that this generalization holds for all the other isothioureas examined in this study.

It is apparent from the experimental results reported here that amino and variously N-substituted aminoalkylisothioureas with an amino nitrogen to sulfur distance of not more than three carbon atoms are rather labile compounds in aqueous solution and that the products obtained may be controlled, to some extent, by a suitable choice of *p*H, time of reaction and nature of the base used for neutralization. These facile transformations make possible the preparation of a wide variety of mercaptoalkylguanidines, 2-aminothiazolines and 2-aminopenthiazolines in good yields from relatively simple and readily available starting materials. In addition, aminoisothioureas such as 4-ABT and GET, which are incapable of the intramolecular reaction, should be useful protein guanylating agents in the neutral *p*H range.

OAK RIDGE, TENN.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

The Structure of Etamycin

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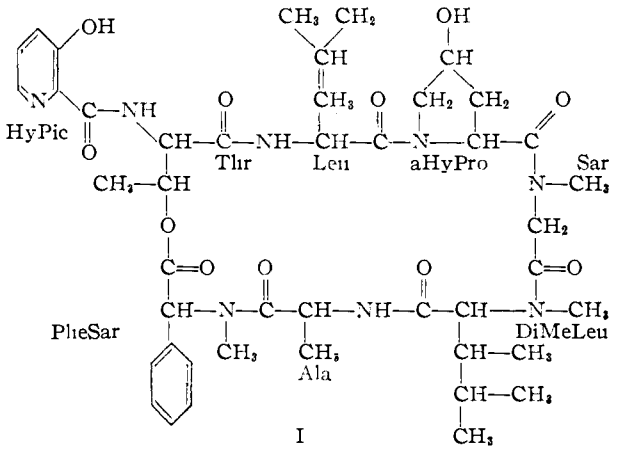
The structure of the antibiotic Etamycin is shown to be a macrocyclic peptide lactone, represented by formula I. Etamycin is an unusual peptide containing eight amino acids, only two of which are known as components of animal protein. Four of the amino acids, 3-hydroxypicolinic acid, *allo*-hydroxy-D-proline, L-α-phenylsarcosine (IIb) and L-β,N-dimethylleucine (III) have not been encountered previously in nature.

The isolation of the peptide antibiotic Etamycin from culture broths of a Streptomyces species was described in 1954 by Heinemann, *et al.*[2] Simultaneously, Bartz, *et al.*,[3] reported an antibiotic termed Viridogrisein, obtained from *Streptomyces griseus*. Etamycin and Viridogrisein were shown subsequently to be identical.[4] The antibiotic exhibits considerable activity against gram-positive



I

organisms and *Mycobacterium tuberculosis;* in addition it causes a reversible leucopenia in dogs.[2,5,6] Etamycin possesses unusual solubility properties for a peptide, being freely soluble in benzene and carbon tetrachloride. The values reported[2,3] for the molecular weight varied from 530 to 982, though most were in the range 800–900. After acid hydrolysis 3-hydroxypicolinic acid (HyPic), D-leucine, *allo*-hydroxy-D-proline and L-alanine were isolated,[3] and threonine was identified (paper chromatography[2]).

This paper reports the isolation from Etamycin hydrolysates of three additional components. These are sarcosine and two previously unknown amino acids, L-α-phenylsarcosine (PheSar, IIb) and L-β,N-dimethylleucine (DiMeLeu, III). On the basis of degradation experiments the structure of Etamycin is formulated as I.[7]

Amino Acid Composition.—Two dimensional paper chromatograms of Etamycin total hydrolysates, developed with ninhydrin at 100°, showed three spots in addition to those corresponding to alanine, threonine, hydroxyproline and leucine. When the color was developed at 60° or below these spots either were very faint or absent. These three spots were due to amino acids rather than to peptides since after elution and vigorous hydrolysis they reappeared at the same places on a two dimensional chromatogram. Two of the amino acids gave the red color test with *p*-nitrobenzoyl chloride–pyridine characteristic of N-alkylamino acids.[8] The third amino acid behaved chromatographically like sarcosine, which is known not to give this color test.[8c] That the three unknown amino acids are N-methylamino acids was confirmed by an N-methyl determination (three N-methyl groups per molecule of Etamycin).

The three N-methylamino acids were isolated by preparative paper chromatography. Deamination of an Etamycin total hydrolysate with nitrous acid[9] destroyed alanine, threonine and leucine. After reconstitution from the nitroso derivatives by heating with hydrochloric acid,[9] the secondary amino acids were separated on Whatman No. 3

(1) (a) Aided by a grant from the National Institutes of Health; (b) National Institutes of Health Postdoctoral Fellow, 1956–1957.

(2) B. Heinemann, *et al.*, *Antibiotics Annual*, **2**, 728 (1954–1955).

(3) Q. R. Bartz, *et al.*, *ibid.*, **2**, 777–784 (1954–1955).

(4) The identity of Viridogrisein with Etamycin was established in the laboratories of the authors of refs. 2 and 3 and at M.I.T. We wish to thank Dr. Murray Goodman for his part in this work.

(5) H. L. Dickison, K. M. Cull and D. E. Tisch, *Antibiotics Annual*, **2**, 733 (1954–1955).

(6) J. Ehrlich, *et al.*, *ibid.*, **2**, 790 (1954–1955).

(7) A preliminary report of the work appeared in THIS JOURNAL, **79**, 3933 (1957).

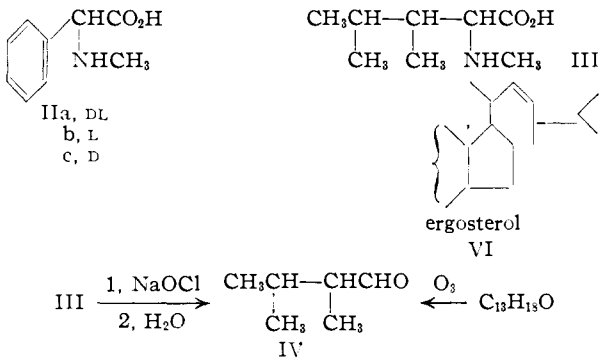
(8) (a) E. Waser, *Mitt. Lebensm. Hyg.*, **20**, 260 (1929); *C. A.*, **24**, 1601 (1930); (b) S. Edlbacher and F. Litvan, *Z. physiol. Chem.*, **265**, 241 (1940); (c) P. A. Plattner and U. Nager, *Helv. Chim. Acta*, **31**, 2203 (1948).

(9) B. Witkop and C. M. Foltz, THIS JOURNAL, **79**, 192 (1957).

paper. Treatment of the paper-strip eluates with Dowex-50 proved to be a good method of freeing the amino acids from soluble paper constituents. One N-methylamino acid was identified as sarcosine by comparison of the 2,4-dinitrophenyl derivative with an authentic sample.

The second N-methylamino acid, $C_9H_{11}NO_2$, sublimation point 246°, had strong infrared peaks at 1500, 730 and 700 cm.$^{-1}$ indicating the presence of a benzene ring. The ultraviolet spectrum was typically benzenoid, having maxima at 252, 257, 262 and 268 mμ (low extinction) and high end absorption from 239 mμ. The amino acid was found to be identical with synthetic D,L-α-phenylsarcosine (IIa), sublimation point 247°, by comparison of infrared and ultraviolet spectra and chromatographic behavior.

The optical inactivity was expected since optically active α-phenylglycine is known[10] to racemize readily in boiling 10% hydrochloric acid, and the standard conditions for total hydrolysis used involved 16 hours heating with 20% hydrochloric acid. However, a second isolation of the phenylsarcosine, employing a contact time of only 45 minutes with boiling 12% hydrochloric acid, gave α-phenylsarcosine (IIb) with $[\alpha]^{31}D$ +118° (in N hydrochloric acid).



Although it seemed probable that the (+)-α-phenylsarcosine isolated was in the L-series,[11] it was convenient to convert D-α-phenylglycine[12] into D-α-phenylsarcosine (IIc). Since the latter had $[\alpha]^{28}D$ −114° (in 5 N hydrochloric acid) the (+)-α-phenylsarcosine in Etamycin belongs to the L-series.[18]

Preparation of D,L-α-phenylsarcosine was accomplished by a modification of the general synthesis used by Fischer and Lipschitz[15] for N-methylamino acids. Tosyl-D,L-α-phenylglycine was methylated with methyl iodide and sodium hydroxide to give tosyl-D,L-α-phenylsarcosine, from which D,L-α-phenylsarcosine (IIa) was obtained on acid hydrolysis. D-α-Phenylsarcosine (IIc) was synthesized by the same method from D-α-phenylglycine, except that the tosyl group was removed in the final step by reduction[16] with sodium in liquid ammonia.

The third N-methylamino acid, $C_8H_{17}NO_2$, appeared to be a homolog of an N-methylleucine on the basis of spectral properties and chromatographic behavior. Degradation with hypochlorite[19,14] gave carbon dioxide from the carboxyl group, an amine from the substituted amino function and an aldehyde from the side chain. The amine was identified as methylamine by conversion to the 2,4-dinitrophenyl derivative. The aldehyde was converted to the 2,4-dinitrophenylhydrazone, m.p. 125.2–126.0°, $[\alpha]^{28}D$ −38.7°. These constants corresponded to those of the DNPH of isopropylmethylacetaldehyde (V) (m.p. 124.0–124.5°, $[\alpha]^{26}D$ −37.7°) which had been obtained by Bergmann and Stansbury[20] by ozonolysis of ergosterol (VI). An authentic sample of the aldehyde IV was prepared by the ozonolysis of ergosterol, following the procedure which Slomp, *et al.*,[21] used for the ozonolysis of 5β-ergost-22-en-3-one, and converted to the DNPH V. The two dinitrophenylhydrazones were identical (m.p., mixed m.p., rotation, infrared spectra), thus establishing β,N-dimethylleucine (III) as the structure of the third amino acid.

The asymmetric center at the α-carbon atom of the dimethylleucine probably was epimerized partially during the acid hydrolysis. A sample, recrystallized three times, had a rotation of $[\alpha]^{29}D$ +33.2° in water and $[\alpha]^{29}D$ +39.2° in 5 N hydrochloric acid.[22] From the change in rotation after acidification one can infer that the amino acid belongs to the L-series.[14,23] The configuration at the β-carbon cannot be assigned, since the aldehyde derived both from the dimethylleucine and from ergosterol has not yet been correlated with D-glyceraldehyde.

Quantitative paper chromatography indicated that in Etamycin Thr, Ala, Leu, Sar, PheSar and DiMeLeu were present in equivalent amounts. However, HyPro could not be estimated accurately by the method used. Microbiological assay of total hydrolysates demonstrated the presence of one mole of L-threonine per mole of Etamycin.[24]

(10) F. Ehrlich, *Biochem. Z.*, **8**, 446 (1908); *Chem. Zentr.*, **79**, **I**, 1632 (1908). Further information on the racemization of phenylglycine is given by J. P. Greenstein, S. M. Birnbaum and M. C. Otey, *J. Biol. Chem.*, **204**, 307 (1953).

(11) L-α-Phenylglycine has a high positive rotation ($[\alpha]^{25}D$ +168° in 5 N hydrochloric acid[18]), and N-methylation would not be expected to change the rotation appreciably. (The changes in rotation after methylation of seven amino acids [Val, Leu, iLeu, Asp, Try, Ala and His] were in the range 4 to 32°—calculated from data in refs. 14, 15, 16 and 17.) For the purpose of assigning the (+)-α-phenylsarcosine from Etamycin to the D- or L-series it was necessary only to synthesize an optical isomer of phenylsarcosine from one of the antipodes of phenylglycine, and compare the rotation of the product with that of the natural material.

(12) (+)-α-Phenylglycine was related chemically to L-alanine by M. Kuna, G. Ovakimian and P. A. Levene, *J. Biol. Chem.*, **137**, 337 (1941).

(13) D. Rudman, A. Meister and J. P. Greenstein, THIS JOURNAL, **74**, 551 (1952).

(14) P. A. Plattner and U. Nager, *Helv. Chim. Acta*, **31**, 2192 (1948).

(15) E. Fischer and W. Lipschitz, *Ber.*, **48**, 360 (1915).

(16) V. du Vigneaud and O. K. Behrens, *J. Biol. Chem.*, **117**, 27 (1937).

(17) J. P. Greenstein, *Adv. in Protein Chem.*, **9**, 121 (1954).

(18) Both the L-PheSar isolated from Etamycin and the synthetic D-PheSar are probably partially racemized.

(19) K. Langheld, *Ber.*, **42**, 2360 (1909).

(20) W. Bergmann and H. A. Stansbury, *J. Org. Chem.*, **9**, 281 (1944).

(21) G. Slomp, *et al.*, THIS JOURNAL, **77**, 1216 (1955).

(22) Hydrolysis of Etamycin under mild conditions gave DiMeLeu which had a lower rotation; *cf.* Experimental section.

(23) O. Lutz and B. Jirgensons, *Ber.*, **63**, 448 (1930); **64**, 1221 (1931).

(24) We are indebted to Merck, Sharp and Dohme Research Laboratories, Rahway, N. J., for these determinations, obtained through the courtesy of Dr. Karl Pfister.

Ultraviolet evidence established that one mole of hydroxypicolinic acid was present in Etamycin.[3] Titration with aqueous sodium hydroxide indicated a molecular weight of 890. Analytical data are in good agreement with an empirical formula, $C_{44}H_{62}N_8O_{10}\cdot H_2O$ for Etamycin dried at 110° (0.01 mm.) or below and with the formula $C_{44}H_{62}N_8O_{10}$ for material dried at 135° (0.01 mm.). All evidence points to the presence of only one of each component in Etamycin.

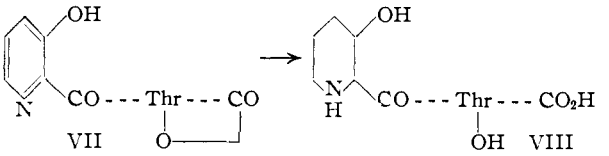
Lactone Structure.—Evidence that Etamycin is a macrocyclic lactone was obtained as follows. The antibiotic has no acidic or basic groups except the phenolic hydroxyl group and tertiary nitrogen of the 3-hydroxypicolinic acid residue. The infrared spectrum of Etamycin possesses a peak at 1745 cm.$^{-1}$ characteristic of an ester function. Treatment with 0.1 *N* sodium hydroxide at room temperature produced as the sole product the salt of an antibiotically inactive acid, Etamycin acid, which contains the hydroxypicolinic acid and all amino acids, and has a peak at 1730 cm.$^{-1}$ attributable to the carboxyl group. No second fragment containing a hydroxyl group could be detected[25] which suggested a lactone structure. Etamycin acid is recovered unchanged on storage in dilute acid solution. Preliminary experiments with N,N-dicyclohexylcarbodiimide gave a product of low biological activity. Under these conditions γ- and δ-hydroxyacids would be expected to form lactones readily. Four times as much periodic acid was consumed by Etamycin acid as by Etamycin under identical and vigorous conditions (45 hr., 37°, in 80% acetic acid). It is evident that the lactone link involves the terminal carboxyl group of the peptide and the hydroxyl group of either threonine or hydroxyproline.

Treatment of Etamycin acid with chromic acid in acetic acid–pyridine destroyed both hydroxyproline and threonine. Under the same conditions the hydroxyproline in Etamycin was destroyed, but threonine was unaffected. Consequently the lactone link joins the terminal carboxyl group with the hydroxyl group of threonine. It is of interest that the change of rotation in going from the macrocyclic lactone Etamycin ($[\alpha]^{26}D$ +31°) to Etamycin acid ($[\alpha]^{25}D$ −7.9°) is in the direction predicted by an extension of Witkop's application[26] (to the case of 5- and 6-membered amino acid lactones) of Hudson's lactone rule.[27]

Amino Acid Sequence.—The sequence of amino acids in Etamycin was deduced by stepwise degradation and independently by partial hydrolysis. Preliminary experiments with enzymatic degradation using carboxypeptidase and papain were unpromising.

The observation that neither Etamycin nor Etamycin acid contains a primary or secondary amino group suggested that the hydroxypicolinic acid was attached to the N-terminal amino acid of the peptide chain. To obtain a compound susceptible to stepwise degradation from the amino end, the hydroxypicolinic acid had to be removed under conditions which left the peptide chain unaffected. After several attempts, this was accomplished by catalytic hydrogenation of the pyridine ring and saponification of the lactone, thus producing 3-hydroxypipecolic acid as the N-terminus of an octapeptide which could be degraded by conventional methods (*cf.* partial formulas VII → VIII). Ultraviolet spectra and microanalyses of the hydrogenated Etamycin revealed that under the conditions applied the hydroxypicolinic acid and part of the phenylsarcosine were hydrogenated.

The hydroetamycin acid VIII was degraded with phenyl isothiocyanate, following the method of Edman,[28] and using some of the modifications of later workers.[29] It was found that for peptides



in this series dioxane–water (1:1) was more convenient than pyridine–water or acetic acid–water, both as a solvent for phenylthiocarbonyl (PTC) peptide formation and for the subsequent acid-catalyzed formation of the phenylthiohydantoins (PTH's). Furthermore, in the later stages of the degradation, homogeneous (by paper chromatography) peptides could be obtained only if each successive PTC peptide was isolated (by extraction from the reaction mixture) before the acid cleavage. The progress of the PTH formation from PTC peptides was followed by ultraviolet absorption measurements.[29a] Four independent methods were used to follow the course of the degradations, as described in the Experimental section.

Six successive Edman degradations revealed the sequence HyPic–L-Thr–D-Leu–D-aHypro–Sar–L-DiMeLeu–. The seventh degradation (carried out on a very small amount of material) indicated but did not establish definitely L-Ala as the next amino acid.

At this point it was necessary only to determine the C-terminal amino acid to obtain the complete sequence of Etamycin. Hydrazinolysis[30] of Etamycin acid showed L-PheSar to be C-terminal, and therefore the structure of Etamycin is represented by I.

The partial hydrolysis of Etamycin was investigated using various concentrations of hydrochloric acid at 37° for periods of time ranging from 3 days to 2 months. The hydrolysates were examined by means of two-dimensional paper chromatography. It was observed that, although the rate of hydrolysis increased with the concentration of acid used, the course of the hydrolysis was qualitatively the same.

A number of fluorescent peptides, containing hydroxypicolinic acid, could be separated from the amino acids using butanol–acetic acid, and from each other by use of the *t*-amyl alcohol–phthalate

(25) Experiments by R. L. Cargill.

(26) B. Witkop, *Experientia*, **12**, 372 (1956).

(27) C. S. Hudson, THIS JOURNAL, **32**, 338 (1910); **61**, 1525 (1939).

(28) P. Edman, *Acta Chem. Scand.*, **4**, 283 (1950); **10**, 761 (1956).

(29) (a) H. Fraenkel-Conrat and J. I. Harris, THIS JOURNAL, **76**, 6058 (1954); (b) T. Wieland and W. Schön, *Ann.*, **593**, 157 (1955).

(30) S. Akabori, K. Ohno and K. Narita, *Bull. Soc. Chem. Japan*, **25**, 214 (1952); K. Ohno, *J. Biochem. Jap.*, **40**, 621 (1953); C. Niu and H. Fraenkel-Conrat, THIS JOURNAL, **77**, 5882 (1955).

buffer system.[31] The amino acid composition of three of these peptides was determined to be HyPic–(Thr, Leu), HyPic–(Thr, Leu)–HyPro–, and HyPic–(Thr, Leu)–HyPro–Sar.

Taking into account the composition of these peptides and noting that Ala and PheSar appear in hydrolysates before other amino acids, that Thr appears last in hydrolysates and that PheSar is C-terminal, the complete amino acid sequence of Etamycin could be deduced as formula I. However, in the present case we regard the Edman degradation as providing the more convincing evidence for the structure of Etamycin.

The Etamycin structure is reminiscent of that of the actinomycins.[32] All have a heterocyclic carboxylic acid as the amino terminus of a macrocycle peptide lactone and contain D- and N-methylamino acids. In addition the amino acid sequences have features in common. Information on the composition of the antibiotic Echinomycin,[33] while incomplete, indicates that it may well be of the same type as Etamycin and the actinomycins.

However, Etamycin is different from the actinomycins in several respects. It contains 3-hydroxypicolinic acid, *allo*-hydroxy-D-proline, L-α-phenylsarcosine and L-β,N-dimethylleucine, none of which has been encountered previously in a natural product. Etamycin does not have the "doubled" character (two 16-membered lactone rings per molecule) of the actinomycins, but possesses one 22-membered ring. Moreover, Etamycin lacks the characteristically high toxicity of the actinomycins.[34]

Acknowledgments.—We are indebted to Bristol Laboratories of Syracuse, N. Y., for our supply of Etamycin, for microbiological assays and for some financial aid. We wish to thank Mrs. Charles F. Howell for her help with the infrared spectra.

Experimental[35]

Purification of Etamycin.—A solution in carbon tetrachloride of partially purified Etamycin (Bristol Lot No. A8002-19-20) was clarified by filtration (Celite). The hydrochloride was precipitated at 0°, washed with petroleum ether and dried over potassium hydroxide and phosphorus pentoxide (under these conditions the hydrochloride is converted slowly to the free base). Treatment with aqueous sodium bicarbonate gave the free base, which was chromatographed on neutral alumina (activity V[36]). The methylene chloride eluate was evaporated and the residue dissolved in methanol and treated with charcoal. Lyophilization from benzene gave pure Etamycin, m.p. 168–170° dec., λ_{max} 304.5 mμ (log ϵ 3.91) in ethanol, $[\alpha]^{26}D$ +62° (*c* 5.0 in chloroform), $[\alpha]^{26}D$ +31° (*c* 5 in ethanol) [reported[3] 162–165°, λ_{max} 304 mμ ($E^{1\%}_{1cm}$ 92, corresponding to log ϵ 3.91), $[\alpha]^{25}D$ +59° (*c* 5 in chloroform), $[\alpha]^{25}D$ +28° (*c* 5 in ethanol)]. This material was used for all experiments unless otherwise stated; microbiological assay for L-threonine in total hydrolysates calcd. 11.6, found 10.7 and 11.2.[24]

Anal. Calcd. for $C_{44}H_{62}N_8O_{10}$: C, 60.12; H, 7.11; N, 12.75. Found: C, 59.95; H, 7.40; N, 13.07 (dried at 135° (0.01 mm.)). Calcd. for $C_{44}H_{62}N_8O_{10}\cdot H_2O$: C, 58.91; H, 7.19; N, 12.49; 3N-CH_3, 4.35; equiv. wt., 897. Found: C, 58.96; H, 7.17; N, 12.54; N-CH_3, 4.63; equiv. wt. (potentiometric titration with 0.01 *N* sodium hydroxide), 890 (dried at 110° (0.01 mm.)).

Etamycin Acid.—A solution of 0.9 (1 mmole) of Etamycin in 30 ml. of 0.1 *N* sodium hydroxide was allowed to stand for 2 hours at room temperature. After treatment with an excess of carbon dioxide, the filtered (Norit and Celite) solution was extracted with three portions of methylene chloride. The acidified solution (*p*H 3) was again extracted 3 times with methylene chloride. Evaporation of the latter extract gave Etamycin acid as a colorless powder, m.p. 140–143° dec., $[\alpha]^{25}D$ −7.9° (*c* 5.0 in ethanol). The ultraviolet spectrum is virtually identical with that of Etamycin. In the infrared (potassium bromide) the carbonyl frequency is shifted from 1745 cm.$^{-1}$ (Etamycin [lactone]) to 1730–1735 cm.$^{-1}$ (Etamycin acid). Potentiometric titration indicated that an equivalent amount of carboxyl and phenolic acidity was present.

Total Hydrolysis of Etamycin.—Etamycin was hydrolyzed by heating at 105° for 16 hours with 6 *N* hydrochloric acid in a sealed tube. Two dimensional chromatograms of the hydrolysate showed seven ninhydrin-positive spots, four of which corresponded to the previously identified Thr, Ala, aHyPro and Leu. The R_f values of these spots were (phenol, butanol–acetic acid)[37]: Thr (0.33, 0.19), Ala (0 47, 0.24), HyPro (0.54, 0.13), Sar[38] (0.67, 0.19), Leu (0.74, 0.60), PheSar[38] (0.88, 0.55), DiMeLeu[38] (0.92, 0.68). The Thr, Ala and Leu spots were purple, the HyPro spot was yellow and the three spots identified as Sar, PheSar and DiMeLeu were brownish-purple; the latter spots did not develop rapidly below 100° and were not as intense as the spots due to Ala, Thr and Leu. The HyPro spot gave a bright red color when subjected to the sensitive isatin–Ehrlich test.[39] This test was applied subsequent to ninhydrin to detect small quantities of HyPro on chromatograms.

The amino acids identified as PheSar and DiMeLeu gave bright red spots with the *p*-nitrobenzoyl chloride–pyridine test, indicative of N-alkylamino acids.[40] A convenient way to perform the test is to dip the chromatogram in an 0.2% solution of *p*-nitrobenzoyl chloride in benzene and, after allowing the benzene to evaporate, in pyridine–petroleum ether (1:10). The color appears immediately after the pyridine treatment and fades in a few minutes. It is possible to use the test before, but not after treatment of the chromatograms with ninhydrin.

Amino Acid Content of Etamycin.—A quantitative paper chromatographic method, similar to that described by Pernis and Wunderly,[41] was used to determine the ratios of amino acids in Etamycin (in conjunction with microanalytical data). The method also was used to follow the Edman degradation and in the examination of partial hydrolysates.

Two-dimensional paper chromatograms of an Etamycin total hydrolysate gave an uncorrected ratio of 1.0:1.0:0.88:0.86:0.32:0.69 for Leu:Ala:Thr:Sar:PheSar:DiMeLeu. After identification of the N-methylamino acids,

(31) S. Blackburn and A. G. Lowther, *Biochem. J.*, **48**, 126 (1951).

(32) H. Brockmann, *et al.*, *Angew. Chem.*, **68**, 66 (1956); E. Bullock and A. W. Johnson, *J. Chem. Soc.*, 3280 (1957).

(33) W. Keller-Schierlein and V. Prelog, *Helv. Chim. Acta*, **40**, 205 (1957).

(34) "Handbook of Toxicology, Vol. 2: Antibiotics," W. S. Spector, Ed., W. B. Saunders Co., Philadelphia, Pa., 1957, p. 7; *cf.* p. 70 for the toxicity of Etamycin.

(35) Melting points are corrected unless otherwise specified. The microanalyses are by Dr. S. M. Nagy and associates at M.I.T., and W. Manser at E.T.H., Zürich.

(36) H. Brockmann and H. Schodder, *Ber.*, **74**, 73 (1941).

(37) The phenol system consisted of phenol–water (4:1). The butanol–acetic acid had the composition 1-butanol–acetic acid–water (4:1:5).

(38) These amino acids were identified after isolation (*vide infra*).

(39) J. B. Jepson and I. Smith, *Nature*, **171**, 43 (1951); **172**, 1100 (1953).

(40) Waser[8a] and Edlbacher[8b] described this as a test for α-amino acids, to be carried out in a test-tube. Plattner and Nager[8c] found that when the test is performed on paper chromatograms, α-methylamino acids (except sarcosine) give intense red colors, while α-amino acids do not give a positive test. In our experience a positive test was given by the N-methyl derivatives of valine, norvaline, α-phenylglycine, β-phenylalanine, leucine, *allo*-isoleucine, isoleucine, norleucine and β-methylleucine, and by pipecolic acid and N-ethylleucine. No color was given by sarcosine, proline, *allo*-hydroxyproline or N-methyl-γ-aminobutyric acid.

(41) B. Pernis and C. Wunderly, *Biochim. et Biophys. Acta*, **11**, 209 (1953). In our procedure, the amino acid spots were located by development at 100° after dipping in 0.05% ninhydrin in acetone. After the treatment with methanolic potassium hydroxide, the cut-out spots and suitable blanks were stored overnight in a vacuum desiccator over sulfuric acid. The colors then were developed fully by the procedure of Moore and Stein,[42] and determined by the use of a Coleman Junior Spectrophotometer at 570 mμ.

(42) S. Moore and W. H. Stein, *J. Biol. Chem.*, **176**, 367 (1948).

color yields were determined (using two-dimensional paper chromatograms prepared from a standard mixture of the pure amino acids) to be Sar:PheSar:DiMeLeu = 0.89:0.32:0.60, with Ala = 1.0 as standard. Application of these color yields and the recorded[42] ones for Leu, Ala and Thr to the uncorrected ratio gives a corrected ratio of 1.0:1.0:0.96:0.97:1.0:1.1 for Leu:Ala:Thr:Sar:PheSar:DiMeLeu in Etamycin. Hydroxyproline could not be determined accurately by this method.

Preparative Paper Chromatography.—An Etamycin total hydrolysate was subjected to the deamination procedure of Witkop and Foltz,[9] which destroyed Thr, Ala and Leu and gave a mixture of the hydrochlorides of the secondary amino acids. The deaminated hydrolysate was chromatographed on Whatman No. 3 paper (*ca.* 25 mg. per sheet), using butanol–acetic acid as the developing solvent. Bands containing HyPro, Sar, PheSar and DiMeLeu were revealed after streaking with 0.1% ninhydrin in acetone and development of the color at 100°.

Isolation of Sarcosine as the Dinitrophenyl Derivative.—Ten "sarcosine" strips from Whatman No. 3 chromatograms were eluted chromatographically with water. The eluted amino acid was converted[43] to the dinitrophenyl derivative. After recrystallization from ethanol–water, 10.3 mg. of 2,4-dinitrophenylsarcosine, m.p. 185–186°, was obtained. A mixed m.p. with authentic DNP-sarcosine[44] (m.p. 186–187°) was 185–186°, and the infrared spectra of the two were identical.

Isolation of α-Phenylsarcosine. A. D,L-α-Phenylsarcosine (IIa).—The concentrated eluate from 35 "phenylsarcosine" strips (Whatman No. 3 chromatograms) was acidified to *p*H 2 and extracted with ethyl acetate. The residue, obtained by evaporation of the aqueous layer, was dissolved in 11 ml. of ethanol and centrifuged to remove a trace of insoluble material. Evaporation of the ethanol gave 610 mg. of light brown solid. The material was dissolved in water and adsorbed on a short column of Dowex 50-X8 (<400 mesh; hydrogen cycle). After the column had been washed thoroughly with water (*ca.* 80 ml.), the amino acid was eluted with N-ammonium hydroxide. The course of the elution was followed easily by means of the red color test.[8] Evaporation of the eluate gave a fluffy tan solid, which was dissolved in a small quantity of water and decolorized with Norit; filtration and evaporation gave 212 mg. of colorless crystalline solid. Recrystallization from aqueous acetone, followed by sublimation at 160–170° (0.01 mm.), produced 123 mg. of D,L-α-phenylsarcosine, sublimation point 245–246°; λ_{max} 252, 257, 262, 268 mμ (log ε's 2.30, 2.42, 2.40, 2.26), λ_{min}. 247 (log ε 1.90) in ethanol. A portion was recrystallized from aqueous acetone for analysis. The infrared and ultraviolet spectra of the D,L-α-phenylsarcosine were identical with those of authentic material.

Anal. Calcd. for $C_9H_{11}NO_2$: C, 65.44; H, 6.71; N, 8.48. Found: C, 65.30; H, 6.66; N, 8.57.

B. **Optically Active α-Phenylsarcosine (IIb).**—Partially purified Etamycin hydrochloride (5 g.) was hydrolyzed by reflux for 25 min. with 30 ml. of 12% hydrochloric acid. The hydrolysate was deaminated by the above procedure[9] except that the hydrolysis of the nitrosoamino acids was performed by refluxing 20 min. with 12% hydrochloric acid. Paper chromatography of the hydrolysate and elution of the amino acid were as described previously (20 sheets of Whatman No. 3). The eluate was chromatographed directly on Dowex 50-X8, and the evaporated eluate was dissolved in water, decolorized with Norit, filtered and evaporated to give 60 mg. of α-phenylsarcosine, $[\alpha]^{31}D$ +118 ± 2° (*c* 4.8 in *N* hydrochloric acid). Recrystallization from aqueous ethanol did not change the rotation appreciably.

Tosyl-D,L-α-phenylglycine.—Tosyl-D,L-α-phenylglycine was prepared by the procedure recommended by Katsoyannis and du Vigneaud[45] for the preparation of tosyl-L-isoleucine. From 2.3 g. of α-phenylglycine there was obtained 2.8 g. (60%) of tosyl-D,L-α-phenylglycine, m.p. 181.5–183.5°. Recrystallization from aqueous ethanol gave an analytical sample, m.p. 182–184°.

Anal. Calcd. for $C_{15}H_{15}NO_4S$: C, 59.00; H, 4.95; N, 4.59. Found: C, 59.26; H, 5.11; N, 4.80.

Tosyl-D,L-α-phenylsarcosine.—A mixture of 1.0 g. of tosyl-D,L-α-phenylglycine, 3.4 ml. of 2 *N* sodium hydroxide and 0.24 ml. of methyl iodide was stirred magnetically in a sealed tube for 30 min. at 65–70°. After acidification with hydrochloric acid the product was extracted into ethyl acetate and back-extracted into *N* potassium bicarbonate. After acidification and transfer into ethyl acetate, evaporation and crystallization from a mixture of ethyl acetate and cyclohexane gave 0.55 g. of tosyl-D,L-α-phenylsarcosine, m.p. 143.5–146.5°. Two recrystallizations from the same mixture of solvents provided an analytical sample, m.p. 145.5–146.5°.

Anal. Calcd. for $C_{16}H_{17}NO_4S$: C, 60.11; H, 5.37; N, 4.39. Found: C, 60.43; H, 5.42; N, 4.25.

D,L-α-Phenylsarcosine.[46]—A solution of 250 mg. of tosyl-D,L-α-phenylsarcosine in a mixture of 3 ml. of glacial acetic acid and 3 ml. of concentrated hydrochloric acid was heated for 16 hours at 105° in a sealed tube. After evaporation of solvent, the residue was dissolved in a small amount of water and adsorbed on Dowex 50-X8 (<400 mesh; hydrogen cycle). The column was washed with water, and the amino acid was eluted with N-ammonium hydroxide. Upon evaporation D,L-α-phenylsarcosine, sublimation point 246–246.5°, was obtained (reported sublimation point 274°,[47a] 270°[47b]). After sublimation at 160–170° (0.01 mm.) and recrystallization from aqueous ethanol the sublimation point was unchanged. The temperature at which sublimation is complete depends on the experimental conditions. In our determinations, loosely packed material was used, and the rate of heating was about 2° per minute.

Tosyl-D-α-phenylglycine.—D-α-Phenylglycine,[48] $[\alpha]^{48}D$ −168° (*c* 2 in 5 *N* hydrochloric acid) [reported[13] $[\alpha]^{25}D$ −169.0° (*c* 1 in 5 *N* hydrochloric acid)] was converted by the usual procedure[45] to the tosyl derivative, which was recrystallized from aqueous ethanol, m.p. 179–180°, $[\alpha]^{28}D$ −114° (*c* 2.1 in ethanol).

Anal. Calcd. for $C_{15}H_{15}NO_4S$: C, 59.00; H, 4.95; N, 4.59. Found: C, 59.06; H, 5.19; N, 4.32.

Tosyl-D-α-phenylsarcosine.—Tosyl-D-α-phenylglycine (900 mg.) was methylated as described for the D,L-compound. Crystallization of the product from ethyl acetate–cyclohexane gave 275 mg. of impure tosyl-D,L-phenylsarcosine, m.p. 134–145°. The residue obtained by evaporation of the mother liquors was crystallized from benzene–cyclohexane to give 335 mg. of tosyl-D-α-phenylsarcosine, m.p. 115–120°. Recrystallization from ethyl acetate–petroleum ether afforded an analytical sample, m.p. 115.5–117.0°, $[\alpha]^{27}D$ −63° (*c* 0.8 in ethanol).

Anal. Calcd. for $C_{16}H_{17}NO_4S$: C, 60.11; H, 5.37; N, 4.39. Found: C, 60.27; H, 5.12; N, 4.41.

D-α-Phenylsarcosine (IIc).—To a solution of 275 mg. of tosyl-D-α-phenylsarcosine in *ca.* 15 ml. of liquid ammonia were added small pieces of sodium until a permanent blue color was obtained. Destruction of the excess sodium (ammonium chloride) and evaporation gave a white solid. A solution of the latter in water was adsorbed on Amberlite IR-400 (hydroxide cycle), and, after thorough washing, the amino acid was eluted with *N* hydrochloric acid. The acidic solution was chromatographed over Dowex 50-X8 hydrogen cycle) to afford (after elution with *N* ammonium hydroxide and evaporation) 140 mg. of D-α-phenylsarcosine. Recrystallization from aqueous acetone provided an analytical sample, sublimation point 249°, $[\alpha]^{28}D$ −114° (*c* 1.4 in 5 *N* hydrochloric acid).[18]

Anal. Calcd. for $C_9H_{11}NO_2$: C, 65.44; H, 6.71; N, 8.48. Found: C, 65.06; H, 6.80; N, 8.14.

(43) F. Sanger, *Biochem. J.*, **39**, 507 (1945).

(44) C. E. Dalgliesh, A. W. Johnson, A. R. Todd and L. C. Vining, *J. Chem. Soc.*, 2946 (1950). The m.p. reported by these authors was 178°.

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(46) During preliminary experiments on the N-methylamino acids of Etamycin, chromatographic samples of a number of N-methylamino acids were prepared by the modification of the Fischer–Lipschitz[14] procedure, exemplified by the preparation of D,L-PheSar. The *Rf* values of these N-methylamino acids in butanol–acetic acid (together with Sar, Ala, Leu and DiMeLeu) were: Sar (0.18), Ala (0.25), N-MeVal (0.49), N-Me-D,L-NVal (0.54), D,L-PheSar (0.53), Leu (0.58), N-MePhe (0.59), N-Me-D-aiLeu (0.61), N-MeiLeu (0.63), N-MeLeu (0.65), N-Me-D,L-NLeu (0.66) and DiMeLeu (0.68).

(47) (a) F. Tiemann and R. Piest, *Ber.*, **14**, 1982 (1881); (b) F. Knoop, *ibid.*, **52**, 2266 (1919).

(48) M. Betti and M. Meyer, *ibid.*, **41**, 2071 (1908).

Isolation of β,N-Dimethylleucine. A.—The dimethylleucine from the paper eluate (57 sheets of Whatman No. 3) was adsorbed on Dowex 50-X8 (<400 mesh), and after thorough washing with water, eluted with 2 *N* ammonium hydroxide. The crude amino acid was purified further by sublimation at 120–155° (0.01 mm.) and charcoal treatment of the sublimate. Two recrystallizations from aqueous acetone gave 208 mg. of dimethylleucine as colorless needles, m.p. 315–316° dec. (uncor.), $[\alpha]^{28}D$ +36.2° (*c* 2.4 in 5 *N* hydrochloric acid). A third recrystallization raised the rotation to $[\alpha]^{29}D$ +39.2° (*c* 2.2 in 5 *N* hydrochloric acid), $[\alpha]^{29}D$ +33.15° (*c* 2.0 in water).

Anal. Calcd. for $C_8H_{17}O_2N$: C, 60.34; H, 10.76; N, 8.80. Found: C, 60.12; H, 10.47; N, 8.77.

B.—Etamycin was hydrolyzed and the hydrolysate chromatographed under the conditions described for the isolation of optically active PheSar. The eluate of 20 sheets of Whatman No. 3 paper was purified through the ion exchange procedure, and the product rechromatographed on 3 sheets of Whatman No. 3 (to remove amino acid impurities). Subsequent chromatography on Dowex 50, recrystallization from water–acetone–ether and drying gave 55 mg. of dimethylleucine with a lower rotation than DiMeLeu isolated by procedure A, $[\alpha]_D^{30}$ +33.2° (*c* 1.9 in 5 *N* hydrochloric acid), $[\alpha]^{30}D$ +26.0° (*c* 1.8 in water).

Degradation of β,N-Dimethylleucine.—Following Plattner and Nager's[13] modification of Langheld's[16] procedure, 80 mg. (0.50 mmole) of DiMeLeu was degraded with sodium hypochlorite to an amine and an aldehyde component. The DNP-amine, after 3 recrystallizations from ethanol, had m.p. 182–183°, undepressed upon admixture of authentic DNP-methylamine. The DNPH of the aldehyde fragment (47 mg.) after three recrystallizations from 95% ethanol had $[\alpha]^{28}D$ −38.7° (*c* 1.5 in chloroform), and m.p. 125.2–126.0°, undepressed upon admixture of (−)-DNPH of isopropylmethylacetaldehyde. Infrared spectra of the DNP-methylamine and of the DNPH were identical with those of authentic samples.

(−)-DNPH of Isopropyl Methyl Acetaldehyde.—By a procedure analogous to that of Slomp, *et al.*,[21] 800 mg. (*ca.* 2 mmoles) of ergosterol was ozonized in methylene chloride–pyridine solution at −60°. Two mmoles of ozone was taken up rapidly and then the rate of reaction slowed down. After decomposition with glacial acetic acid–zinc dust, 150 ml. of water was added, and the methylene chloride and 100 ml. of aqueous phase were distilled off under nitrogen. From the distillate the volatile aldehyde was isolated as the DNPH, which, after chromatography[14] over alumina (neutral Woelm, activity V)[36] and 2 recrystallizations from 95% ethanol had m.p. 124–125°, $[\alpha]^{32.5}D$ −34.5° (*c* 1.8 in chloroform) (reported[20] m.p. 124–124.5°, $[\alpha]^{26}D$ −37.7°).

Partial Hydrolyses of Etamycin.[49]—Etamycin was subjected to 10%, 20% and concentrated hydrochloric acid at 37°. Samples were withdrawn after various lengths of time ranging from 3 days to 2 months, and examined by means of two dimension paper chromatography (phenol, butanol–acetic acid). In the course of a hydrolysis the order of appearance of individual amino acids was (PheSar, Ala)–(other amino acids)–Thr. A ninhydrin-positive spot (R_f between those of Ala and Leu in butanol–acetic acid) was detected in the latter stages of several hydrolyses. It was presumably due to a peptide (or peptides), but preliminary attempts at characterization were unpromising.

A number of fluorescent peptides (containing hydroxypicolinic acid) were observed in partial hydrolysates. A hydrolysate obtained by treatment of Etamycin for one week at 37° with 20% hydrochloric acid proved advantageous for the separation of fluorescent peptides. The partial hydrolysate was chromatographed on Whatman No. 1 paper using butanol–acetic acid. Bands of fluorescent peptides near the solvent front were cut out, eluted with water and re-chromatographed on buffered paper using the *t*-amyl alcohol–*p*H 6 phthalate buffer system.[31] The peptides separated into five fluorescent bands (R_f's 0.42, 0.51, 0.60, 0.81 and 0.88). Strips containing peptides were sprayed with concentrated hydrochloric acid–methanol (1:9), and extracted continuously with acetone for 6 hours (Soxhlet extractor). Evaporation of the extract and acid hydrolysis (16 hours, 6 *N* HCl, 105°) gave a mixture of amino acids from the peptide and a (comparatively) large amount of phthalic acid. After several extractions with ethyl acetate (to remove the phthalic acid) the hydrolysate was evaporated and chromatographed (phenol–butanol–acetic acid). The peptides having R_f's of 0.81, 0.60 and 0.51 were shown to contain (Thr and Leu), (Thr, Leu and HyPro) and (Thr, Leu, HyPro and Sar), respectively. Therefore, they may be formulated as Pic-(Thr, Leu), Pic-(Thr, Leu)-HyPro and Pic-(Thr,Leu)-HyPro-Sar. By the quantitative ninhydrin method it was possible to discriminate between peptide constituents and trace impurities.

Chromic Acid Oxidation of Etamycin and Etamycin Acid.—A chromic acid solution was prepared from 100 mg. of chromic acid, 0.10 ml. of pyridine and 3.0 ml. of acetic acid. Etamycin and Etamycin acid were oxidized under identical conditions as follows. A solution of 10 mg. of peptide in 0.15 ml. of chromic acid solution was allowed to stand for 16 hours at room temperature. The reaction mixture, diluted with 5 ml. of water, was extracted with four 5-ml. portions of chloroform, and the extract was dried (sodium sulfate), and evaporated to dryness. The residue was hydrolyzed for 16 hours in 3 ml. of 6 *N* hydrochloric acid at 105°, and the hydrolysate was evaporated, flushed with water and dissolved in 0.50 ml. of water. This solution was subjected to two dimensional paper chromatography (phenol–butanol–acetic acid).

When the chromatogram from oxidized Etamycin was developed with ninhydrin, strong spots corresponding to all amino acids except hydroxyproline appeared. The isatin–Ehrlich test[39] for hydroxyproline also was negative. The chromatogram from oxidized Etamycin acid, developed with ninhydrin, showed only a trace of threonine and strong spots corresponding to all other amino acids except hydroxyproline. A very faint isatin–Ehrlich test for hydroxyproline was obtained. An earlier experiment was carried out under milder conditions which did not oxidize completely hydroxyproline in either Etamycin or Etamycin acid. In this case it was shown by quantitative paper chromatography that in Etamycin acid about 80% of the threonine was destroyed, while in Etamycin no detectable destruction of threonine had occurred.

The Hydrogenation of Etamycin.—A solution of 2 g. of Etamycin in 15 ml. of glacial acetic acid was hydrogenated over 0.2 g. of platinum oxide (Adams catalyst) at 50 atm. and 50° for 16 hours. After removal of the catalyst by filtration, the solution was freeze-dried to give the acetate salt of the hydrogenated peptide, which was converted (sodium bicarbonate) to the free base (2 g.).

The product gives no color with ferric chloride and shows no absorption at 304 mμ. The extinction of the band at 268 mμ indicated that approximately one-half of the phenylsarcosine was hydrogenated. No detectable acidic group was present, but 0.97 equivalent of a basic function per mole of peptide could be titrated potentiometrically with 0.01 *N* hydrochloric acid.

Edman Degradation.—In the following description, the course of the Edman degradation of hydroetamycin is outlined insofar as the standard procedures[28,29] were modified to adapt them to the special properties of the peptide.

A solution of 90 mg. (0.1 mmole) of hydroetamycin in 4 ml. of *N* sodium hydroxide–dioxane (1:1) was allowed to stand overnight at room temperature. The *p*H of the solution was adjusted to 8.9 with *N* hydrochloric acid–dioxane (1:1) using a *p*H meter, and 0.35 ml. of phenyl isothiocyanate was added. While the mixture was stirred magnetically for 2.5 hours at 40°, 0.1 *N* sodium hydroxide–dioxane (1:1) was added slowly to keep the *p*H at 8.7–9.0. After several extractions with cyclohexane and benzene (using a centrifuge), the solution was acidified to *p*H 4.5 and the PTC peptide extracted with ethyl acetate. The organic layer was dried with sodium sulfate, filtered and evaporated under reduced pressure to give the PTC hydroetamycin acid. A solution of this in 6 *N* hydrochloric acid–dioxane (1:1) was kept at room temperature. The progress of the reaction was followed by ultraviolet measurements between 240 and 270 mμ.[29a] After 4 hours, absorption in the broad maximum at 264 mμ did not increase, indicating that the formation of the hydroxypipecolyl-PTH was complete. The PTH was extracted with ethyl acetate and the second peptide was obtained by evaporation of the aqueous layer under reduced pressure.

Following the aforementioned procedure seven subsequent degradations were carried out. The time required for the acid degradation of the PTC peptides varied from 2 hours

(49) Some preliminary experiments were by Y. L. Yeh.

for the threonyl peptide to 5 hours for the sarcosyl peptide. The results of the degradations were determined by four methods: A, paper chromatography of the PTH's[50]; B, paper chromatography of the amino acids obtained by acid hydrolysis of the PTH's at 150°[51]; C, dinitrophenylation and hydrolysis of each successive peptide, followed by paper chromatography[31] of the resulting DNP-amino acids, and D, quantitative estimation of the amino acid content of each successive peptide after total hydrolysis and two dimensional paper chromatography.

Individual Steps of the Degradation. Hydroxypipecolic Acid.—The PTH (method A) had approximately the same R_f value in pyridine–heptane (3:7) as hydroxyproline-PTH and threonine-PTH. Methods B and C gave only decomposition products.

Threonine.—The PTH (method A) had the same R_f as authentic Thr-PTH, and the spot showed the characteristic pink center. Method B gave only decomposition products. DNP-Thr (method C) could be obtained when the DNP peptide was hydrolyzed for 6 hours instead of the usual 12–14 hours. Method D gave a ratio Leu:Thr of 10:1 showing the disappearance of Thr in this degradation step.

Leucine.—In this and the following steps method A gave no clear-cut results. Methods B and C clearly established Leu as the 3rd amino acid, and this was confirmed by method D, which gave a ratio Ala:DiMeLeu:Leu of 82:79:2.

Hydroxyproline.—The best evidence for the position of aHyPro is the disappearance of this amino acid in the 4th degradation step (method D). Hydrolysis of the PTH (method B) gave a small amount of HyPro, in spite of the vigorous conditions. The DNP-aHyPro was destroyed under the normal conditions used for hydrolysis of the DNP peptide (method C).

Sarcosine.—The only conclusive evidence was given by method D, which gave the ratio Ala:DiMeLeu:PheSar:Sar = 24:12:13:0.

Dimethylleucine.—Method D gave the ratio Ala:PheSar:DiMeLeu = 16:20:0.

Alanine.—Hydrolysis of the PTH produced Ala (method B), but this could not be regarded as conclusive because small amounts of Ala appeared in PTH hydrolysates of previous degradation steps.

The Hydrazinolysis[30] of Etamycin Acid.—A solution of 10 mg. of Etamycin acid in 0.3 ml. of anhydrous hydrazine (freshly distilled from barium oxide) was heated in a sealed tube at 105° for 16 hours. After evaporation of the hydrazine in a desiccator over sulfuric acid, the residue was dissolved in 1.0 ml. of water and shaken for 2 hours with 0.20 ml. of benzaldehyde. The aqueous layer was separated, extracted with 2–3 ml. portions of ether, and subjected to paper chromatography using butanol–acetic acid as the developing solvent. The only detectable amino acid spot was one due to α-phenylsarcosine, although a small spot with a very high R_f value also was observed (most likely a C-terminal peptide fragment).

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE]

Synthesis of the Pressor-Antidiuretic Hormone, Arginine-Vasopressin

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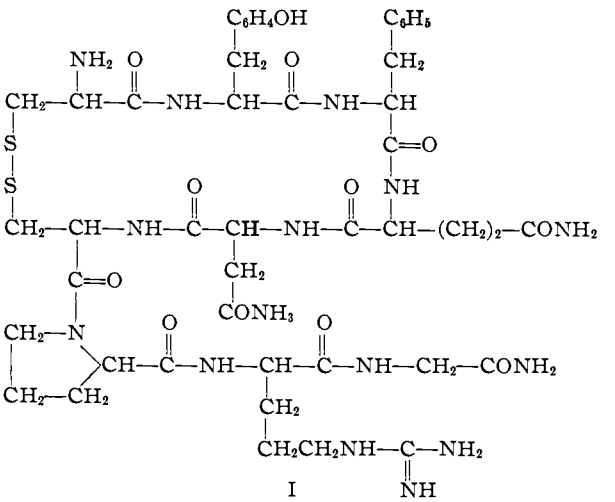
A product synthesized according to the structure proposed for arginine-vasopressin has been found to possess a potency approximately equivalent to that of the pressor-antidiuretic hormone isolated from the posterior pituitary glands of beef. The key intermediate in the synthesis of this hormone was the protected hexapeptide amide, carbobenzoxy-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-arginylglycinamide, which, after cleavage of the carbobenzoxy group with HBr–acetic acid, was coupled with the azide of S-benzyl-N-tosyl-L-cysteinyl-L-tyrosyl-L-phenylalanine. The resulting crude protected nonapeptide hydrobromide was treated with sodium in liquid ammonia to remove the protecting groups and then oxidized by aeration. The biologically active synthetic material thus obtained was purified by countercurrent distribution followed by electrophoresis and compared with natural arginine-vasopressin as to potency, partition coefficient, electrophoretic mobility, amino acid content and chromatography on the resin IRC-50. In all of these comparisons the synthetic material behaved the same as natural arginine-vasopressin.

Degradative studies[2–4] on highly purified preparations of arginine-vasopressin, the principal pressor and antidiuretic hormone of the posterior pituitary gland of beef, allowed structure I to be postulated for this hormone.[4,5]

Synthesis of the hormone according to this structure was undertaken[6] by a route which resembled the synthesis of oxytocin[7,8] in that each involved the preparation of a protected nonapeptide amide, in the case of vasopressin S-benzyl-N-carbobenzoxy - L - cysteinyl - L - tyrosyl - L - phenylalanyl - L - glutaminyl - L - asparaginyl-S - benzyl - L - cysteinyl - L - prolyl - L - arginyl-



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