residue, crystallized from methanol, gave methyl 6-ethyl-4-oxo-1,4-dihydrocinnolin-3-ylpropionate (13), mp 173–174 °C. In a similar manner, starting with the appropriate propionic acid, there was obtained the following esters (13–25) described in Table II.

N-1 Alkylated Esters of Substituted 4-Oxo-1,4-dihydrocinnolin-3-ylpropionic Acids (26–32). Sodium hydride in oil (60%, 0.33 g) was washed free from oil with 3 × 5 ml of petroleum ether (bp 40–60 °C) and then suspended in 10 ml of dimethylformamide. Ethyl 6-ethyl-4-oxo-1,4-dihydrocinnolin-3-ylpropionate (14) (1 g) was added and the mixture was stirred at room temperature for 15 min after which time 0.5 ml of methyl iodide was added and the mixture stirred at 60–70 °C for 2 h. The mixture was cooled and poured into 100 ml of water and the resulting suspension filtered. The solid residue was crystallized from petroleum ether (bp 60–80 °C) and gave ethyl 6-ethyl-1-methyl-4-oxo-1,4-dihydrocinnolin-3-ylpropionate (26), mp 92–94 °C. The following N-alkylated derivatives (27–32) were obtained in a similar manner from the appropriate starting materials except that when the reaction was poured into 100 ml of water, instead of being filtered, the mixture was extracted with 3 × 100 ml of diethyl ether; the combined extracts were washed with saturated sodium chloride solution, dried ($MgSO_4$), and evaporated to dyness. The residue was crystallized from the appropriate solvent and in this way compounds 27–32 reported in Table II were obtained.

Catalytic Reduction of 4-Oxo-1,4-dihydrocinnolin-3-ylpropionic Acid (2). A solution of 1 g of 4-oxo-1,4-dihydrocinnolin-3-ylpropionic acid (2) in 140 ml of methanol and 40 ml of water was hydrogenated at STP using 1 g of 5% palladium/carbon and 0.4 g of platinum oxide as catalyst for 5 h. The catalyst was filtered off and the solvent evaporated. Crystallization of the residue from water gave 4-oxo-1,4,5,6,7,8-hexahydrocinnolin-3-ylpropionic acid (33): mp 226–228° (0.81 g); ν_{max} 1715 cm^{-1} (acid C=O); δ (Me_2SO-d_6) 2.9–2.1 (8 H, complex, benzylic CH_2 and CH_2 adjacent to carboxylic group) and 1.9–1.7 (4 H, complex, other $-CH_2-$); pK_a values in 50% aqueous acetone of 6.00 and 10.84. Anal. ($C_{11}H_{14}N_2O_3$) C, H, N.

Pharmacological Methods. The antiallergic activity of the test compounds shown in Tables I and II was assessed by their ability to inhibit passive cutaneous anaphylaxis (PCA) in rats. All compounds were initially tested by the intravenous route at 20 mg/kg, while only the more active members were further evaluated by the oral route. Groups of three rats received an intradermal injection on one shaved flank of 0.1 ml of a suitable dilution of rat reaginic antiserum. After 48 h the animals were challenged with 1 ml of an equal volume of antigen (egg albumin, 10 mg/ml) and Evans blue 0.5% w/v. The intensity of the reaction was assessed 30 min later by awarding an arbitrary score from 0 to 10.[14] Compounds were administered either intravenously at the same time as antigen or orally 15 and 60 min prior to antigenic challenge. The normal screening doses are 20 mg/kg intravenously or 40 mg/kg per os. Results are expressed in terms of the ID_{50} as that dose required to produce a 50% inhibition of PCA compared with a control group of rats receiving no drug.

Metabolic Studies.[15] A capsule of the ethyl ester 14 was dosed to a rhesus monkey at 20 mg/kg and the 0–24 h urine collected, and the chloroform extract examined by TLC using the solvent systems chloroform–ethanol–formic acid (85:15:1 v/v) and 2-propanol–ammonia–water (7:3:1 v/v). No 6-ethyl-4-oxo-1,4-dihydrocinnolin-3-ylcarboxylic acid was detected colorimetrically or by removal from the plate by extraction with solvent and determination of the ultraviolet spectrum of the area where authentic 3-carboxylic acid was known to cochromatograph. The dosed ester 14 was mainly excreted in the urine as the corresponding acid 3.

References and Notes

(1) J. B. L. Howell and R. E. C. Altounyan, *Lancet*, **2**, 539 (1967).
(2) (a) R. W. Ferraresi, A. P. Roszkowski, and E. Kepel, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **33**, 762 (1974); J. R. Pfister, R. W. Ferraresi, I. T. Harrison, W. H. Rooks, A. P. Roszkowski, A. van Horn, and J. H. Fried, *J. Med. Chem.*, **15**, 1032 (1972); (b) E. S. K. Assem, J. A. Evans, and M. McAllen, *Br. Med. J.*, **2**, 93 (1974).
(3) S. P. Haydu, J. L. Bradley, and D. T. D. Hughes, *Br. Med. J.*, **3**, 283 (1975).
(4) B. J. Broughton, P. Chaplen, P. Knowles, E. Lunt, D. L. Pain, K. R. H. Wooldridge, R. Ford, S. Marshall, J. L. Walker, and D. R. Maxwell, *Nature (London)*, **251**, 650 (1974).
(5) B. A. Spicer, J. W. Ross, and H. Smith, *Clin. Exp. Immunol.*, **21**, 419 (1975).
(6) A. Nohara, H. Kuriki, T. Saijo, K. Ukawa, T. Murata, M. Kanno, and Y. Sanno, *J. Med. Chem.*, 18, 34 (1975).
(7) D. J. Gilman, D. S. Thomson, and W. S. Waring, *Nature (London)*, **250**, 592 (1974), and references cited therein.
(8) D. J. Gilman, U.K. Patent 1306839; *Chem. Abstr.*, 77, 34545m (1972).
(9) J. Goose and A. M. J. N. Blair, *Immunology*, **16**, 749 (1969).
(10) G. Jones and G. D. Tringham, *J. Chem. Soc., Perkin Trans. 1*, 1280 (1975).
(11) W. Borsche and A. Herbert, *Justus Liebigs Ann. Chem.*, **546**, 293 (1941).
(12) D. E. Ames, R. F. Chapman, and D. Waite, *J. Chem. Soc. C*, 470 (1966).
(13) D. E. Ames and A. C. Lovesay, *J. Chem. Soc.*, 6036 (1965).
(14) D. S. Thomson and D. P. Evans, *Clin. Exp. Immunol.*, **13**, 537 (1973).
(15) This experiment was performed by Dr. J. M. Fromson of these laboratories.

Structure of the Peptide Antibiotic Polypeptin

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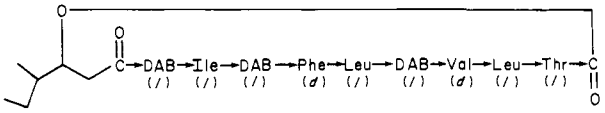
Polypeptin, a basic peptide antibiotic isolated from *Bacillus circulans*, was separated into two components by countercurrent distribution. The two components, polypeptin A and polypeptin B, had identical amino acid compositions but varied in the structure of the hydroxy acid constituent attached to the α-amino group of the peptide chain. Polypeptin A contained 3-hydroxy-4-methylhexanoic acid and polypeptin B contained 3-hydroxy-5-methylhexanoic acid. The stereochemistry of these hydroxy acids was not determined. Studies involving partial acid hydrolysis and chemical synthesis are consistent with the lactone structure for polypeptin A. Polypeptin B differs only in the position of the methyl group in the hydroxyacyl moiety.

Polypeptin is a basic peptide antibiotic isolated[1] in 1948 from a variant of *Bacillus circulans*. It was originally given the name circulin after the producing organism but the name was later changed[2] because of a name conflict with another natural product from *B. circulans*. Polypeptin has a wide spectrum of antimicrobial action. It is active against many gram-positive and gram-negative bacteria and most fungi and originally attracted some interest because of its

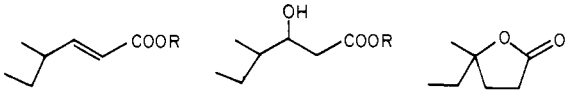
moderate activity against *M. tuberculosis*. However, it is toxic when administered parenterally and has not found clinical application. Polypeptin is related both through taxonomy and general structure to the polymyxin family of peptides. Some chemical studies of polypeptin have appeared,[3,4] but the complete structure has not previously been determined.

Amino Acid and Hydroxy Acid Constituents of Polypeptin. Crude polypeptin was separated by countercurrent distribution into two components, polypeptins A and B. These components were subjected to amino acid



analysis and the results obtained were in agreement with those of Hausmann and Craig.[4] Both peptides have the amino acid composition: *l*-Thr, *d*-Val, *l*-Ile, *l*-Leu_2, *d*-Phe, *l*-DAB_3, where DAB represents α,γ-diaminobutyric acid.

The difference between polypeptins A and B is the nature of their organic acid moieties. Acid hydrolysis of polypeptin A gave three ether-extractable compounds, identified as their methyl esters by mass spectrometry and ^1H NMR as

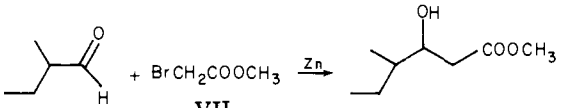


Ia, R = H
b, R = CH_3

IIa, R = H
b, R = CH_3

III

Results obtained from a brief acid hydrolysis and from a base hydrolysis established that IIa is the natural product, while Ia and III are formed by dehydration of IIa. Compound IIa has two asymmetric centers, the configurations of which were not determined. Compound VIII, which has the same covalent structure as IIb but which is racemic at the two asymmetric centers, was synthesized via the Reformatsky reaction.



VI VII VIII (mixed isomers)

Compound VIII was indistinguishable from IIb by several criteria and differed from the natural material by ^1H NMR only because ^1H NMR resolved signals from the two pairs of diastereomers. Thus all protons within two bonds of an asymmetric center gave rise to two resolvable signals and all others to one. In each case one of the doubled resonances corresponded to the resonance observed in IIb and decoupling experiments established the expected pattern of spin coupling.

Both acid and base hydrolysis of polypeptin B yielded only two ether-extractable components, identified as their methyl esters as

IVa, R = H
b, R = CH_3

Va, R = H
b, R = CH_3

The time course of hydrolysis indicated that IVa was the natural product.

General Structural Considerations. The molecular weight of polypeptin has been established by the method

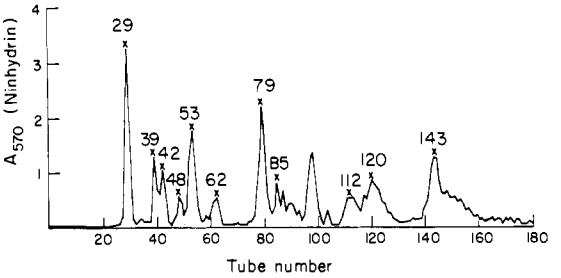
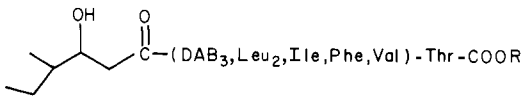


Figure 1. Elution of a partial acid hydrolyzate of polypeptin A from Bio-Rad AG50W-X4 200–400 mesh (2.0 × 60 cm) with the pyridine acetate gradient described. Fractions are 7.5 ml. Peaks marked with an x and a tube number were further characterized.

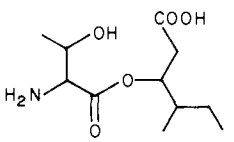
of partial substitution to be 1150 ± 100[4]. This agrees well with the molecular weight expected for the observed composition. Since polypeptins A and B have the same amino acids and isomeric hydroxy acids, they have the same molecular weight.

Paper electrophoresis and dansylation analysis of polypeptin A or B revealed three free amino groups, which were identified as the γ-amino groups of the three DAB residues. No carboxyl group was demonstrable. Since the nine amino acids and the hydroxy acid together contain 12 amino and 10 carboxyl groups, the electrophoresis results indicate that the peptide chain is unbranched and that the terminal carboxyl group is involved in a nonamide linkage. The documented acid and base lability[3,4] of polypeptin suggested that the linkage was an ester, and this was confirmed by mild base hydrolysis (10% 1 N NaOH in CH_3OH). After hydrolysis, a free carboxyl group was demonstrable by electrophoresis and Thr was detectable as the C-terminal amino acid. Hydrolysis also caused the disappearance of a characteristic infrared absorption (5.75 μ) of polypeptin. The partial structure of polypeptin derived from these data is



IX

Amino Acid Sequence. The amino acid sequence of polypeptin was determined by partial acid hydrolysis because no proteolytic enzyme was found which would degrade the antibiotic. Sequence results were identical whether polypeptin A or B or a mixture of the two was used, establishing the identity of the two components with respect to the peptide portion of the molecule. The elution profile obtained with the partial hydrolyzate of polypeptin A on Bio-Rad AG50W-X4 is shown in Figure 1. Each significant peak was examined by amino acid analysis and electrophoresis at pH 1.7 and 5.6 for heterogeneity. The sequence of peptides in fractions found to be homogeneous by these criteria is shown in Table I. Fraction 62 yielded a small amount of a compound whose chemical properties and ^1H NMR spectrum were consistent with the structure



X

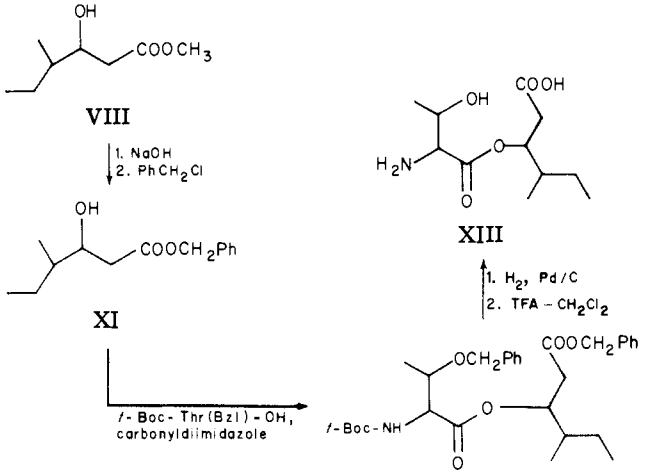
To establish this structure with more certainty compound

Table I

Fraction	Amino acid composition	Structure
29	Thr	Thr
39	Leu	Leu
42	DAB	H.A.[a]-DAB
48	Phe	Phe
53	$Val_{1.0}$, $Leu_{1.0}$	Val-Leu
62	Thr	Thr-H.A.[a,b]
79	$DAB_{3.4}$, $Leu_{1.0}$, $Phe_{1.0}$	DAB, Phe-Leu[c]
85	$Ile_{1.0}$, $DAB_{2.1}$	H.A.[a]-DAB-Ile-DAB
112	$Val_{1.0}$, $Leu_{1.0}$, $DAB_{1.0}$	DAB-Val-Leu
120	$Val_{1.0}$, $Leu_{1.9}$, $DAB_{1.0}$	Leu-DAB-Val-Leu
143	$Val_{1.0}$, $Leu_{2.0}$, $Phe_{0.9}$, $DAB_{1.1}$	Phe-Leu-DAB-Val-Leu

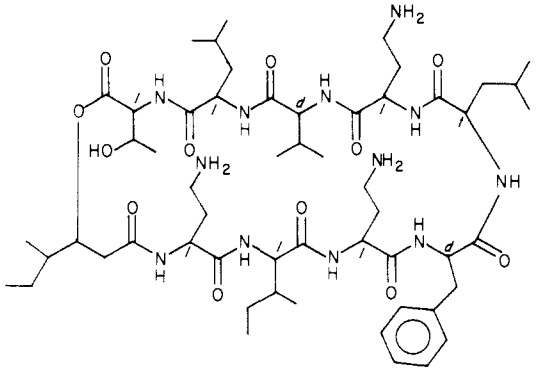
[a] Hydroxy acid IIa for polypeptin A or IVa for polypeptin B. [b] Ester involving the Thr carboxyl group and the hydroxyl group of the hydroxy acid. [c] Mixture separable by electrophoresis.

XIII, which contains hydroxy acid VIII in place of IIa, was synthesized via



The synthetic material XIII was identical with X with respect to electrophoretic mobility, R_f on TLC, and ^1H NMR, except where the configurational heterogeneity of the synthetic hydroxy acid led to doubled signals.

Complete Structure. The only formula for polypeptin A compatible with the general structural information and the sequence data is XIV.



XIV

This structure must be regarded as tentative because two amino acid overlaps have not been established by partial hydrolysis and because the configuration of the hydroxy acid is not known. Ring closure via the macrocyclic lactone is supported by the structure of hydrolysis fragment 62 (X) and by the fact that the antibiotic appears to have no hydroxyl group other than that of the hydroxy acid with which to esterify the carboxyl group of Thr. The presence of any volatile alcohol in the structure of polypeptin was ruled out by base hydrolysis followed by gas chromatography.[5]

The relationship of structure XIV to that of the polymyxins is substantial. Both are presumably biosynthesized as linear peptides with the amino terminus blocked with an aliphatic acid. The peptide chain contains nine amino acids in the case of the polypeptins and ten in the case of the polymyxins. The carboxyl groups of both types of peptides are blocked by ring closure, via ester formation in the polypeptins and amide formation in the polymyxins. The final structure of the polypeptins contains a 31-membered ring, reminiscent of the very stable 30-membered ring structure of cyclic decapeptides such as gramicidin S.[6] Investigations of the secondary structure of polypeptin A in solution are in progress.

Experimental Section

Amino acid analyses were performed on a Beckman Model MS amino acid analyzer. Hydrolyses for amino acid analyses were done for 24 h in 6 N HCl at 110° in evacuated sealed tubes. Analysis of the optical series of amino acids was done by the method of Manning and Moore.[7] High-voltage electrophoresis was done at 0° in a Pherograph flat bed apparatus on MN paper No. 214 (Brinkman) at 100 V/cm. Buffers were 50% acetic acid (pH 1.7) and pH 5.6 pyridine acetate. Gramicidin S and bacitracin were used as standards in electrophoresis of polypeptin. Thin-layer chromatography of peptides was performed on Eastman Chromagram sheets, Type K301R2 (silica gel G). Dansylation was according to Gray[8] and dansyl amino acids were identified by electrophoresis at pH 4.4[8] or by TLC[9] on polyamide layers. Anhydrous hydrazine (MCB) was used as received for C-terminal analysis.[10] Subtractive Edman degradation was done according to Konigsberg.[11] Except where specified all chemicals were reagent grade and used without further purification.

Infrared spectra of polypeptin were recorded on a PE237 grating spectrophotometer in KBr pellets. High-resolution ^1H NMR spectra were obtained on a Varian HR220 spectrometer. Analytical gas chromatography of hydroxy esters was done using an F&M Model 402 instrument with a 6 ft × 2 mm column of DC-560 on Gaschrom Q with a temperature program from 45 to 220° at 5°/min. The carrier gas flow was 50 ml/min. Preparative separation was accomplished on an Aerograph Model A-90-P3 instrument with a 10 ft × 3/8 in. column of DEGS on Chromosorb W at 150° with a carrier gas flow of 135 ml/min. Isobutane chemical ionization mass spectrometry was performed by the Rockefeller University Mass Spectrometry Service Lab on a Du Pont Model 21-492 machine modified for chemical ionization under license from Scientific Research Instruments of Baltimore, Md. The mass spectrometer was operated at a resolution of 2000 with a source pressure of approximately 0.5 Torr.

Countercurrent Distribution of Polypeptin. Countercurrent distribution of polypeptin (a generous gift of Dr. S. F. Howell) in a 1000-tube apparatus with the solvent system 2-BuOH–isopropyl ether–0.1 N HCl (2:1:3) yielded two components, polypeptin A (K = 0.895) and polypeptin B (K = 0.592). Higher acid concentrations resulted in slow transformation of the antibiotic and peak broadening.

Isolation of Hydroxy Acids. (a) Acid Hydrolysis. The hydrochloride of polypeptin A or B (200 mg) was refluxed under N_2 for 24 h in 15 ml of 18% H_2SO_4 which had been deaerated with N_2. The hydrolyzate was extracted five times with ether; the ether extract was dried over Na_2SO_4 and evaporated to an oil through a Vigreux column. The oil was methylated with an ether solution of diazomethane (prepared from *N,N'*-dinitroso-*N,N'*-dimethylterephthalamide). Analytical gas chromatography on DC-560 on Gaschrom Q yielded peaks at 6, 9, and 11 min for polypeptin A, identified as compounds III, IIb, and Ib, respectively. The mixture was separated preparatively on DEGS

on Chromosorb W with retention times of 5, 20, and 31 min (15, 35, and 50%), respectively. Hydrolysis for 6 h yielded the same components but in different ratios (5, 80, 15%).

Compound III was identified by chemical ionization mass spectrometry (CIMS) (M + 1 = 129), infrared (5.67 μ), and NMR [($CDCl_3$) τ 8.62 (s, 3 H), 9.03 (t, 3 H, J = 7.2 Hz), 8.34, 8.00, and 7.44 (3 m, each 2 H)]. Anal. ($C_7H_{12}O_2$) C, H, N. Compound IIb was characterized by CIMS (M + 1 = 161), infrared (5.80 μ), and NMR [(CCl_4) τ 9.13 (d, 3 H, J = 6.4 Hz), 9.09 (t, 3 H, J = 7.5 Hz), 8.4–9.0 (m, 3 H), 7.66 (m, 2 H), 7.36 (br s, 1 OH), 6.35 (s, 3 H, OCH_3), 6.19 (m, 1 H)]. Anal. ($C_8H_{16}O_3$) C, H, N. Compound Ib was identified by CIMS (M + 1 = 143) and NMR [($CDCl_3$) τ 4.25 (dd, 1 H, J_{trans} = 16 Hz), 3.12 (d, 1 H, J = 16 Hz)]. Anal. ($C_8H_{14}O_2$) C, H, N.

(b) Base Hydrolysis. Polypeptin A or B (200 mg of free base) was refluxed under N_2 for 6 h in 15 ml of deaerated 2.5 N NaOH. The hydrolyzate was cooled in ice, acidified to pH 2 with concentrated H_2SO_4, and extracted with ether. The ether extract was dried, evaporated, and esterified as above. Polypeptin A yielded a mixture of 90% IIb and 10% Ib, while polypeptin B yielded 90% IVb and 10% Vb.

Methyl 3-Hydroxy-4-methylhexanoate (VIII). Dry benzene (50 ml) and active[12] 20 mesh zinc (6.54 g, 100 mmol) were added to a dry three-necked 250-ml flask fitted with a pressure equalizing dropping funnel, a reflux condenser, and a N_2 inlet. The dropping funnel was filled with a mixture of 2-methylbutyraldehyde (VI) (8.6 g, 100 mmol) and methyl bromoacetate (VII) (15.3 g, 100 mmol) diluted to 50 ml with dry benzene. This mixture (10 ml) was added to the zinc and heating was begun. As the benzene approached reflux temperature a vigorous reaction began. The remaining contents of the dropping funnel was added dropwise over the course of 30 min and reflux was continued for a further 45 min. When cool, the reaction mixture was poured into 150 ml of ice-cold 20% H_2SO_4 and stirred at 0° for 30 min. The organic layer was removed and the aqueous layer extracted four times with ether–benzene (1:1). The combined organic layers were washed with water, saturated $NaHCO_3$, and water and dried over $MgSO_4$. The solvent was removed by rotary evaporation and the oil was distilled at 10-mm pressure to give 8.87 g (56%) of a clear liquid, boiling at 97–103°: infrared 5.80 μ. Anal. ($C_8H_{16}O_3$) C, H, N.

Partial Acid Hydrolysis. Two 250-mg portions of polypeptin hydrochloride were incubated at 37° in 30 ml of 6 N HCl each, one portion for 3 days and the other for 8 days. The pooled sample was extracted with ether and evaporated to dryness. The residue was taken up in 5 ml of 0.2 N pyridine acetate buffer, pH 3.1, applied to a 2.0 × 60 cm column of Bio-Rad AG50W-X4 200–400 mesh and eluted[13] with a 1:2 gradient of 0.2 N pyridine acetate (pH 3.1)–2 N pyridine acetate (pH 5.0), 500:1000 ml, until 900 ml had been collected. The larger chamber was then emptied and refilled with 8.5 N pyridine acetate, pH 5.6, and elution was continued. The flow rate was maintained at 30 ml/h.

3-[(2-Amino-3-hydroxybutyryl)oxy]-4-methylhexanoic Acid (Thr-H.A., XIII). (a) VIII (800 mg, 5 mmol) was saponified with 10% 1 N NaOH in MeOH and the free acid was converted to the benzyl ester by the method of Gisin.[14] The benzyl ester XI (774 mg, 3.1 mmol) was obtained as an oil. Anal. ($C_{14}H_{20}O_3$) C, H, N.

(b) Carbonyldiimidazole (535 mg, 3.3 mmol) was dissolved in 6 ml of CH_2Cl_2 and cooled in an ice bath. After 15 min, XI (3.1 mmol) was added and washed in with 2 ml of CH_2Cl_2. The solution was stirred at 0° for 45 min and then at room temperature for 80 h. Water was added to destroy any excess activated acid and the solution was taken to dryness. The residue was dissolved in 30 ml of ether and washed successively with water, saturated citric acid, water, saturated $NaHCO_3$, and water. The ether solution was dried over $MgSO_4$ and evaporated to give 1.49 g (2.8 mmol, 90%) of an oil (XII) which was carried on without characterization.

(c) XII (2.8 mmol) was taken up in 10 ml of methanol and hydrogenated overnight in a Parr apparatus at 36 psi, with 0.5 g of 5% Pd on powdered charcoal as catalyst. After filtration, the solution was evaporated to dryness and the residue was taken up in 30 ml of saturated $NaHCO_3$, washed twice with ether, acidified to pH 2.5 with solid citric acid, and extracted six times with ethyl acetate. The extract was dried over $MgSO_4$ and evaporated and the residue was dissolved in 6 ml of 50% trifluoroacetic acid (TFA) in CH_2Cl_2. After 30 min this solution was taken to dryness to yield 945 mg (2.6 mmol, 94%) of XIII as its TFA salt. This material was further purified and converted to its acetate salt by dissolving it in 2 ml of H_2O and applying it to a 2.0 × 30 cm column of Bio-Rad AG50W-X4 200–400 mesh in water, washing with 400 ml of water, and eluting with 0.3 M ammonium acetate buffer, pH 4.5. Repeated lyophilization from water yielded XIII acetate as an amorphous solid (790 mg, 2.6 mmol). Anal. ($C_{13}H_{25}NO_7$) C, H, N.

XIII was compared to the natural material X by electrophoresis, TLC, and ^1H NMR. Both gave a purple color with ninhydrin, both on paper and on silica gel TLC. Neither had any mobility on paper at pH 5.6 and both moved as cations at pH 1.7. They had identical R_f values on TLC of 0.43 in n-BuOH–HOAc–H_2O (4:1:1), 0.57 in 2-BuOH–88% HCOOH–H_2O (15:3:2), and 0.00 in EtOAc–pyridine–HOAc–H_2O (60:20:6:11). The ^1H NMR spectra were identical except for small differences attributable to the lack of configurational homogeneity in the hydroxy acid component of XIII: NMR (D_2O) τ 9.14 (d, 3 H, J = 6.5 Hz), 9.10 (t, 3 H, J = 7.4 Hz), 8.69 (d, 3 H, J = 6.9 Hz), 7.52 (m, 2 H), 5.95 (d, 1 H, J = 7.0 Hz), 5.65 (m, 1 H).

Test for a Volatile Alcohol in Polypeptin. Polypeptin hydrochloride (125 mg, 0.1 mmol) was dried for 24 h at 56° in vacuo. The dried peptide was transferred to a test tube, suspended in 1 ml of 1.3 N NaOH, and sealed in the tube in vacuo. After heating for 1 h at 110°, the tube was chilled and opened and the contents were transferred to a short-path distillation apparatus and diluted to 5 ml with H_2O. This solution was neutralized with concentrated H_2SO_4 in an ice bath, 3 ml of ethylene glycol was added, and the solution was distilled into a double liquid nitrogen trap. Distillation was carried out to a bath temperature of 150°. This procedure gave a recovery of 74% in control experiments with added ethanol. The distillate was diluted to 5 ml and examined for alcohols by gas chromatography on a column of Porapak Q at 115°.[5] No volatile material was detectable at a sensitivity sufficient to quantitate a yield of 1%. Retention times of standard alcohols were MeOH, 1 min; EtOH, 2.5 min; n-PrOH, 6 min; n-BuOH, 8.5 min; 1-hexanol, 27 min.

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References and Notes

(1) C. McLeod, *J. Bacteriol.*, **56**, 749 (1948).
(2) W. Carson, C. McLeod, P. A. Tetrault, H. Koffler, D. H. Peterson, and D. R. Colingsworth, *J. Bacteriol.*, **58**, 115 (1949).
(3) S. F. Howell, *J. Biol. Chem.*, **186**, 863 (1950).
(4) W. Hausmann and L. C. Craig, *J. Am. Chem. Soc.*, **76**, 4892 (1954).
(5) J. T. Piechocki, *J. Pharm. Sci.*, **57**, 134 (1968).
(6) A. Stern, W. A. Gibbons, and L. C. Craig, *Proc. Natl. Acad. Sci. U.S.A.*, **61**, 734 (1968).
(7) J. M. Manning and S. Moore, *J. Biol. Chem.*, **243**, 5591 (1968).
(8) W. R. Gray, *Methods Enzymol.*, 11, 139 (1969).
(9) K. R. Woods and K. T. Wang, *Biochim. Biophys. Acta*, **133**, 369 (1967).
(10) H. Fraenkel-Conrat and C. M. Tsung, *Methods Enzymol.*, 11, 151 (1967).
(11) W. Konigsberg, *Methods Enzymol.*, 11, 46 (1967).
(12) L. F. Fieser and W. S. Johnson, *J. Am. Chem. Soc.*, **62**, 575 (1940).
(13) W. A. Schroeder, *Methods Enzymol.*, 11, 351 (1967).
(14) B. F. Gisin, *Helv. Chim. Acta*, **56**, 1476 (1973).