distribution in the toluene system for 100 transfers. A single peak with $K = 0.94$ was observed. The material isolated from this peak behaved as a single component in paper chromatography: $R_{f\,BAW}$ 0.77, $R_{f\,SBA}$ 0.79, yield 1.6 g. (87.9%), m.p. 104–110°, $[\alpha]^{25}D$ −30.0° (*c* 1, methanol).

Anal. Calcd. for $C_{61}H_{89}N_{13}S_4O_{14}$ (1357): C, 54.0; H, 6.61; N, 13.4. Found: C, 54.2; H, 6.69; N, 13.7

Carbobenzoxy-seryl-tyrosyl-seryl-methionyl-γ-benzyl-glutamyl-histidyl-phenylalanyl-N^G-tosyl-arginyl-tryptophyl-glycyl-N$^\epsilon$-*t*-butyloxycarbonyl-lysyl-N$^\epsilon$-*t*-butyloxycarbonyl-lysyl-N^G-tosyl-arginyl-N^G-tosyl-arginyl-proline *t*-Butyl Ester (XIII).—The decapeptide[9] XII (0.35 g., 200 mmoles) was dissolved in 2 cc. of dimethylformamide, and the solution was cooled in ice and stirred with 0.1 cc. of triethylamine. Compound II (0.076 g., 200 mmoles) was added and stirring was continued for 1 hr. at 0°. Compound VIII (0.25 g., 200 mmoles) was then added and the mixture stirred at room temperature for 24 hr. The reaction mixture was then kept at 37° for another 24 hr. The solvent was removed *in vacuo* at 40°, and the residue was dissolved in the lower phase of the carbon tetrachloride system and submitted to countercurrent distribution for 70 transfers. The material in tubes 0–20 was pooled, evaporated to dryness, washed with water, and triturated with methanol to yield 0.305 g. of XIII, which, however, was contaminated with traces of ninhydrin-positive material (unreacted VIII). A sample of XIII was prepared for analysis by exhaustive washing with methanol until all traces of VIII were removed; $[\alpha]^{25}D$ −51.5° (*c* 2.4, dimethylformamide).

Anal. Calcd. for $C_{138}H_{187}N_{29}S_4O_{33}$ (2908): C, 57.0; H, 6.48; N, 14.0. Found: C, 56.6; H, 6.17; N, 13.8.

Seryl-tyrosyl-seryl-methionyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophyl-glycyl-lysyl-lysyl-arginyl-arginyl-proline (XV). —Compound XIII (0.305 g.) was dissolved in 15 cc. of trifluoroacetic acid in a nitrogen atmosphere and kept at room temperature for 1.5 hr. The trifluoroacetic acid was removed *in vacuo* at room temperature and the residue was dried overnight over P_2O_5–NaOH. The residue was then submitted to countercurrent distribution in the system composed of 1-butanol–acetic acid–water (4:1:5, by volume) for 80 transfers. A fast moving peak, $K = 6.3$ (ultraviolet, Pauly and ninhydrin positive), and a slower moving peak, $K = 0.7$ (ninhydrin positive, ultraviolet and Pauly negative), were seen. The peak with $K = 6.3$ (XIV) was isolated and found homogeneous in paper chromatography; $R_{f\,BAW}$ 0.66, wt. 0.175 g. (yield based on XII is 33%).

Compound XIV (0.16 g.) was dissolved in 200 cc. of freshly distilled liquid ammonia and treated with small pieces of sodium until the blue color persisted for 30–40 min. The ammonia was allowed to evaporate and the residue dried *in vacuo* over P_2O_5–concentrated H_2SO_4. The peptide was desalted[9] on IRC-50 cation exchange resin and eluted with pyridine–acetic acid–water (30:4:66, by volume). The solvent was removed *in vacuo* at room temperature and the residue was dissolved in water and lyophilized to yield 0.11 g. of crude XV. This material was purified by chromatography on a carboxymethylcellulose column (1 × 55 cm.) using continuous gradient elution with ammonium acetate (Fig. 3). The major peak was isolated and rechromatographed on CMC (Fig. 4) to yield, after three lyophilizations, 71 mg. of XV (peptide content determined by ultraviolet absorption at 280 mμ, 87%). Compound XV was found to be homogeneous by electrophoresis on paper at pH 3.7 (mobility relative to lysine, 0.81) and on polyacrylamide gel at pH 4.5 (see Fig. 5). Amino acid analysis is given in Table II; $[\alpha]^{25}D$ −56.8 (*c* 0.5, 0.1 *M* acetic acid).

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[CONTRIBUTION FROM THE HORMONE RESEARCH LABORATORY, UNIVERSITY OF CALIFORNIA, BERKELEY, CALIF.]

A New Synthesis of a Biologically Active Nonadecapeptide Corresponding to the First Nineteen Amino Acid Residues of Adrenocorticotropins

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A completely new method for the synthesis of a nonadecapeptide with an amino acid sequence corresponding to the first nineteen residues of ACTH has been described. The biological and chemical properties of the product are in agreement with those of the synthetic peptide reported by us in 1960.

The synthesis of the nonadecapeptide seryl-tyrosyl-seryl-methionyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophyl-glycyl-lysyl-prolyl-valyl-glycyl-lysyl-lysyl-arginyl-arginyl-proline,[1,2] in 1960, not only served to establish the structural features of the ACTH molecule essential for eliciting adrenal response, but also proved conclusively that the melanocyte-stimulating activity exhibited by the adrenocorticotropins is an intrinsic property. Subsequent work from our laboratory[3,4] as well as the efforts of other investigators[5–7] has shown that the nonadecapeptide corresponding to the first nineteen residues of ACTH probably represents the active core of this hormone. Hence it appeared important to synthesize this peptide by a completely different route and obtain confirmation of the biological properties previously observed.[1]

In our original synthesis, the final steps involved the reaction of the NH_2-terminal tetrapeptide with the C-terminal pentadecapeptide by the azide procedure. With the availability of the crystalline NH_2-terminal decapeptide carbobenzoxy-seryl-tyrosyl-seryl-methionyl γ-benzyl-glutamyl-histidyl-phenylalanyl-N^G-tosyl-arginyl-tryptophyl-glycine[8] (V), the protected nonadecapeptide was prepared by the reaction with a suitably protected nonapeptide derivative.

Two different derivatives of the nonapeptide were synthesized. In one, the ε-amino groups of lysine were blocked with the *t*-butyloxycarbonyl group and the guanidino functions of the arginine residues were masked with the *p*-toluenesulfonyl group. The protected nonapeptide N$^\alpha$-carbobenzoxy-N$^\epsilon$-*t*-butyloxycarbonyl-lysyl-prolyl-valyl-glycyl-N$^\epsilon$-*t*-butyloxycarbonyl-lysyl-N$^\epsilon$-*t*-butyloxycarbonyl-lysyl-N^G-tosyl-ar-

(1) C. H. Li, J. Meienhofer, E. Schnabel, D. Chung, T-B. Lo, and J. Ramachandran, *J. Am. Chem. Soc.*, **82**, 5760 (1960); **83**, 4449 (1961).

(2) All amino acids occurring in the peptides mentioned in this paper are of the L-configuration with the exception of glycine.

(3) C. H. Li, D. Chung, J. Ramachandran, and B. Gorup, *J. Am. Chem. Soc.*, **84**, 2460 (1962).

(4) C. H. Li, J. Ramachandran, and D. Chung, *ibid.*, **85**, 1895 (1963).

(5) K. Hofmann, H. Yajima, T-Y. Liu, and N. Yanaihara, *ibid.*, **84**, 4475 (1962).

(6) K. Hofmann, H. Yajima, T-Y. Liu, N. Yanaihara, C. Yanaihara, and J. L. Humes, *ibid.*, **84**, 4481 (1962).

(7) R. Schwyzer and H. Kappeler, *Helv. Chim. Acta*, **46**, 1550 (1963).

(8) C. H. Li, J. Ramachandran, D. Chung, and B. Gorup, *J. Am. Chem. Soc.*, **86**, 2703 (1964).

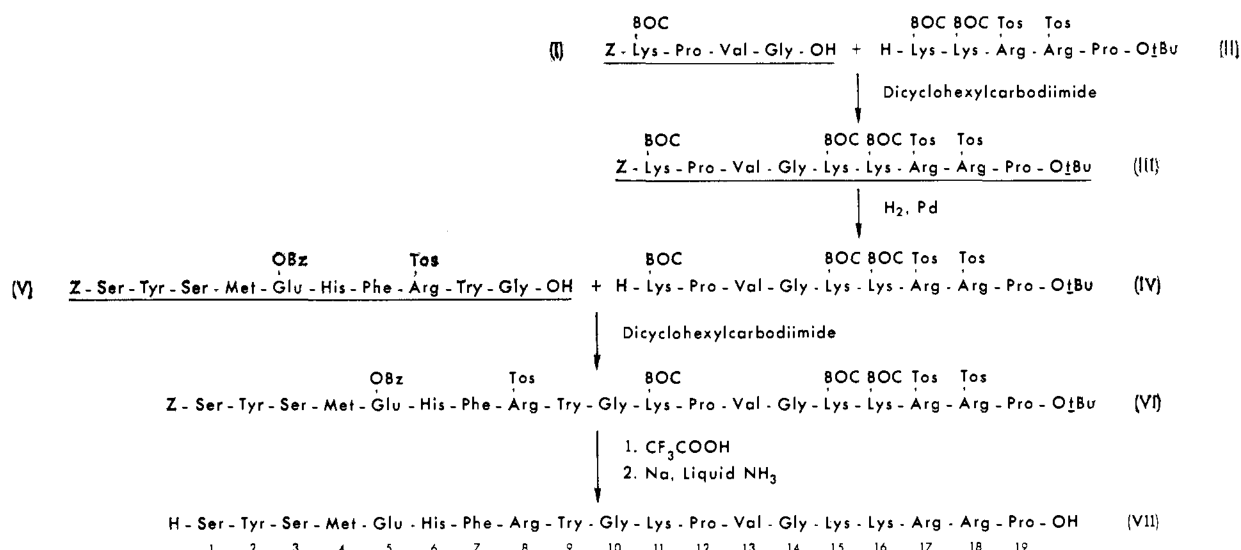

Fig. 1.—Outline of the synthesis of α^{1-19}-ACTH: Z, carbobenzoxy; TOS, p-toluenesulfonyl; BOC, t-butyloxycarbonyl; Bz benzyl.

ginyl-N^G-tosyl-arginyl-proline t-butyl ester (III) was synthesized by the reaction of N$^\alpha$-carbobenzoxy-N$^\epsilon$-t-butyloxycarbonyl-lysyl-prolyl-valyl-glycine (I) with N$^\epsilon$-t-butyloxycarbonyl-lysyl-N$^\epsilon$-t-butyloxycarbonyl-lysyl-N^G-tosyl-arginyl-N^G-tosyl-arginyl-proline t-butyl ester[9a]

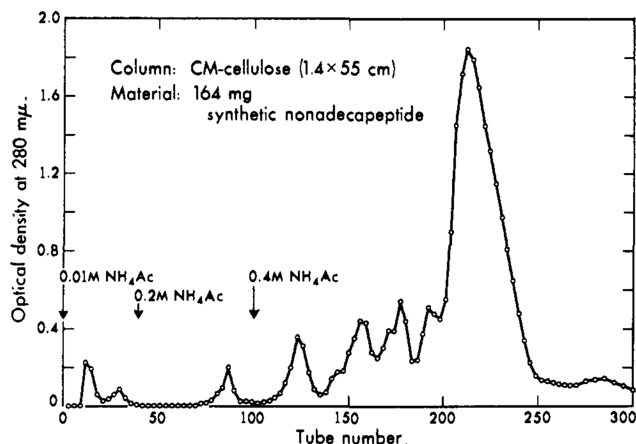

Fig. 2.—CMC chromatography of crude α^{1-19}-ACTH (VII). The initial buffer was 0.01 M ammonium acetate of pH 4.5. After 3–4 hold-up volumes (4 cc./tube) had been collected, a gradient with respect to pH and concentration was started by introducing 0.2 M ammonia buffer of pH 6.7 through a 500-ml. mixing flask containing the starting buffer. Later, the gradient was increased by substituting 0.4 M ammonium acetate of pH 6.7 as the solution flowing into the mixing flask.

(II), with the use of dicyclohexylcarbodiimide.[9b] Compound III could be crystallized in the form of beautiful needles by slow evaporation of a methanol solution and was isolated in 78% yield. The crystalline tetrapeptide I was prepared by the reaction of N$^\alpha$-carbobenzoxy-N$^\epsilon$-t-butyloxycarbonyl-lysine p-nitrophenyl ester[10] with prolyl-valyl-glycine methyl ester[8] followed by saponification of the product.

The nonapeptide derivative in which all the basic side chain functions were blocked with the p-toluenesulfonyl group, namely, N$^\alpha$-carbobenzoxy-N$^\epsilon$-tosyl-lysyl-prolyl-valyl-glycyl-N$^\epsilon$-tosyl-lysyl-N$^\epsilon$-tosyl-lysyl-N^G-tosyl-arginyl-N^G-tosyl-arginyl-proline t-butyl ester (VIII) was prepared by the reaction of the crystalline tetrapeptide N$^\alpha$-carbobenzoxy-N$^\epsilon$-tosyl-lysyl-prolyl-valyl-glycine[1] (IX) with N$^\epsilon$-tosyl-lysyl-N$^\epsilon$-tosyl-lysyl-N^G-tosyl-arginyl-N^G-tosyl-arginyl-proline t-butyl ester[9] (X) using N-ethyl 5-phenylisoxazolium 3′-sulfonate.[11] Compound VIII was purified by countercurrent distribution in the toluene system. However, all attempts at crystallization of VIII failed.

The carbobenzoxy group was removed from III by catalytic hydrogenolysis in the presence of Pd to yield IV. Peptide IV was purified by countercurrent distribution in the toluene system. For the synthesis of the nonadecapeptide, IV was linked to V by the use of dicyclohexylcarbodiimide. These reactions are schematically presented in Fig. 1. The protected nonadecapeptide VI was isolated by countercurrent distribution in the carbon tetrachloride system followed by thorough washing with methanol to remove any unreacted IV. VI was obtained in 38% yield.

Compound VI was first treated with trifluoroacetic acid to remove the t-butyloxycarbonyl groups and the t-butyl ester group. The product was purified[9] by countercurrent distribution in the system 1-butanol–acetic acid–water (4:1:5 by volume) for 100 transfers. The nonadecapeptide derivative (K = 3) was isolated and the remaining protecting groups were removed by reduction with sodium in liquid ammonia.[12] The nonadecapeptide VII was isolated by desalting on IRC-50 resin[13a] followed by chromatography on carboxymethylcellulose[13b] using continuous gradient elution with ammonium acetate. A typical chromatogram is shown in Fig. 2. Rechromatography on CMC (Fig. 3) yielded VII in 43% yield; VII was found to be homogeneous by electrophoresis on paper and on polyacrylamide gel.[14]

The amino acid composition of an acid hydrolysate of VII was determined by the chromatographic method[15]

(9) (a) C. H. Li, J. Ramachandran, and D. Chung, *J. Am. Chem. Soc.*, **86**, 2711 (1964); (b) J. C. Sheehan and G. P. Hess, *ibid.*, **77**, 1067 (1955).
(10) R. Schwyzer and W. Rittel, *Helv. Chim. Acta*, **44**, 159 (1961).
(11) R. B. Woodward, R. A. Olofson, and H. Mayer, *J. Am. Chem. Soc.*, **83**, 1010 (1961).
(12) V. du Vigneaud and O. K. Behrens, *J. Biol. Chem.*, **117**, 27 (1937).
(13) (a) H. B. F. Dixon and M. B. Stack-Dunne, *Biochem. J.*, **61**, 483 (1955); (b) E. A. Peterson and H. A. Sober, *J. Am. Chem. Soc.*, **78**, 751 (1956).
(14) S. Raymond and L. Weintraub, *Science*, **130**, 711 (1959); R. A. Reisfeld, U. J. Lewis, and D. E. Williams, *Nature*, **195**, 281 (1962).

TABLE I

AMINO ACID COMPOSITION OF THE SYNTHETIC α^{1-19}-ACTH

	Ser	Tyr	Met	Glu	His	Phe	Arg	Try	Gly	Lys	Pro	Val
Theoretical	2	1	1	1	1	1	3	1	2	3	2	1
Chromatographic	1.81	0.95	0.95	1.02	1.06	0.99	3.13	1.00[a]	2.12	2.95	2.01	0.91
Microbiological	1.99	0.98	0.94	1.05	0.95	1.01	3.11	...	2.10	2.95	2.02	0.93

[a] Determined by a spectrophotometric method.[17]

as well as by microbiological means.[16] The results are shown in Table I and are seen to be in excellent agreement with theory. Since the intermediates were purified by crystallization and the final peptide-forming step involved a glycine residue as the C-terminal, the nonadecapeptide may be considered to be optically homogeneous, as is indeed shown by the microbiological data.

Investigation of the various biological activities revealed that VII possessed the same potency in several assays as the original nonadecapeptide[1] within the limits of error of the procedures. Thus the melanocyte-stimulating activity of VII was comparable to that of native α_s-ACTH as assayed both *in vitro*[18] and *in vivo*.[19] It may also be seen in Table II that the

TABLE II

BIOLOGICAL ACTIVITIES OF TWO SYNTHETIC α^{1-19}-ACTH

Biological activity	1960 prepn.	1963 prepn.
1. *In vitro* steroidogenesis, I.U./mg.	40	33
2. *In vitro* MSH activity,[a] units/g.	1.4×10^7	7×10^7
3. *In vivo* MSH activity,[b] units/g.	0.1	0.1

[a] The same preparation of α_s-ACTH was assayed *in vitro* for MSH activity in 1960 and 1963 and found, respectively, to possess 1.4×10^7 and 6×10^7 units/g. [b] The dose produces a change in melanophore index in hypophysectomized *Rana pipiens* of from 1+ to 3+ within an hour.

potencies of VII in eliciting adrenal response *in vitro*[20] and *in vivo*[21] are in agreement with that of the peptide[1,22] synthesized in 1960.

Experimental[23]

N$^\alpha$-Carbobenzoxy-N$^\epsilon$-*t*-butyloxycarbonyl-lysyl-prolyl-valyl-glycine Methyl Ester.—Prolyl-valyl-glycine methyl ester[8] (1.6 g., 5.6 mmoles) was dissolved in 50 cc. of ethyl acetate and allowed to react at room temperature with 3.0 g. (6 mmoles) of N$^\alpha$-carbobenzoxy-N$^\epsilon$-*t*-butyloxycarbonyl-lysine *p*-nitrophenyl ester[10] for 2 days. The tetrapeptide methyl ester crystallized from the reaction mixture. The crystals (2.134 g.) were filtered off, the filtrate was evaporated to dryness, and the residue was crystallized from warm ethyl acetate to yield an additional 0.633 g. of the tetrapeptide; total yield 2.767 g. (76%), m.p. 137–138°. Recrystallization from ethyl acetate did not alter the melting point, $[\alpha]^{25}D$ −86.6° (*c* 0.5, methanol). The tetrapeptide methyl ester was found to be homogeneous in paper chromatography in two solvents, $R_{f\ BAW}$ 0.93 and $R_{f\ SBA}$ 0.94.

Anal. Calcd. for $C_{32}H_{49}N_5O_9$ (647.8): C, 59.3; H, 7.62; N, 10.8. Found: C, 59.2; H, 7.83; N, 10.6.

N$^\alpha$-Carbobenzoxy-N$^\epsilon$-*t*-butyloxycarbonyl-lysyl-prolyl-valyl-glycine (I).—The tetrapeptide methyl ester described above (2.23 g., 3.45 mmoles) was dissolved in 30 cc. of acetone and cooled to 0°. A solution of 1 *N* NaOH (7 cc., 7 mmoles) was slowly added and the mixture was then stirred at room temperature for 1 hr. The solution was poured into 150 cc. of ice water and the pH adjusted to 3 with ice-cold 10% citric acid. The acetone was removed *in vacuo* and the aqueous phase was ex-

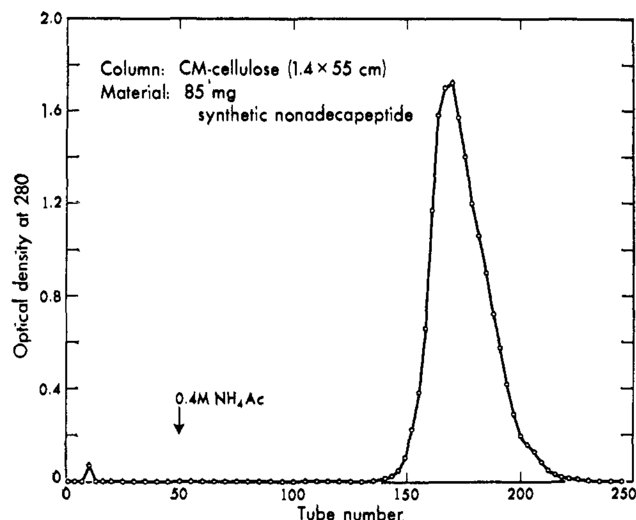

Fig. 3.—Rechromatography of VII on CMC; conditions as Fig. 2.

tracted twice with ethyl acetate. The ethyl acetate extracts were pooled, washed with water until neutral, and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* to yield a residue which was homogeneous in paper chromatography in two solvents, $R_{f\ BAW}$ 0.83 and $R_{f\ SBA}$ 0.51. The residue was triturated with ethyl acetate to yield 2.13 g. (98%) of I in the form of fine platelets, m.p. 117–118°, $[\alpha]^{25}D$ −72.8° (*c* 1, methanol).

Anal. Calcd. for $C_{31}H_{47}N_5O_9$ (633.7): C, 58.8; H, 7.48; N, 11.1. Found: C, 58.6; H, 7.34; N, 11.1.

N$^\alpha$-Carbobenzoxy-N$^\epsilon$-*t*-butyloxycarbonyl-lysyl-prolyl-valyl-glycyl-N$^\epsilon$-*t*-butyloxycarbonyl-lysyl-N$^\epsilon$-*t*-butyloxycarbonyl-lysyl-N^G-tosyl-arginyl-N^G-tosyl-arginyl-proline *t*-Butyl Ester (III).—Compound I (0.79 g., 1.24 mmoles) and II[9] (1.55 g., 1.24 mmoles) were dissolved in a mixture of 25 cc. of ethyl acetate and 3 cc. of dimethylformamide. The solution was cooled to 0° and 0.256 g. (1.24 mmoles) of dicyclochexylcarbodiimide[9a] was added, the mixture stirred at 0° for 2 hr., and stored at 4° for 24 hr. Glacial acetic acid (1 cc.) was added and after 2 hr. the solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate and the insoluble dicyclohexylurea was removed by filtration. The ethyl acetate solution was washed with ice-cold 5% citric acid, water, 5% sodium bicarbonate, and water. The organic phase was dried over anhydrous sodium sulfate, the solvent removed *in vacuo*, and the residue redissolved in methanol. Slow evaporation of the methanol resulted in the formation of long needles of crystalline III, 1.80 g. (78%), m.p. 120–125°. A sample was recrystallized from methanol, m.p. 123–125°, $[\alpha]^{25}D$ −48.0° (*c* 1, methanol). Compound III

(15) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).

(16) The microbiological assay was carried out by the Shankman Laboratories, Los Angeles, Calif.

(17) T. W. Goodwin and R. A. Morton, *Biochem. J.*, **40**, 628 (1946).

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(19) L. T. Hogben and D. Slome, *Proc. Roy. Soc.* (London), **B108**, 10 (1931).

(20) M. Saffran and A. V. Schally, *Endocrinology*, **56**, 523 (1955); C. Rerup, *Acta Endocrinol.*, **29**, 83 (1958).

(21) H. S. Lipscomb and D. H. Nelson, *Endocrinology*, **71**, 13 (1962).

(22) C. H. Li, *Recent Progr. Hormone Res.*, **18**, 1 (1962).

(23) All melting points were determined on a Fisher–Johns block and are uncorrected. Microanalyses were performed by the Microanalytical Laboratory of the Department of Chemistry, University of California, Berkeley. All samples for microanalysis were dried in an Abderhalden pistol with P_2O_5 at 77° for 16 hr. at 3 mm. pressure. Paper chromatography was performed on Whatman No. 1 filter paper at room temperature. The solvents employed were 1-butanol–acetic acid–water (4:1:1, BAW) and 2-butanol–10% ammonia (85:15, SBA), both by volume. Peptide spots were detected by ninhydrin reagent and by the chlorine method.[24] Countercurrent distribution was carried out using the toluene system (toluene–chloroform–methanol–water, 5:5:8:2) or the carbon tetrachloride system (carbon tetrachloride–chloroform–methanol–0.01 *M* ammonium acetate, 1:3:3:1), both by volume.

(24) H. Zahn and E. Rexroth, *Z. Anal. Chem.*, **148**, 181 (1955).

was found to be homogeneous in paper chromatography in two solvents, $R_{f\ \mathrm{BAW}}$ 0.81 and $R_{f\ \mathrm{SBA}}$ 0.81.

Anal. Calcd. for $C_{88}H_{138}N_{18}S_2O_{22}$ (1864): C, 56.7; H, 7.46; N, 13.5. Found: C, 56.4; H, 7.62; N, 13.3.

N$^{\epsilon}$-*t*-Butyloxycarbonyl-lysyl-prolyl-valyl-glycyl-N$^{\epsilon}$-*t*-butyloxycarbonyl-lysyl-N$^{\epsilon}$-*t*-butyloxycarbonyl-lysyl-N^{G}-tosyl-arginyl-N^{G}-tosyl-arginyl-proline *t*-Butyl Ester (IV).—Compound III, (1.86 g., 1 mmole) was dissolved in 40 cc. of methanol and submitted to catalytic hydrogenolysis using a Vibro-mixer[25] in the presence of Pd freshly prepared from 1 g. of $PdCl_2$ for 8 hr. The catalyst was filtered off and the filtrate and washings were evaporated to dryness. The residue was purified by countercurrent distribution in the toluene system for 100 transfers. A single peak with K = 1 was seen and isolated to yield 1.4 g. (81%) of IV, m.p. 120–130°, $[\alpha]^{25}D$ −42.7° (c 1, methanol). Compound IV was found to be homogeneous in paper chromatography, $R_{f\ \mathrm{BAW}}$ 0.76 and $R_{f\ \mathrm{SBA}}$ 0.81.

Anal. Calcd. for $C_{80}H_{132}N_{18}S_2O_{20}$ (1730): C, 55.6; H, 7.70; N, 14.6. Found: C, 55.9; N, 7.59; N, 14.4.

N$^{\alpha}$-Carbobenzoxy-N$^{\epsilon}$-tosyl-lysyl-prolyl-valyl-glycyl-N$^{\epsilon}$-tosyl-lysyl-N$^{\epsilon}$-tosyl-lysyl-N^{G}-tosyl-arginyl-N^{G}-tosyl-arginyl-proline *t*-Butyl Ester (VIII).—N$^{\alpha}$-Carbobenzoxy-N$^{\epsilon}$-tosyl-lysyl-prolyl-valyl-glycine[1] (IX, 0.69 g., 1 mmole) was dissolved in 30 cc. of acetonitrile. The solution was cooled to 0° and 0.14 cc. (1 mmole) of triethylamine and 0.254 g. (1 mmole) of N-ethyl-5-phenylisoxazolium 3′-sulfonate[11] were added. The mixture was stirred at 0° for 1 hr., 1.36 g. (1 mmole) of N$^{\epsilon}$-tosyl-lysyl-N$^{\epsilon}$-tosyl-lysyl-N^{G}-tosyl-arginyl-N^{G}-tosyl-arginyl-proline *t*-butyl ester[9] (X) was added and the stirring continued at room temperature for 36 hr. The solvent was removed *in vacuo* and the residue submitted to countercurrent distribution in the toluene system for 100 transfers. A large peak with K = 0.22 corresponding to the desired nonadecapeptide and two small peaks, K = 1.22 (unreacted X) and K = 4.56 (unreacted IX, were observed and were well separated from one another. The major peak (K = 0.22) was pooled, evaporated to dryness, the residue dissolved in methanol, filtered free of traces of insoluble material, and evaporated to dryness to yield 1.45 g. (71.5%) of the protected nonapeptide VIII. The peptide VIII was found to be homogeneous in paper chromatography, $R_{f\ \mathrm{BAW}}$ 0.91 and $R_{f\ \mathrm{SBA}}$ 0.82, $[\alpha]^{25}D$ −45.9° (c 1, methanol), m.p. 115–120°.

Anal. Calcd. for $C_{94}H_{135}N_{18}S_5O_{22}$ (2030): C, 55.7; H, 6.56; N, 12.4. Found: C, 55.5; H, 6.41; N, 12.6.

N$^{\epsilon}$-Tosyl-lysyl-prolyl-valyl-glycyl-N$^{\epsilon}$-tosyl-lysyl-N$^{\epsilon}$-tosyl-lysyl-N^{G}-tosyl-arginyl-N^{G}-tosyl-arginyl-proline *t*-Butyl Ester (XI).—Compound VIII (1.4 g., 0.69 mmole) was dissolved in 30 cc. of methanol and decarbobenzoxylated by catalytic hydrogenolysis in the presence of Pd freshly prepared from 1 g. of $PdCl_2$. After CO_2 evolution had stopped (8 hr.), the catalyst was filtered off, washed with methanol, and the filtrate and washings evaporated to dryness *in vacuo*. The residue was purified by countercurrent distribution in the toluene system for 100 transfers. A single symmetrical peak with K = 0.76 was seen. The material in the peak was pooled, evaporated to dryness, redissolved in methanol, and precipitated from anhydrous ether to yield 1.04 g. (80%) of XI, m.p. 120–130° $[\alpha]^{25}D$ −37.1° (c 1, methanol). Peptide XI was found to be homogeneous in paper chromatography, $R_{f\ \mathrm{BAW}}$ 0.86 and $R_{f\ \mathrm{SBA}}$ 0.88.

Anal. Calcd. for $C_{86}H_{129}N_{18}S_5O_{20}$ (1895): C, 54.5; H, 6.86; N, 13.3. Found: C, 54.1; H, 6.51; N, 13.1.

Carbobenzoxy-seryl-tyrosyl-seryl-methionyl-γ-benzyl-glutamyl-histidyl-phenylalanyl-N^{G}-tosyl-arginyl-tryptophyl-glycyl-N$^{\epsilon}$-*t*-butyloxycarbonyl-lysyl-prolyl-valyl-glycyl-N$^{\epsilon}$-*t*-butyloxycarbonyl-lysyl-N$^{\epsilon}$-*t*-butyloxycarbonyl-lysyl-N^{G}-tosyl-arginyl-N^{G}-tosyl-arginyl-proline *t*-Butyl Ester (VI).—The decapeptide[9] V (0.336 g., 0.2 mmole) and IV (0.346 g., 0.2 mmole) were dissolved in 2 cc. of dimethylformamide and cooled to 0°. Dicyclohexylcarbodiimide (0.046 g., 0.22 mmole) was added and the mixture was stirred at 0° for 1 hr. and kept at 4° for 3 days. Glacial acetic acid (0.5 cc.) was added, and after 2 hr. the solution was evaporated to dryness *in vacuo*. The residue was submitted to countercurrent distribution in the carbon tetrachloride system for 80 transfers to remove unreacted V (K = 1). The material from tubes 0 to 14 was isolated and washed exhaustively with water followed by methanol to remove unreacted IV. Peptide VI was obtained in 38% yield (0.255 g.).

Seryl-tyrosyl-seryl-methionyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophyl-glycyl-lysyl-prolyl-valyl-glycyl-lysyl-lysyl-arginyl-arginyl-proline (VII).—Compound VI (0.362 g., 0.107 mmole) was dissolved in 25 cc. of trifluoroacetic acid and stirred at room temperature for 2 hr. The solvent was removed *in vacuo* and the residue purified by countercurrent distribution for 100 transfers in the system 1-butanol–acetic acid–water (4:1:5 by volume). The peak with K = 3 (ultraviolet and ninhydrin positive) was isolated to yield 0.205 g. (63%) of the partially protected nonadecapeptide. This material was dissolved in 150 cc. of freshly distilled liquid ammonia and treated with small pieces of sodium until the blue color persisted for 30–40 min. The ammonia was then allowed to evaporate and the residue dried over concentrated H_2SO_4 and P_2O_5. The residue was dissolved in 20 cc. of 0.1 N acetic acid and desalted on IRC-50 resin as described earlier.[9] The crude nonadecapeptide was eluted with pyridine–acetic acid–water (30:4:66 by volume) and isolated by lyophilization to yield 0.164 g. of material. This was applied on a carboxymethylcellulose column and chromatographed using continuous gradient elution with ammonium acetate (Fig. 2). The major peak was isolated and rechromatographed on CMC (Fig. 3). Peptide VII was then isolated by lyophilization (3×) in a yield of 43% (0.085 g.; peptide content based on ultraviolet, 82%). Peptide VII was found to be homogeneous by electrophoresis on paper (mobility relative to lysine, 0.81; pH 3.7, 400 volts, 4 hr.) and polyacrylamide gel. The amino acid composition of VII as determined by the chromatographic and microbiological procedures was found to be in excellent agreement with theoretically expected values (Table I); $[\alpha]^{25}D$ −84.9° (c 0.5, 0.1 M acetic acid).

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(25) Vibro-mixer, A. G. Fuer Chemie-Apparatebau, Zurich, Model E1.

COMMUNICATIONS TO THE EDITOR

Ion Pairs in Reactions of Trityl Benzoate[1]

Sir:

While R^+X^- ion pairs have been invoked in discussions of solvolysis and exchange reactions of trityl chloride,[2] no actual attempt was made until very recently to estimate for any trityl system the importance of ion pair return under any particular set of conditions. For carbonyl-^{18}O-labeled trityl benzoate (RX) in dry acetone at 60°, Swain and Tsuchihashi[3] recently reported a first-order rate constant of 3.8×10^{-6} sec.$^{-1}$ for ^{18}O equilibration, this equilibration being completely suppressed initially in the presence of LiN_3. First-order rate constants (10^6k) from following disappearance of azide ion were 4.4 ± 0.4 and 6.3 ± 0.6 for 0.006 and 0.010 M LiN_3, respectively. A nearly quantitative

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(2) (a) R. F. Hudson and B. Saville, *Chem. Ind.* (London), 1423 (1954); (b) C. G. Swain and M. M. Kreevoy, *J. Am. Chem. Soc.*, **77**, 1122 (1955); (c) E. D. Hughes, C. K. Ingold, *et al.*, *J. Chem. Soc.*, 1265 (1957); (d) C. G. Swain and E. E. Pegues, *J. Am. Chem. Soc.*, **80**, 812 (1958).

(3) C. G. Swain and G. Tsuchihashi, *ibid.*, **84**, 2021 (1962).