applied in 10 ml of 50% formic acid to a 0.9 × 20-cm column of phosphocellulose (Whatman, Ltd.) and was eluted with a linear gradient of pyridine-formate buffer (Figure 4B). The main peak contained 42 mg of material (42% of the starting material). Amino acid analysis of this peak gave the values shown in Table I. The fact that a single cleavage yielded a single linear decapeptide proved conclusively the cyclic nature of tyrocidine C.

The main peak material from the phosphocellulose chromatography was dissolved in 1 ml of 50% ethanol. Four ml of 0.01 N HCl was added together with 1 mg of pepsin. The peptic digestion was allowed to proceed for 16 hours at 37°. At the end of this time, the material was lyophilized and the dried product was dissolved in 2 ml of 50% acetic acid for chromatography on a 0.9 × 150-cm column of Sephadex G-25. The pattern obtained is shown in Figure 5A. Cut B con-

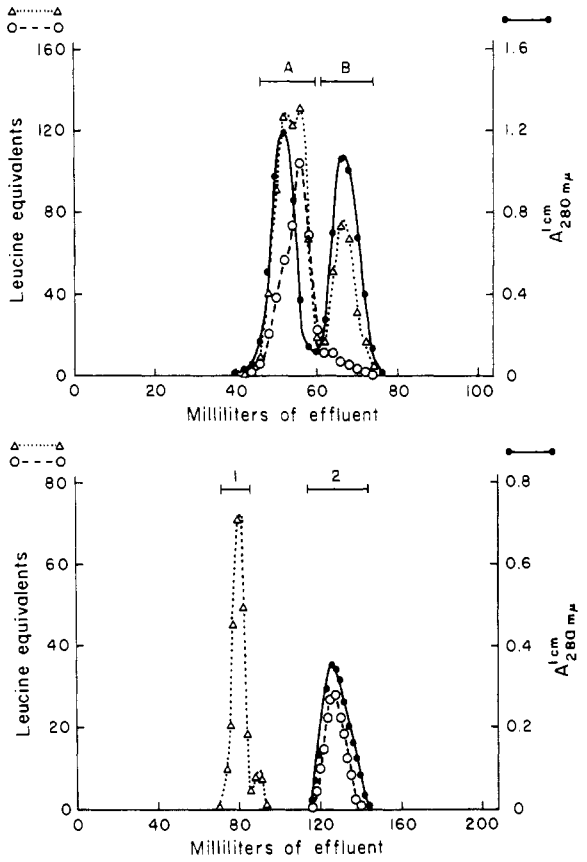


FIGURE 5: (A) Sephadex chromatography of a 16-hour peptic digest of reduced tyrocidine C. The material was eluted with 50% acetic acid from a 0.9 × 150-cm column of Sephadex G-25. Two ml fractions were collected at a flow rate of 6 ml/hour. ●——●, $A^{1\,cm}_{280\,m\mu}$; ○-------○, leucine equivalents before alkaline hydrolysis; Δ-------Δ, leucine equivalents following alkaline hydrolysis. (B) Rechromatography of the material from peak A of (A). The material was eluted with 10% acetic acid from a 0.9 × 150-cm column of Sephadex G-25. Two-ml fractions were collected at a flow rate of 6 ml/hour. ●——●, $A^{1cm}_{280m\mu}$; ○-------○, Δ——Δ, leucine equivalents.

tained a hexapeptide, apparently pure and subsequently shown to be Pro-Try-Try-AspNH_2-GluNH_2-Tyr. Cut A was shown by comparison of absorbancy at 280 mμ and ninhydrin analysis to be heterogeneous. Rechromatography on a 0.9 × 150-cm column of Sephadex G-25 equilibrated with 10% acetic acid gave the pattern in Figure 5B. Cut 1 of this chromatogram had no absorbance at 280 mμ and was subsequently shown to be the tetrapeptide Val-Orn-Leu-phenylalaninol; Cut 2 was found to be undigested peptide which was subsequently digested by retreatment with pepsin; the overall recovery of peptic digest was then quantitative. The results of the various degradative procedures used to determine the sequence of the hexapeptide and the tetrapeptide are given and are represented schematically in Figure 6.

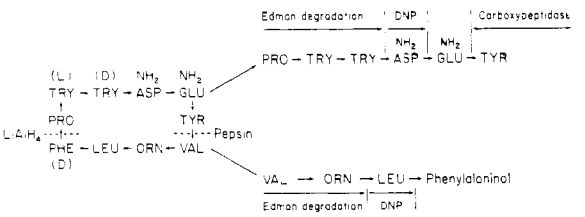


FIGURE 6: Schematic representation of the steps employed in the determination of the amino acid sequence of tyrocidine C.

Determination of the Amino Acid Sequence of the Hexa- and Tetrapeptides. In the outline of the degradative steps given, the amino acid compositions for each step are expressed as mole ratios. The peptides were not purified after each step except as indicated in the footnote. The yields in parentheses were calculated from one step to the next and are not cumulative. The N-terminal amino acid indicated by the analysis is italicized.

HEXAPEPTIDE. Digestion with carboxypeptidase A for 24 hours gave only Tyr, 0.96. Three stages of the Edman degradation were performed on the hexapeptide to yield a tripeptide which was subsequently treated with FDNB.

Intact peptide:	Pro, 0.97; Try, 1.96, Asp, 1.00; Glu, 1.02; Tyr, 1.00; NH_3, 2.08
Edman degradation, stage 1 (71%):[1]	*Pro, 0.29;* Try, 1.98; Asp, 0.98; Glu, 1.00; Tyr, 0.99
Edman degradation, stage 2 (80%):	Pro, 0.00; *Try, 1.23;* Asp, 1.00; Glu, 1.00; Tyr, 0.97
Edman degradation, stage 3 (85%):	Pro, 0.00; *Try, 0.55;* Asp, 1.01; Glu, 1.00; Tyr, 1.00
Treatment with FDNB (90%):	Pro, 0.00; Try, 0.00; *Asp, 0.58;* Glu, 1.00

The presence of 2 moles of ammonia in the analysis of the intact peptide indicated that aspartic and glutamic acids were present as asparagine and glutamine. The foregoing data established the sequence, Pro-Try-Try-AspNH_2-GluNH_2-Tyr.

TETRAPEPTIDE. Two stages of the Edman degradation were performed to yield a dipeptide. The removal of ornithine in the second stage could not be detected by the subtractive technique since it had been converted in the first stage to the phenylthiocarbamyl derivative. For this reason it was not possible to estimate the yield in the second stage; therefore the resulting dipeptide was treated with FDNB. The nearly

[1] This peptide was purified by chromatography on Sephadex G-25 in 10% acetic acid prior to the second stage of the Edman degradation.

TABLE II: Specific Rotations for DNP Derivatives of the Aromatic Amino Acids from Tyrocidine B and C.

DNP-Amino Acid	Tyrocidine B				Tyrocidine C			
	No. of Residues	Configuration	Reported α_D	Found α_D	No. of Residues	Configuration	Reported α_D	Found α_D
Tyrosine (*O,N*-di)	1	L	−11.7°	−10 ± 3°	1	L	−11.7°	−14 ± 3°
Phenylalanine	2	D	+103°	+72 ± 7°	1	D	+103°	+88 ± 8°
Tryptophan	1	L	−181°	−161 ± 1°	2	L+D	0°	−2 ± 2°
Tryptophan in position adjacent to proline in intact tyrocidine C:						L	−181°	−117 ± 12°
Tryptophan in position adjacent to asparagine in intact tyrocidine C:						D	+181°	+71 ± 14°

quantitative disappearance of leucine established the sequence, Val-Orn-Leu-phenylalaninol.

Intact peptide:	Val, 1.02; Orn, 1.01; Leu, 1.00; phenylalaninol, 0.91
Edman degradation, stage 1 (90%):	*Val, 0.07;* Leu, 1.00; phenylalaninol, 0.85
Edman degradation, stage 2:	Val, 0.00; Leu, 1.00; phenylalaninol, 0.88
Treatment with FDNB:	Val, 0.00; *Leu, 0.21;* phenylalaninol, 0.85

Determination of Optical Configuration of the Tryptophans in Tyrocidine C. As a preliminary step in determining the optical configuration of the tryptophans in tyrocidine C, the acid hydrolysate of 12 μmoles of tyrocidine C was chromatographed on a 0.9 × 150-cm column of Sephadex G-25 in 0.2 M acetic acid (Figure 7). The tryptophan, tyrosine, and phenylalanine were separated because of differences in aromatic character (Porath, 1962); each was isolated and treated with FDNB. For comparison a similar procedure was applied to tyrocidine B. The specific rotations determined on these compounds are given in Table II. After it had been shown by this method that tyrocidine C contained D- and L-tryptophan, it was necessary to determine their position in the peptide. This was accomplished by performing a single Edman degradation on the hexapeptide in order to make the tryptophan adjacent to proline amino terminal. Reaction of the resultant pentapeptide with FDNB succeeded in labeling this tryptophan with a DNP group and acid hydrolysis of the DNP peptide followed by ether extraction made possible the isolation of this DNP-tryptophan. The amino acids remaining in the aqueous phase were chromatographed on Sephadex G-25 in 0.2 M acetic acid. The free tryptophan isolated by this method was the one which had been adjacent to the asparagine in the intact tyrocidine C. It was treated with FDNB and the specific rotation of the DNP-tryptophan was determined. A summary of the results of these rotation studies is given in Table II.

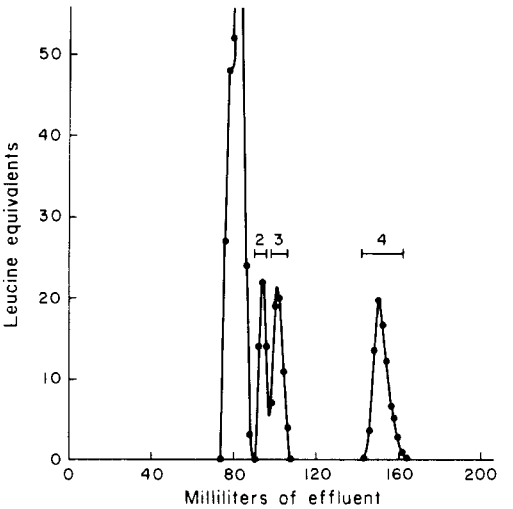


FIGURE 7: Sephadex chromatography of an acid hydrolysate of tyrocidine C. The amino acids were eluted with 0.2 M acetic acid from a 0.9 × 150-cm column of Sephadex G-25. Two-ml fractions were collected at a flow rate of 10 ml/hour. Peak 2 was phenylalanine, peak 3 was tyrosine, and peak 4 was tryptophan. The first peak contained all the nonaromatic amino acids plus ammonia.

In order to determine the optical rotation of the remaining amino acids, the hydrolysate of 77 mg of tyrocidine was chromatographed on columns of Aminex Q-15 and Q-50 ion-exchange resins using volatile buffers of pyridine-formate. The amino acids emerged in the same order as on the amino acid analyzer employing citrate buffers, with the exception of proline, which emerged between aspartic and glutamic acid. The free amino acids were isolated and each was dissolved in 1 ml of 5 N HCl. The concentrations were determined by chromatography of samples on the automatic amino acid analyzer. The rotations were determined at 435 mμ using a mercury arc light source. The results are given in Table III.

TABLE III: Specific Rotations for the Nonaromatic Amino Acids from Tyrocidine C.[a]

Amino Acid	Amount (μmoles/ml)[b]	Reported $\alpha_{435m\mu}$[c]	Found $\alpha_{435m\mu}$
L-Aspartic acid	7.9	+52.0°	+22 ± 5°
L-Glutamic acid	32.3	+67.9°	+37 ± 1°
L-Leucine	38.2	+37.5°	+29 ± 1°
L-Ornithine	30.5	+59.2°	+41 ± 1°
L-Proline	29.3	−105.0°	−65 ± 2°
L-Valine	33.0	+60.3°	+56 ± 1°

[a] All readings were made at 20° in 5 N HCl using a 1-dm polarimeter cell of 0.6-ml capacity. The instrument used was a Rudolph Model 80 spectropolarimeter employing a mercury arc light source. [b] Determined by chromatography of a sample on the Spinco amino acid analyzer. [c] From Greenstein and Winitz (1961).

Discussion

Treatment of tyrocidine C with $LiAlH_4$ under mild conditions has caused a specific cleavage to occur at the acyl-proline linkage, thereby converting the cyclic structure to a linear peptide. This linear peptide, in contrast to its cyclic precursor, could be digested with pepsin which gave a single split to yield a hexapeptide and a tetrapeptide. Determination of the amino acid sequence was then a straightforward application of well-established N-terminal and C-terminal degradative techniques. The data obtained as reported in the Experimental section are sufficient to assign a unique amino acid sequence for tyrocidine C as shown in Figure 6.

The $LiAlH_4$ cleavage generated amino-terminal proline and converted the adjacent phenylalanine to a residue of 2-amino-3-phenylpropionaldehyde. In order to manipulate this linear peptide without the danger of condensation reaction occurring at the aldehyde group, it was desirable to convert it to a less reactive species. This was the purpose of the subsequent reduction with $NaBH_4$, which converted the aldehyde to a residue of phenylalaninol.

It is interesting to note that carboxypeptidase digestion of the hexapeptide did not go beyond the first residue. Normally one would expect carboxypeptidase A to hydrolyze carboxyl-terminal glutamine. A possible explanation for the failure to observe any removal of this residue is that the second residue from the glutamine is a D-amino acid.

An observation made during amino acid analysis of the tyrocidine peptides was that good recovery of tryptophan was frequently obtained with direct acid hydrolysis. This was brought about in part by carefully flushing the hydrolysis tube with nitrogen to remove every trace of oxygen prior to sealing under vacuum. However, it has been observed (King and Craig, 1955a) that acid hydrolysis of tyrocidine B even without careful nitrogen flushing gives good tryptophan recovery. Since this is in contrast to what one usually experiences with protein hydrolysates (Moore and Stein, 1963) it suggests that tryptophan breakdown occurs to a large extent only when the breakdown products of other amino acids not present in tyrocidine such as serine, threonine, cysteine, or glycine can interact with the indole nucleus in some way to cause it to be degraded.

An interesting property of the tyrocidine peptides is the high degree of aggregation which they show. One example of this phenomenon is the deviation of the experimental countercurrent-distribution pattern (Figure 2) from the theoretical. This type of skewing is characteristic of substances which associate. A detailed investigation of the aggregation of the tyrocidines is currently under way in this laboratory.

A phenomenon which simplified many of the isolation procedures that were employed in this study was the affinity of aromatic amino acids for the dextran gel of Sephadex (Porath, 1962). Since the aromatic amino acids are eluted in the order shown in Figure 7 and are much retarded with respect to the nonaromatic amino acids, it was not surprising to find that the tyrocidines could be eluted from Sephadex in the order of increasing tryptophan content as shown in Figure 3. This simple and rapid method for separating tyrocidines A, B, and C has made it feasible to study the genetics of *B. brevis* with respect to the synthesis of the different tyrocidines as well as the mechanism for biosynthesis of these polypeptides.

The occurrence of racemization observed in the measurement of the rotations of the amino acids as shown in Tables II and III is not excessive in view of the hydrolytic treatment to which the tyrocidine was subjected. Partial racemization of certain residues has been observed previously in the tyrocidine work (King and Craig, 1955a) and may be owing in part to events occurring during acid hydrolysis. Nevertheless, the predominating configuration of each amino acid residue can be derived from the results obtained in these studies.

In tyrocidine A there are two residues of D-phenylalanine and one of L-phenylalanine. If the same basic sequence exists for all the tyrocidines, as now appears to be the case, then there is only one possible structure for tyrocidine B, since in this case L-phenylalanine is replaced by L-tryptophan. In tyrocidine C, however, a D-phenylalanine is replaced by D-tryptophan, and the question arises as to why one is found and not both of the possible tyrocidine C isomers. It is indeed possible that other strains of *B. brevis* can synthesize the other isomer.[2] Part of the answer to this question may lie in the fact that the pentapeptide Val-Orn-Leu-Phe-Pro indicated by the dotted lines in Figure 1, which has

[2] It has been observed by Mach *et al.* (1963) that *B. brevis* grown in the absence of phenylalanine and in a medium containing large amounts of tryptophan is able to replace all of the phenylalanines in tyrocidine by tryptophan.

not been observed to be modified in the tyrocidines which we have studied, also occurs unmodified in the cyclic decapeptide gramicidin S which actually is a dimer of it. The reoccurrence of this pentapeptide fragment may have some important implication as regards the biosynthesis or the function of these cyclic peptides in the organism.

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Enzymatic Modification of Myosin by Disulfide Exchange*

David Hartshorne† and Manuel Morales‡

ABSTRACT: With various compounds, and under various conditions, there is studied the exchange between simple disulfides and the SH groups of myosin, and between disulfide myosin and various thiols. The first of these exchanges "modifies" myosin, as regards its nucleoside triphosphatase activity, and the second exchange partially "regenerates" the original myosin. Using ^{35}S-labeled dithiopropionate to modify myosin, and "cold" thiopropionate to regenerate it, one finds that when maximal ATPase activity is reached during modification there is a much greater number of reacted SH groups than when a similar maximal ATPase activity is reached on regeneration. This result is interpretable on the assumption that SH reaction and modification are distinct processes, and therefore not always "tightly coupled."

There is evidence (e.g., Gilmour and Gellert, 1961; Rainford *et al.*, 1964) that, as regards rate of reaction with organic mercurials, the SH groups of myosin [8.6 moles SH/10^5 g myosin, by amino acid analysis (Kominz *et al.*, 1954)] fall into various classes (X, very fast; Y, moderately fast; Z, very slow). It is to be expected that the reactions of myosin SH groups with other reagents will show a similar heterogeneity in reaction rate. In this paper we study, in both directions, reaction by disulfide exchange (Barany, 1959; Stracher, 1963, 1964),

$$R_t\text{—S—S—}R_t + \text{myosin—S}^- \rightarrow \text{myosin—S—S—}R_t + R_t\text{—S}^- \quad (1)$$

We also studied the possible reaction of myosin with thiols (Morales *et al.*, 1957),

* From the Cardiovascular Research Institute, University of California, San Francisco. *Received July 31, 1964; revised October 19, 1964.* This investigation was supported by grants CI-8 of the American Heart Association and G-19442 of the National Science Foundation.

† Postdoctoral Trainee, U.S.P.H.S. training grant HTS-5251; present address: Department of Biochemistry, State University of New York, Downstate Medical Center, Brooklyn, N.Y.

‡ Career Investigator, American Heart Association.