Adrenocorticotropins. XXXVI. Synthesis of a Biologically Active Prolinol Analog of the Nonadecapeptide Corresponding to the Amino-Terminal Portion of the ACTH Molecule[1a]

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Abstract: The synthesis is described of a nonadecapeptide, L-seryl-L-tyrosyl-L-seryl-L-methionyl-L-glutamyl-L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycyl-L-lysyl-L-prolyl-L-valylglycyl-L-lysyl-L-lysyl-L-arginyl-L-arginyl-L-prolinol, representing the NH_2-terminal 19 amino acid residues in adrenocorticotropin (ACTH), but in which a COOH-terminal prolinol has been substituted for the proline residue occurring at position 19 in the native hormone. The synthetic nonadecapeptide alcohol, which represents the first instance in the ACTH series of a peptide terminating in a primary alcoholic function, has been shown to possess a melanocyte-stimulating potency similar to that of either the corresponding nonadecapeptide amide or of native ACTH on a molar basis. The *in vitro* adrenal steroidogenic potency (338 IU/mole) corresponded to about 50% of the activity of native sheep ACTH, but was significantly higher than that of the corresponding nonadecapeptide amide.

During a series of synthetic studies carried out in this laboratory over the past 7 years, our attention was directed toward the biological significance of the basic sequence of amino acid residues occurring at positions 15–18 (see Figure 1) in the adrenocorticotropin (ACTH) molecule. Whereas the heptadecapeptide corresponding to the NH_2-terminal 17 amino acid residues in the hormone appeared to be the smallest unit which still possesses a low but significant adrenal-stimulating potency,[1b] a notably higher activity was noted in the case of the corresponding heptadecapeptide amide,[2] which exhibited a potency very similar to that of the nonadecapeptide[3,4] corresponding to the first 19 amino acid residues in ACTH. Finally, the recently synthesized amides of the octadeca- and nonadecapeptides[2] were found to be considerably more active by the *in vivo* steroidogenesis assay[5] than any of the peptides mentioned above. In view of this apparent relationship between the net positive charge associated with the basic sequence (positions 15–18) in synthetic peptides related to ACTH and the adrenal-stimulating potency, the synthesis of the nonadecapeptide alcohol seryltyrosylserylmethionylglutamylhistidylphenylalanylarginyltryptophylglycyllysylprolylvalylglycyllysyllysylarginylarginylprolinol (XIII)[6] was undertaken. In this peptide the negative charge associated with the COOH-terminal portion of the basic core is blocked in an unnatural fashion by substitution of a prolinol residue for the proline normally occurring at position 19 in ACTH. It was hoped that this analog would enable us to distinguish between the functional importance of the net positive charge *per se* and that of the COOH-terminal amide groups in the manifestation of high adrenal-stimulating activity in the above-mentioned peptides. The synthesis and properties of the nonadecapeptide alcohol, designated as prolinol^{19}-α^{1-19}-ACTH, is described in the present paper.

L-Prolinol (I), which served as the starting point for the present synthesis (see Figure 2), was obtained by means of the reduction of L-proline methyl ester[7] with lithium borohydride;[8] it was characterized as the crystalline neutral oxalate salt.[9,10] The fully protected COOH-terminal pentapeptide N^{α}-carbobenzoxy-N^{ϵ}-*t*-butyloxycarbonyllysyl-N^{ϵ}-*t*-butyloxycarbonyllysyl-N^{G}-tosylarginyl-N^{G}-tosylarginylprolinol (VIII) was synthesized in a stepwise manner. N^{α}-Carbobenzoxy-N^{G}-tosylarginine[11] was coupled to L-prolinol in two consecutive steps using N-ethyl-5-phenylisoxazolium 3′-sulfonate[12] for carboxyl activation. The resulting fully protected tripeptide N^{α}-carbobenzoxy-N^{G}-tosylarginyl-N^{G}-tosylarginylprolinol (IV) was decarbobenzoxylated to give the corresponding free base (V) which was condensed in the two subsequent steps with N^{α}-carbobenzoxy-N^{ϵ}-*t*-butyloxycarbonyllysine *p*-nitrophenyl ester.[13] The fully protected pentapeptide (VIII) obtained in this manner was subjected to catalytic hydrogenolysis for removal of the α-carbobenzoxy group, and yielded the partially protected pentapeptide N^{ϵ}-*t*-butyloxycarbonyllysyl-N^{ϵ}-*t*-butyloxycarbonyllysyl-N^{G}-tosylarginyl-N^{G}-tosylarginylprolinol (IX).

As was observed previously[14] during the synthesis of similar peptides, the intermediates in the synthesis of IX resisted crystallization; hence extensive use was made of countercurrent distribution in the purification of these peptides. The apparent homogeneity of the various intermediates in the synthesis of XIII was further verified by means of paper chromatography and thin layer chromatography in several solvent systems.

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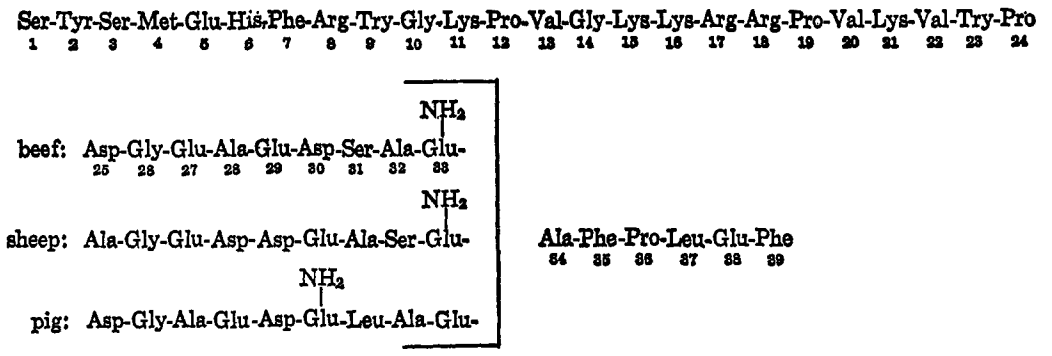

Figure 1. Structure of adrenocorticotropins.

Peptide IX was investigated in terms of its stereochemical properties by means of digestion with leucine aminopeptidase (LAP). Because of its low solubility in aqueous media, IX was dissolved in a mixture composed of Tris buffer (pH 8.5) containing 35% (v/v) of dimethylformamide before digestion. The LAP preparation tended, however, to precipitate from this mixture during incubation and was therefore added in three equal portions at intervals during the incubation period. At the completion of the digestion, paper chromatography in the BAW system revealed the presence in significant amounts of three ninhydrin-positive components corresponding in R_f to authentic N^G-tosylarginine,[11] N^ϵ-*t*-butyloxycarbonyllysine,[13] and N^G-tosylarginylprolinol (III). The mixture was subjected to quantitative analysis on the short column of the Beckman–Spinco automatic amino acid analyzer and the amounts of N^G-tosylarginine and N^ϵ-*t*-butyloxycarbonyllysine present were found to be, respectively, 0.98 and 1.98 moles per mole of IX digested.

The resistance of the arginyl–prolinol bond in peptide III to further digestion with LAP is not unexpected in view of a similar resistance offered by peptide bonds linking the imino group of L-proline to the carboxyl groups of either L-arginine[4,15] or L-lysine.[16] Due apparently to an unusual binding affinity for the resin, III could not be removed from the short column of the amino acid analyzer even after a prolonged elution with the buffer, and hence the amounts present in the digest could not be determined directly. For the determination of III in the digestion mixture an alternative approach, based on the conversion of III to its dinitrophenyl (DNP) derivative N^α-2,4-dinitrophenyl-N^G-tosylarginylprolinol (IIIa), was employed. An aliquot of the digest was treated with 1-fluoro-2,4-dinitrobenzene (DNFB)[16] according to a procedure[17] for the conversion of amino alcohols to their corresponding DNP derivatives. After the separation of the IIIa formed in the mixture by means of thin layer chromatography, the amount present was determined quantitatively from a standard curve relating the optical density at the wavelength of maximal absorption (345 mμ) to the theoretical amount of authentic IIIa present in the appropriate control samples. According to this procedure, the average amount of IIIa present in the reaction mixture derived from the digest of peptide IX after dinitrophenylation was found to be 0.97 mole per mole of IX digested.

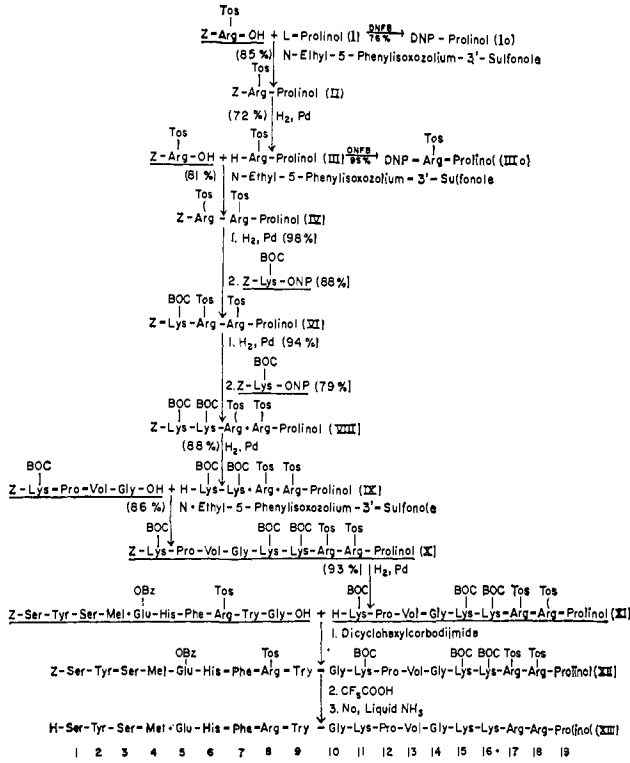

Figure 2. Outline of the synthesis of prolinol^{19}-α^{1-19}-ACTH: Z, carbobenzoxy; Tos, *p*-toluenesulfonyl; BOC, *t*-butyloxycarbonyl; Bz, benzyl; ONP, *p*-nitrophenoxy; DNP, 2,4-dinitrophenyl; DNFB, 1-fluoro-2,4-dinitrobenzene.

For the condensation of the previously synthesized protected tetrapeptide N^α-carbobenzoxy-N^ϵ-*t*-butyloxycarbonyllysylprolylvalylglycine[3] with IX, N-ethyl-5-phenylisoxazolium 3′-sulfonate[12] was utilized to give the crystalline fully protected nonapeptide N^α-carbobenzoxy-N^ϵ-*t*-butyloxycarbonyllysylprolylvalylglycyl-N^ϵ-*t*-butyloxycarbonyllysyl-N^ϵ-*t*-butyloxycarbonyllysyl-N^G-tosylarginyl-N^G-tosylarginylprolinol (X), in good yield. Removal of the carbobenzoxy group gave rise to the corresponding free base XI in crystalline form. Both X and XI appeared homogeneous by the criteria of countercurrent distribution and paper and thin layer chromatography in various solvent systems. In the final step the crystalline decapeptide N^α-carbobenzoxyseryltyrosylserylmethionyl-γ-benzylglutamylhistidylphenylalanyl-N^G-tosylarginyltryptophylglycine[1] was coupled to XI

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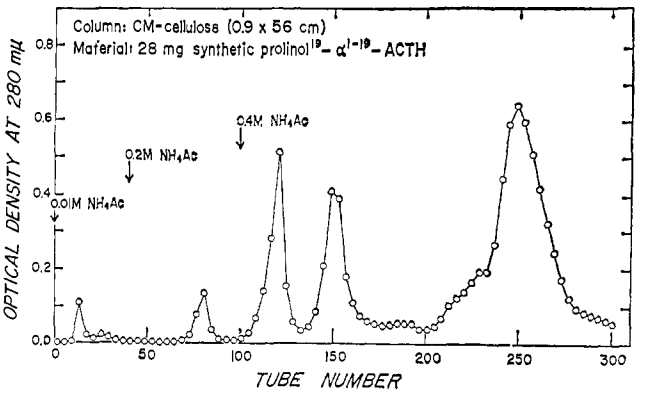

Figure 3. CMC chromatography of crude prolinol^{19}-α^{1-19}-ACTH (XIII). The material was applied to the column in 0.01 *M* ammonium acetate buffer of pH 4.5. After 40 aliquots (4 ml per aliquot) had been collected, a pH and concentration gradient was started by introducing a 0.2 *M* ammonium acetate solution (pH 6.7) into the column through a mixing chamber containing 500 ml of the starting buffer. When a total of 100 aliquots of eluate had been collected, the gradient was further increased by introducing a 0.4 *M* ammonium acetate solution through the mixing chamber for the remainder of the experiment.

using the dicyclohexylcarbodiimide (DCCI)[18] method. The fully protected nonadecapeptide N^{α}-carbobenzoxyseryltyrosylserylmethionyl-γ-benzylglutamylhistidylphenylalanyl-N^{G}-tosylarginyltryptophylglycyl-N^{ϵ}-*t*-butyloxycarbonyllysylprolylvalylglycyl-N^{ϵ}-*t*-butyloxycarbonyllysyl-N^{ϵ}-*t*-butyloxycarbonyllysyl-N^{G}-tosylarginyl-N^{G}-tosylarginylprolinol (XII) was recovered in crude form and the *t*-butyloxycarbonyl groups were removed directly by treating the product with trifluoroacetic acid. The partially deblocked material was submitted to countercurrent distribution for 200 transfers in the BAW system and gave rise to three distinct components. The material recovered from the second peak (K = 2.8), representing the partially deblocked nonadecapeptide, was treated with sodium in liquid ammonia[19] to remove the remaining protecting groups. The product was desalted on an IRC-50 cation exchange column[20] and subjected to chromatography on carboxymethylcellulose (CMC)[21] in a continuous ammonium acetate concentration gradient. The results of the chromatography are shown in Figure 3. In several experiments XIII was recovered from the main peak in an over-all yield varying between 9 and 10% for the final coupling step, based on the peptide content as measured by ultraviolet absorption.[22] The over-all yield based on L-prolinol was on the order of 2.2% through 12 steps (Figure 2).

The nonadecapeptide alcohol obtained after CMC chromatography behaved as a single component in paper electrophoresis at pH 3.7. An acid hydrolysate of XIII was submitted to quantitative amino acid analysis according to the chromatographic method[23] and the values found were in good agreement with the expected ratio of the individual amino acid residues in prolinol^{19}-α^{1-19}-ACTH (see Table I). The tryptophan content was determined by means of a spectrophotometric method.[22]

Table I. Amino Acid Composition of Synthetic Prolinol^{19}-α^{1-19} ACTH

Amino acid	Theoretical	Found
Serine	2	1.71
Tyrosine	1	1.08
Methionine	1	0.96
Glutamic acid	1	0.92
Histidine	1	1.06
Phenylalanine	1	1.09
Arginine	3	3.02
Tryptophan	1	0.95[a]
Glycine	2	2.00
Lysine	3	3.21
Proline	1	1.02
Valine	1	0.99
Prolinol	1	0.94[b]

[a] Determined by a spectrophotometric method.[22] [b] Determined by the DNP method.[17]

For the quantitative determination of prolinol in an acid hydrolysate of XIII, the acid hydrolyzate was treated with DNFB for the conversion of the amino alcohol to its DNP derivative. The DNP-prolinol was extracted into ether from the basic reaction mixture after dinitrophenylation, and was further purified in a quantitative manner by means of thin layer chromatography. The amount present was then determined from a standard curve relating the optical density at the wavelength of maximal absorption (370 mμ) to the theoretical amount of authentic DNP-prolinol present in the appropriate control samples. According to this procedure two separate preparations of XIII were each found to give a value of 0.94 mole of DNP-prolinol recovered per mole of peptide XIII hydrolyzed.

The biological properties of the synthetic nonadecapeptide alcohol are compared in Table II with those of the sheep hormone (α_s-ACTH) and the corresponding nonadecapeptide amide.[2] The melanocyte-stimulating activity of the nonadecapeptide alcohol, when assayed by both the *in vitro*[24] and *in vivo*[25] methods, was found to be on the same order of magnitude (on a molar basis) as either that of the corresponding amide or the native hormone. The average adrenal-stimulating potency of the alcohol, as measured by the *in vitro* steroidogenesis procedure[26] was found to be 338 IU/μmole, compared to 122 IU/μmole for the amide and 617 IU/μmole for native sheep ACTH.[2] Furthermore, when the synthetic nonadecapeptide alcohol was assayed in a comparative *in vitro* system against the nonadecapeptide amide, the alcohol was shown to possess a potency of 338 IU/μmole relative to a value of 117 IU/μmole for the amide. The significantly higher activity of the alcohol as compared to the corresponding amide *in vitro*, seems to indicate that in addition to the effect of the net charge associated with the basic core of peptides related to ACTH, other factors such as the

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Table II. Biological Activities of ACTH and Related Synthetic Nonadecapeptides

Biological effect	α_9-ACTH	$\alpha^{1-19}NH_2$-ACTH	Prolinol[19]-α^{1-19}-ACTH
In vitro adrenal steroidogenesis,[a] IU/μmole	617 (917–418)	122 (178–84)	338 (749–124)
In vitro MSH activity,[b] units/μmole	4.5×10^5 ($2.6–10.2 \times 10^5$)	4.2×10^5	6.8×10^5 ($2.3–12.3 \times 10^5$)
In vivo MSH activity,[c] μmole	4.4×10^{-6}	8.5×10^{-6}	8.6×10^{-6}

[a] Average of at least three assays; 95% confidence limits are given in parentheses. [b] The range found after at least seven assays is shown in parentheses. [c] The dose produces a change in melanophore index from 1+ to 3+ in hypophysectomized *Rana pipiens* within 1 hr.

structural features at the COOH-terminus of the basic core, are also operative in the manifestation of adrenal-stimulating activity.

Experimental Section[27]

L-Prolinol (I). L-Proline methyl ester hydrochloride[7] (17 g, 102.3 mmoles), after being dried thoroughly over P_2O_5 and NaOH pellets *in vacuo*, was suspended in 250 ml of dry, peroxide-free tetrahydrofuran,[35] and while stirring at room temperature, successive portions of powdered $LiBH_4$ (about 0.3 g per portion) were added directly to the mixture with minimal exposure to air moisture. The reaction was controlled by periodic cooling of the flask in an ice bath, until the initially vigorous effervescence had ceased. The remainder of the $LiBH_4$ was then added to bring the total amount to approximately 5.5 g (256 mmoles). Stirring was continued under anhydrous conditions for 18 hr at room temperature. The mixture was cooled to −10° and 2 *N* HCl (190 ml) was added; the remaining solution was distinctly acidic after 8 hr of stirring at room temperature. After the acidic mixture was stored at 0° for 2 days, it was concentrated to a paste at 30° *in vacuo*. A cold, 4 *N* NaOH solution was added to the mixture at 0° until pH 10–11 was reached. The alkaline mixture (270 ml) was saturated with NaCl and extracted repeatedly with 100-ml portions of chloroform (25 times) until the final extract upon removal of the solvent furnished less than 1% of the theoretical yield of prolinol. The combined chloroform extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to furnish 15 g of a yellow syrup, which, after distillation under reduced pressure, yielded 7.4 g (71%) of L-prolinol as an almost colorless oil: bp 98° (10 mm), $[\alpha]^{23}D$ +27.4° (*c* 1.1, 0.2 *N* HCl) (lit.[9] bp 98° (10 mm)). The product was homogeneous in paper chromatography in four solvents: R_f (BAW) 0.42, R_f (SBA) 0.55, R_f (BPAW) 0.56, R_f (nBA) 0.34.

A sample of the distillate was converted to the neutral oxalate and recrystallized twice from absolute ethanol and once from 99% ethanol: mp 152–155° (sintering at 145°), $[\alpha]^{24}D$ +20.2° (*c* 2, water) (lit.[9,10] mp 158–160°, $[\alpha]^{18}D$ +21.5° (*c* 2, water)).

Anal. Calcd for $C_{12}H_{24}O_6N_2$ (292.3): C, 49.3; H, 8.18; N, 9.59. Found: C, 49.1; H, 7.92; N, 9.35.

N-2,4-Dinitrophenylprolinol (Ia). L-Prolinol (0.1 g, 1 mmole) was dissolved in 1.5 ml of 10% $NaHCO_3$ solution and a solution of 0.205 g (1.1 mmoles) of 1-fluoro-2,4-dinitrobenzene (DNFB)[16] in 3 ml of acetone was added. After the mixture was shaken for 2 hr at room temperature, 0.05 g (0.56 mmoles) of DL-alanine was added and shaking was continued for another 2 hr. Acetone was removed from the reaction mixture *in vacuo* and the remaining aqueous phase was diluted with a few milliliters of water before extraction with ether. After the third extraction, the ether phase was practically colorless. The ether extracts were combined, extracted three times with portions of a 2% $NaHCO_3$ solution, and dried (anhydrous Na_2SO_4) at 0°. The product obtained after removal of the solvent *in vacuo* was dried over P_2O_5, dissolved in 4 ml of ethyl acetate and precipitated from 200 ml of petroleum ether (bp 30–60°) to give 0.203 g (76%) of a reddish yellow, viscous oil, $[\alpha]^{24}D$ −1275° (*c* 0.1, methanol). The product was homogeneous in several paper and thin layer chromatographic systems: R_f (BAW) 0.87, R_f (SBA) 0.83, R_f (BPAW) 0.89 (on paper) and R_f (BAW) 0.63, R_f (SBA) 0.61, R_f (BPAW) 0.66, R_f (CM)(8:2) 0.64 (on silica gel).

Anal. Calcd for $C_{11}H_{13}O_5N_3$ (267.2): C, 49.4; H, 4.91; N, 15.8. Found: C, 49.6; H, 4.94; N, 15.6.

N^{α}-Carbobenzoxy-N^{G}-tosylarginylprolinol (II). N^{α}-Carbobenzoxy-N^{G}-tosylarginine[11] (9.26 g, 20 mmoles) was dissolved in 190 ml of acetonitrile with slight warming in a water bath. After the solution was cooled to 0°, triethylamine (2.8 ml, 20 mmoles) followed by N-ethyl-5-phenylisoxazolium 3′-sulfonate[12] (5.3 g, 21 mmoles) was added and the mixture stirred at 0° for 1.5 hr. To the slightly turbid solution was added a solution of 2.02 g (20 mmoles) of L-prolinol (I) in 10 ml of acetonitrile, and the mixture was stirred for another 2.5 hr in the cold and for 24 hr at room temperature. Acetonitrile was removed *in vacuo*, the resulting glassy product was dissolved in moist ethyl acetate (250 ml), and the solution was extracted successively with 100-ml portions of water, 0.1 *N* HCl, water, 5% $NaHCO_3$ solution, water, and saturated NaCl solution. The organic layer was dried over anhydrous sodium sulfate, and the solvent was removed *in vacuo*. After being dried *in vacuo* over P_2O_5, the product was dissolved in 190 ml of ethyl acetate with slight warming, and precipitated from 850 ml of petroleum ether (bp 30–60°), to yield 9.3 g (85%) of peptide II as an amorphous powder, mp 75–80°, $[\alpha]^{25}D$ −12.1° (*c* 4, methanol). The product resisted all attempts at crystallization but behaved as a single component in paper chromatography in four solvents: R_f (BAW) 0.80, R_f (SBA) 0.78, R_f (BPAW) 0.88, R_f (nBA) 0.83, as well as in thin layer chromatography in one solvent system: R_f (CM)(1:1) 0.71.

Anal. Calcd for $C_{26}H_{35}O_6N_5S$ (545.5): C, 57.2; H, 6.47; N, 12.8. Found: C, 57.5; H, 6.62; N, 12.6.

(27) Melting points were determined on a Fischer-Johns melting block and are uncorrected. Microanalyses were performed by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley, Calif. All samples for microanalysis were dried in an Abderhalden drying pistol with P_2O_5 under reduced pressure at 77 or 40° depending on the melting point of the sample. For paper chromatography the following solvent systems were employed using the descending technique on Whatman No. 1 filter paper at room temperature: BAW = 1-butanol–acetic acid–water (4:1:1 by volume), SBA = 2-butanol–10% ammonia (85:15 by volume), BPAW = 1-butanol–pyridine–acetic acid–water (30:20:6:24 by volume), and nBA = 1-butanol saturated with 0.1% aqueous ammonia, using the lower phase for saturation of the tank atmosphere and the upper phase for development. Thin layer chromatography was carried out according to the procedure of Stahl.[28] In addition to the systems used for the development of paper chromatograms, the following solvent systems were also used for the development of thin layer chromatograms: CM (8:2) = chloroform–methanol (8:2 by volume) and CM (1:1) = chloroform–methanol (1:1 by volume). Spots on paper chromatograms or thin layer plates were located by means of the ninhydrin reagent, Pauly reagent,[29] the chlorine procedure,[30] isatin,[31] the Sakaguchi reagent,[32] and ultraviolet fluorescence quenching on thin layer plates. Paper electrophoresis was performed in a Beckman paper electrophoresis apparatus (Durrum type) on Whatman No. 3 filter paper using a pyridinium acetate buffer (pH 3.7) consisting of pyridine–glacial acetic acid–water in a ratio of 1:10:289 by volume. All hydrogenolytic operations were carried out in the presence of Pd catalyst prepared freshly[33] from $PdCl_2$ with a Vibro mixer[34] (Model El, A. G. Fuer Chemie-Apparatebau, Zurich). For countercurrent distribution the following solvent systems were employed: chloroform–water = chloroform saturated with distilled water, butanol–ammonia = 1-butanol saturated with 0.1% ammonia, toluene system = chloroform–toluene–methanol–water (5:5:8:2 by volume), carbon tetrachloride system = chloroform–carbon tetrachloride–methanol–water (3:1:3:1 by volume), and the BAW system = 1-butanol–glacial acetic acid–water (4:1:5 by volume). Distribution patterns were determined either by weighing the material recovered from aliquots after drying or, when applicable, by means of ultraviolet absorption at 280 mμ.

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(35) The commercial solvent, stabilized with 0.1% hydroquinone, was refluxed for 24 hr over Na metal, distilled, and refluxed for another 12 hr over $LiBH_4$. The final distillate was stored in the dark over Na metal and used within 2 weeks.

N^G-Tosylarginylprolinol (III). Compound II (9.2 g, 16.8 mmoles) was dissolved in methanol (150 ml) and hydrogenolyzed for 6.5 hr in the presence of Pd catalyst prepared from 2 g of $PdCl_2$. The catalyst was removed by filtration and the solvent was removed *in vacuo*. The resulting syrup (6.9 g) in paper chromatography revealed the presence of three minor contaminating spots in addition to the major component.

The material was subjected to countercurrent distribution in the chloroform–water system for 200 transfers. The material (5.23 g) obtained from the main peak (K = 4.0) was subjected to a second countercurrent distribution for 100 transfers in the butanol–ammonia system. From the main peak (K = 7), which closely approached the theoretical distribution, peptide III was recovered in a yield of 5 g (72%), as a hygroscopic glassy product: mp 55–60° $[\alpha]^{24}D$ −1.89° (*c* 5, methanol). The product was homogeneous in four paper chromatographic systems: R_f (BAW) 0.42, R_f (SBA) 0.50, R_f (BPAW) 0.69, R_f (nBA) 0.79, as well as in two thin layer systems: R_f (CM) (1:1) 0.20, R_f (CM) (8:2) 0.14.

Anal. Calcd for $C_{18}H_{29}O_4N_5S$ (411.5): C, 52.6; H, 7.10; N, 17.1; S, 7.81. Found: C, 52.4; H, 7.17; N, 16.8; S, 7.58.

N^α-2,4-Dinitrophenyl-N^G-tosylarginylprolinol (IIIa). To a solution of 0.168 g (0.41 mmole) of peptide III in a mixture of 1 ml of acetone and 4 ml of 5% $NaHCO_3$ solution, 1-fluoro-2,4-dinitrobenzene (0.084 g, 0.45 mmole) dissolved in 1 ml of acetone was added. After the suspension was stirred at room temperature for 2 hr, 0.036 g (0.4 mmole) of DL-alanine was added and stirring was continued at room temperature for an additional 2 hr. The solvents were removed *in vacuo* and the remaining yellow product, after drying, was dissolved in approximately 20 ml of the CM (8:2) solvent system and applied to a column (1.2 × 23 cm) of silica gel (Merck and Co., 0.05–0.02 mm granule size, "for chromatography"), packed and equilibrated with the same solvent mixture. The column was developed by using the CM (8:2) solvent system and the first 5 ml of yellow eluate was discarded. The remaining portion of the weakly adsorbed yellow band, which completely separated from a band of strongly adsorbed material, was eluted in a total volume of 40 ml.

The material recovered from the eluate was rechromatographed on a silica gel column of larger capacity (1.25 × 57 cm) using the same conditions for elution as before, but with the eluate collected in aliquots of 5 ml each. The yellow band emerged from the column between tubes 11–23. The contents of tubes 13–20, which exhibited an infinite optical density at 345 mμ, were combined and yielded, after removal of the solvents, 0.218 g (93%) of a glassy product: mp 110–115°, $[\alpha]^{24}D$ −38.2° (*c* 1.5, methanol). This material, which resisted all attempts at crystallization, behaved as a single component in paper chromatography in three solvents: R_f (BAW) 0.83, R_f (SBA) 0.78, R_f (BPAW) 0.88, as well as in four thin layer chromatographic systems: R_f (BAW) 0.52, R_f (SBA) 0.43, R_f (BPAW) 0.61, R_f (CM) (8:2) 0.49.

Anal. Calcd for $C_{24}H_{31}O_8N_7S$ (577.6): C, 49.9; H, 5.42; N, 17.0; S, 5.55. Found: C, 49.8; H, 5.28; N, 16.9; S, 5.36.

N^α-Carbobenzoxy-N^G-tosylarginyl-N^G-tosylarginylprolinol (IV). Triethylamine (1.65 ml, 11.8 mmoles) was added to a solution of N^α-carbobenzoxy-N^G-tosylarginine (5.47 g, 11.8 mmoles) in acetonitrile (100 ml) at 0°. The solution was then stirred vigorously and N-ethyl-5-phenylisoxazolium 3′-sulfonate (3.15 g, 12.4 mmoles) was added. The mixture was stirred at 0° for 1.75 hr, and a solution of III (4.85 g, 11.8 mmoles), obtained by slightly warming the latter in 40 ml of acetonitrile, was added. After the mixture was stirred at room temperature for 21 hr, some insoluble material was filtered off and the solvent was removed *in vacuo;* the residual glassy product was dissolved in 300 ml of moist chloroform. The solution was extracted with 150 ml of water, and then successively with 100-ml portions of 0.1 *N* HCl, water, 5% $NaHCO_3$ solution, water, and finally with a saturated solution of NaCl. After the chloroform phase was dried over anhydrous Na_2SO_4, the solvent was removed *in vacuo*, and the dried product was dissolved in 60 ml of methanol. Precipitation from anhydrous ether (800 ml) yielded 8.2 g (81%) of the amorphous compound IV; mp 118–120°, $[\alpha]^{25}D$ −16.1° (*c* 5, methanol). The product, which resisted crystallization, was found to be homogeneous in paper chromatography in four solvents: R_f (BAW) 0.85, R_f (SBA) 0.80, R_f (BPAW) 0.89, R_f (nBA) 0.90, as well as in thin layer chromatography in one system: R_f (CM) (1:1) 0.67.

Countercurrent distribution of a sample in the carbon tetrachloride system for 240 transfers gave a single peak (K = 0.24) which was in close agreement with the theortical distribution pattern.

Anal. Calcd for $C_{39}H_{53}O_9N_9S_2$ (856): C, 54.8; H, 6.25; N, 14.7; S, 7.48. Found: C, 54.5; H, 6.16; N, 14.4; S, 7.32.

N^G-Tosylarginyl-N^G-tosylarginylprolinol (V). A solution of peptide IV (8 g, 9.35 mmoles) in methanol (150 ml) was hydrogenolyzed for 4.5 hr in the presence of Pd catalyst prepared from 2 g of $PdCl_2$. After removal of the catalyst by filtration and of the solvent by evaporation *in vacuo*, 6.6 g (98%) of the tripeptide free base (V) was obtained, which behaved as a single component in thin layer chromatography: R_f (CM) (1:1) 0.40, $[\alpha]^{23}D$ −8.8° (*c* 5, methanol).

Distribution of a sample for 100 transfers in the carbon tetrachloride system gave a single peak (K = 0.67). The material recovered from the peak was precipitated from a small volume of methanol into anhydrous ether to yield an amorphous powder, mp 104–109°, which was homogeneous in paper chromatography in four solvents: R_f (BAW) 0.49, R_f (SBA) 0.46, R_f (BPAW) 0.75, R_f (nBA) 0.74.

Anal. Calcd for $C_{31}H_{47}O_7N_9S_2$ (621.9): C, 51.6; H, 6.57; N, 17.5. Found: C, 51.7; H, 6.62; N, 17.2.

N^α-Carbobenzoxy-N^ϵ-*t*-butyloxycarbonyllysyl-N^G-tosylarginyl-N^G-tosylarginylprolinol (VI). Protected tripeptide free base V (5.31 g, 7.35 mmoles) was dissolved in 35 ml of dimethylformamide, and 4.06 g (8.1 mmoles) of N^α-carbobenzoxy-N^ϵ-*t*-butyloxycarbonyllysine *p*-nitrophenyl ester[13] was added with stirring at room temperature. After 43 hr at room temperature the reaction was complete, as judged from thin layer chromatography. The entire mixture was precipitated from 1 l. of anhydrous ether and the oily precipitate (9 g) was subjected to countercurrent distribution for 100 transfers in the toluene system. The yellowish material recovered from the main peak (K = 1.0) was dissolved in methanol (45 ml) and precipitated from anhydrous ether (1000 ml) to obtain 7 g (88%) of peptide VI as a white, amorphous powder: mp 106–109°, $[\alpha]^{23}D$ −22.2° (*c* 2, methanol). In paper chromatography compound VI behaved as a single component in four solvent systems: R_f (BAW) 0.88, R_f (SBA) 0.86, R_f (BPAW) 0.91, R_f (nBA) 0.91. The peptide was also homogeneous in thin layer chromatography: R_f (CM) (1:1) 0.69.

Anal. Calcd for $C_{50}H_{73}O_{12}N_{11}S_2$ (1084.3): C, 55.3; H, 6.78; N, 14.2; S, 5.91. Found: C, 55.6; H, 6.52; N, 13.9; S, 6.00.

N^ϵ-*t*-Butyloxycarbonyllysyl-N^G-tosylarginyl-N^G-tosylarginylprolinol (VII). A solution of 6.1 g (5.62 mmoles) of fully protected tetrapeptide VI in 150 ml of methanol was hydrogenolyzed for 5.5 hr in the presence of a Pd catalyst prepared from 2 g of $PdCl_2$. The solution obtained after removal of the catalyst by filtration was concentrated *in vacuo* to give a glassy product which was dissolved in 40 ml of methanol and precipitated with anhydrous ether (700 ml). A yield of 5 g (94%) of compound VII was obtained in amorphous form: mp 105–110°, $[\alpha]^{24}D$ −14.0° (*c* 1, methanol). The material was homogeneous in four paper chromatographic systems: R_f (BAW) 0.68, R_f (SBA) 0.75, R_f (BPAW) 0.85, R_f (nBA) 0.84, as well as in two thin layer systems: R_f (BAW) 0.39, R_f (CM) (8:2) 0.16. Countercurrent distribution of a sample for 80 transfers in the carbon tetrachloride system gave a single peak (K = 0.33) which conformed closely to the theoretical distribution pattern.

Anal. Calcd for $C_{42}H_{67}O_{10}N_{11}S_2$ (950.2): C, 53.1; H, 7.11; N, 16.2. Found: C, 53.0; H, 6.94; N, 16.5.

N^α-Carbobenzoxy-N^ϵ-*t*-butyloxycarbonyllysyl-N^ϵ-*t*-butyloxycarbonyllysyl-N^G-tosylarginyl-N^G-tosylarginylprolinol (VIII). Acetonitrile (15 ml) was added to a solution of VII (2.38 g, 2.5 mmoles) in dimethylformamide (15 ml) followed by N^α-carbobenzoxy-N^ϵ-*t*-butyloxycarbonyllysine *p*-nitrophenyl ester with stirring at room temperature. After the yellow solution was kept at room temperature for 48 hr, followed by a further incubation at 37° for 24 hr, only a trace of unreacted VII could be detected by paper chromatography in the BAW system. The entire mixture was concentrated to a syrup *in vacuo*, dissolved in a small volume of acetone, and precipitated by an addition of 700 ml of anhydrous ether. The yellowish precipitate (3.3 g) was dissolved in 400 ml of chloroform, and the solution was extracted once with 10% citric acid solution (130 ml), then with 130-ml portions of 5% $NaHCO_3$ solution until yellow color no longer appeared in the aqueous phase, and finally with 130-ml portions of water and saturated NaCl solution. After the organic phase was dried over anhydrous Na_2SO_4, the solvent was removed *in vacuo* and the product was subjected to countercurrent distribution for 116 transfers in the carbon tetrachloride system. The material (2.6 g) recovered from the single peak (K = 0.064) was again subjected to countercurrent distribution for 100 transfers in the toluene system. After drying, the material obtained from the main peak (K = 0.88) was dissolved in methanol (10 ml) and precipitated from anhydrous ether (600 ml) to yield 2.57 g (78.5%) of amorphous VIII: mp 105–110°, $[\alpha]^{23}D$ −24.6°

(*c* 2, methanol). The product was homogeneous in four paper chromatographic systems: R_f (BAW) 0.94, R_f (SBA) 0.90, R_f (BPAW) 0.93, R_f (nBA) 0.92 as well as in thin layer chromatography: R_f (CM) (8:2) 0.66.

Anal. Calcd for $C_{61}H_{93}O_{15}N_{13}S_2$ (1312.6): C, 55.8; H, 7.14; N, 13.9; S, 4.89. Found: C, 56.1; H, 7.11; N, 14.0; S, 4.74.

N^{ϵ}-*t*-Butyloxycarbonyllysyl-N^{ϵ}-*t*-butyloxycarbonyllysyl-N^{G}-tosylarginyl-N^{G}-tosylarginylprolinol (IX). Fully protected pentapeptide VIII (2.37 g, 1.83 mmoles) was dissolved in methanol and hydrogenolyzed as before for 5.5 hr in the presence of a Pd catalyst prepared from 2 g of $PdCl_2$. After the catalyst was removed by filtration, methanol was removed *in vacuo* and the product was subjected to countercurrent distribution for 100 transfers in the toluene system. A single peak (K = 2.33), closely approaching the theoretical distribution, was obtained. The material recovered from this peak was dried, dissolved in methanol (10 ml), and precipitated from anhydrous ether (600 ml) to yield 1.89 g (88%) of the amorphous peptide IX: mp 105–109°, $[\alpha]^{24}D$ −21.3° (*c* 2, methanol). Peptide IX behaved as a single component in thin layer chromatography in four solvents: R_f (BAW) 0.52, R_f (BPAW) 0.68, R_f (CM) (8:2) 0.28, R_f (CM) (1:1) 0.64, as well as in four paper chromatographic systems: R_f (BAW) 0.82, R_f (SBA) 0.83, R_f (BPAW) 0.92, R_f (nBA) 0.89.

Anal. Calcd for $C_{53}H_{87}O_{13}N_{13}S_2$ (1178.5): C, 54.0; H, 7.44; N, 15.5; S, 5.44. Found: C, 53.7; H, 7.35; N, 15.4; S, 5.66.

Peptide IX (5.1 mg, 4.3 μmoles) was dissolved in 0.15 ml of dimethylformamide and to the solution was added 0.28 ml of 0.01 *M* Tris buffer (pH 8.5) containing 0.01 *M* $MgCl_2$. Leucine aminopeptidase (Worthington Lot No. 5917) was added to this solution in three equal portions by dissolving 0.3 mg of the enzyme in 0.03 ml of distilled water in each case and adding the fresh enzyme solution at zero time, and after 29 and 68 hr of incubation at 37°. After a total incubation period of 72 hr, the digestion was complete as judged by paper chromatography in the BAW system which revealed the presence in the mixture of N^{G}-tosylarginine,[11] N^{ϵ}-*t*-butyloxycarbonyllysine,[13] and compound III. An aliquot of the digest was subjected to quantitative analysis in the automatic amino acid analyzer[23] against the authentic compounds. The amounts of N^{G}-tosylarginine and N^{ϵ}-*t*-butyloxycarbonyllysine present were found to be 0.98 and 1.98 moles, respectively, per mole of peptide IX digested.

To two test tubes (a and b) each containing 0.8 ml of a 5% $NaHCO_3$ solution, the following additions were made: (a) 0.25 ml of the incubation mixture of III with LAP, followed by 0.13 ml (35 μmoles) of a 5% (w/v) solution of DNFB in acetone; (b) 0.25 ml of the incubation mixture of IX with LAP, followed by 0.15 ml (40 μmoles) of the DNFB solution. Both tubes were shaken at room temperature for 2 hr after the addition of 0.5 ml of acetone to each. DL-Alanine (11 mg, 120 μmoles) was added to each tube and shaking continued for an additional 2 hr at room temperature. After adjusting the total volume to 3.5 ml with water and sufficient acetone to give a clear solution in each case, different aliquots of a (0.1, 0.2, 0.3, and 0.4 ml) and of b (0.5 and 0.8 ml) were applied quantitatively to separate thin layer plates, which were then developed in the CM (8:2) system. The yellow band (R_f 0.50–0.58) was recovered quantitatively from each plate and eluted in separate centrifuge tubes into a total volume of 4 ml absolute ethanol. After the solutions were cleared by means of centrifugation, the absorption spectrum was determined in the region of 230–450 mμ, and from the optical density values at the wavelength of maximal absorption (345 mμ), a standard curve was constructed, giving the optical density as a function of the theoretical concentration of IIIa in eluates derived from a.[36] The corrected amounts of IIIa present in the eluate derived from b were determined directly from the standard curve and corresponded to 0.95 and 0.99 mole per mole of peptide IX digested, respectively.

N^{α}-Carbobenzoxy-N^{ϵ}-*t*-butyloxycarbonyllysylprolylvalylglycyl-N^{ϵ}-*t*-butyloxycarbonyllysyl-N^{ϵ}-*t*-butyloxycarbonyllysyl-N^{G}-tosylarginyl-N^{G}-tosylarginylprolinol (X). N^{α}-Carbobenzoxy-N^{ϵ}-*t*-butyloxycarbonyllysylprolylvalylglycine[3] (0.83 g, 1.3 mmoles) was dissolved in 20 ml of acetonitrile by slightly warming. After cooling the solution to 0°, 0.182 ml (1.3 mmoles) of triethylamine was added, followed by N-ethyl-5-phenylisoxazolium 3′-sulfonate (0.348 g, 1.37 mmoles) and the mixture was stirred at 0° for 1 hr. The pentapeptide free base IX (1.53 g, 1.3 mmoles) was added to the almost clear solution and stirring was continued at room temperature. After 24 hr the reaction was complete as judged by thin layer chromatography of the mixture, and a substantial amount of crystalline material which separated during the reaction was filtered off, washed with some acetonitrile, and dried. The washing solvent was combined with the mother liquor and concentrated *in vacuo* to yield a glassy product, which was dissolved in 100 ml of chloroform. The chloroform solution was extracted twice with water (30 ml), once with saturated NaCl solution (40 ml) and dried over anhydrous Na_2SO_4. After removal of the solvent *in vacuo*, the glassy product was combined with the crystalline material filtered off initially, and subjected to countercurrent distribution for 100 transfers in the toluene system. The main peak (K = 0.59) closely approached the theoretical distribution pattern and yielded 2.1 g of a glassy product after removal of the solvents. Just enough methanol was added to this material to produce a heavy syrup from which peptide X separated in crystalline form upon trituration with ethyl acetate. The total volume was adjusted to about 30 ml by adding more ethyl acetate and after several hours at 0°, 2 g (86%) of crystalline material was collected by filtration: mp 129–131°, $[\alpha]^{24}D$ −43.7° (*c* 1, methanol). The product behaved as a single component in paper chromatography in three solvent systems: R_f (BAW) 0.91, R_f (SBA) 0.90, R_f (BPAW) 0.94. It was homogeneous in three thin layer systems: R_f (BAW) 0.66, R_f (BPAW) 0.74, R_f (CM) (8:2) 0.60.

Anal. Calcd for $C_{84}H_{132}O_{21}N_{18}S_2$ (1794.2): C, 56.2; H, 7.42; N, 14.1; S, 3.57. Found: C, 56.4; H, 7.41; N, 14.3; S, 3.36.

N^{ϵ}-*t*-Butyloxycarbonyllysylprolylvalylglycyl-N^{ϵ}-*t*-butyloxycarbonyllysyl-N^{ϵ}-*t*-butyloxycarbonyllysyl-N^{G}-tosylarginyl-N^{G}-tosylarginylprolinol (XI). Fully protected nonapeptide X (1.8 g, 1 mmole) was dissolved in methanol (30 ml) and subjected to catalytic hydrogenolysis for 5 hr in the presence of Pd prepared from 1 g of $PdCl_2$. The catalyst was filtered off and after removal of the solvent *in vacuo*, the product was subjected directly to countercurrent distribution for 100 transfers in the toluene system. A single peak (K = 2.12), which was in good agreement with the theoretical distribution, was obtained. The material recovered from the peak was obtained in crystalline form by triturating a methanolic syrup with ethyl acetate. After a few hours at 0°, 1.55 g (93%) of crystalline peptide XI was collected: mp 131–133°, $[\alpha]^{24}D$ −36.4° (*c* 1, methanol). It was homogeneous in three paper chromatographic systems: R_f (BAW) 0.84, R_f (SBA) 0.91, R_f (BPAW) 0.93, and in three thin layer solvent systems: R_f (BAW) 0.3, R_f (BPAW) 0.72, R_f (CM) (8:2) 0.22.

Anal. Calcd for $C_{76}H_{126}O_{19}N_{18}S_2$ (1660): C, 55.0; H, 7.65; N, 15.2. Found: C, 54.7; H, 7.71; N, 15.3.

Seryltyrosylserylmethionylglutamylhistidylphenylalanylarginyltryptophylglycyllysylprolylvalylglycyllysyllysylarginylarginylprolinol XIII). N^{α}-Carbobenzoxyseryltyrosylserylmethionyl-γ-benzylglutamylhistidylphenylalanyl-N^{G}-tosylarginyltryptophylglycine[1] (0.168 g, 0.1 mmole), together with 0.166 g (0.1 mmole) of the nonapeptide free base XI, was dissolved in 1.4 ml of dimethylformamide at room temperature. Dicyclohexylcarbodiimide (DCCI)[18] (0.042 g, 0.2 mmole) was added to the clear solution, and the mixture was kept stirring at 0° for 2 days. An additional amount of DCCI (0.04 g) was added, and stirring at the same temperature was continued for 7 additional days. Glacial acetic acid (0.5 ml) was then added to the mixture and stirring was continued for another hour at the same temperature. The solvent was removed *in vacuo*, the product dried over P_2O_5, and 13 ml of trifluoroacetic acid (TFA) was added in a nitrogen atmosphere. After the mixture was stirred for 2 hr at room temperature, the TFA was removed *in vacuo* and the purple, syrupy product was subjected to countercurrent distribution in the BAW system for 200 transfers.

The ninhydrin, ultraviolet, and Pauly-positive peak (K = 2.78) yielded 0.1 g of material, which was dried thoroughly over P_2O_5 *in vacuo*. This material was dissolved in approximately 200 ml of anhydrous liquid ammonia, and small pieces of sodium were added to the solution with stirring under anhydrous conditions at the boiling point of liquid ammonia. In order to retain the blue color for 1 hr, a slight excess of Na was added in the solution. After the ammonia had been allowed to evaporate at room temperature, the residual material was dried *in vacuo* over P_2O_5 and concentrated H_2SO_4. It was then dissolved in 0.1 *N* acetic acid (20 ml) and applied to an IRC-50 cation-exchange column (3 × 5 cm) for desalting.[20] The column was washed successively with 250-ml portions of 0.25% (v/v) acetic acid and water before the band of adsorbed Pauly-positive material was eluted with a mixture of pyridine–glacial acetic acid–water (30:4:66, v/v). Most of the solvent was

(36) A standard curve was also constructed with a reaction mixture derived from a sample of peptide III which was subjected to an identical treatment as (a), but without exposure to LAP. The two curves were completely identical.

removed *in vacuo*, and the remaining solution was lyophilized to produce 0.07 g of material which was submitted to chromatography on a carboxymethylcellulose (CMC) column[21] (0.9 × 56 cm) using a continuous ammonium acetate concentration gradient for elution (Figure 3). The most retarded peak (tubes 237–280) was lyophilized three times and yielded[37] compound XIII in a yield of 0.028 g. Peptide XIII behaved as a single component in paper electrophoresis (mobility relative to L-lysine, 0.95; pH 3.7, 400 v, 5 hr). The amino acid composition of XIII was determined according to the method of Spackman, Stein, and Moore[23] and was found to be in good agreement with the theoretically expected values (Table I); $[\alpha]^{24}D$ −76.7° (*c* 0.4, 0.1 *M* acetic acid). Prolinol content was determined by the dinitrophenylation procedure.

(37) In several experiments the over-all yield of peptide, calculated on the basis of the starting materials used for the final coupling step, was approximately 10% based on the peptide content (about 75%) as determined by the optical density of a solution of the synthetic product in 0.01 *N* HCl at 280 mμ.

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Communications to the Editor

Homogeneous Electron Transfer to Primary Alkyl Halides and Radicals

Sir:

The reactions of sodium naphthalene (0.01–0.1 *M*) with 5-hexenyl and cyclopentylmethyl bromides and chlorides in 1,2-dimethoxyethane at room temperature yield substantial quantities of hydrocarbon products. While the nature of the products indicates that radicals are intermediates in these reactions, the major portion of the product cannot arise in radical–radical or radical–solvent reactions. The evidence indicates that carbanions, instead, are the immediate precursors of the major hydrocarbon products. For the similar reactions of alkyl iodides, evidence that radical–radical reactions, and perhaps radical–solvent reactions, are important has been presented recently.[1]

When sodium naphthalene is used in excess, small quantities of dimeric (C_{12}) hydrocarbons are produced (*ca.* 3% for the chlorides, *ca.* 7% for the bromides), while substantial yields (40–70%) of C_6 hydrocarbons (1-hexene and methylcyclopentane) are obtained.[2] No 1,5-hexadiene, potentially a product, *via* E2 or radical disproportionation reactions, was produced in detectable quantities.

The major product from either of the 5-hexenyl halides is 1-hexene, formed in 40–70% yields. This hydrocarbon cannot arise in a radical–solvent reaction (eq 2) since hydrogen abstraction from solvent does not

$$CH_2{=}CHCH_2CH_2CH_2CH_2X + Naph\cdot^- \longrightarrow CH_2{=}CHCH_2CH_2CH_2CH_2\cdot + Naph + X^- \quad (1)$$

$$CH_2{=}CHCH_2CH_2CH_2CH_2\cdot + SH \longrightarrow CH_2{=}CHCH_2CH_2CH_2CH_3 + S\cdot \quad (2)$$

$$CH_2{=}CHCH_2CH_2CH_2CH_2\cdot \longrightarrow (\text{cyclo-}C_5H_9)CH_2\cdot \quad (3)$$

compete effectively with the cyclization of the 5-hexenyl radical to the cyclopentylmethyl radical (eq 3).[3] The 1-hexene is therefore best interpreted as arising directly from the 5-hexenyl anion.

The most reasonable sources of the 5-hexenyl anion are reduction of the 5-hexenyl radical (eq 4) and opening

$$CH_2{=}CHCH_2CH_2CH_2CH_2\cdot + Naph\cdot^- \longrightarrow CH_2{=}CHCH_2CH_2CH_2CH_2^- + Naph \quad (4)$$

of the cyclopentylmethyl anion. The latter route is excluded by the fact that reductions of cyclopentylmethyl bromide and chloride with sodium naphthalene yield methylcyclopentane as the only C_6 hydrocarbon.[6] Since we have not been able to obtain convincing evidence that C_6 anions persist in solution for times detectable on a laboratory time scale, it is probable that they obtain protons from the solvent before quenching.

Methylcyclopentane is formed in reductions of 5-hexenyl bromide and chloride in yields which depend on concentration conditions and method of mixing reagents. Since the yield can be reduced to near zero when large and excess concentrations of sodium naphthalene are used, it cannot be formed through cyclization of the 5-hexenyl anion, but is best viewed as arising through reaction 3 (followed by reduction, as in reaction 4) in a competition with reaction 4.

(1) G. D. Sargent and S. Bank, Abstracts of the Middle Atlantic Regional Meeting of the American Chemical Society, Feb 3–4, 1966, Philadelphia, Pa., p 120.

(2) Alkyl halides were admitted by means of a breakseal to a solution of sodium naphthalene. Mixing was accomplished by vigorous shaking. The reaction was instantaneous on a laboratory time scale. Reaction mixtures were quenched with acetic acid within 5 min. The products do not isomerize under these conditions. Analyses were by vapor phase chromatography.

(3) This is indicated by considerations of the data of Lamb[4] and Walling,[5] applying reasonable solvent and temperature corrections. As control experiments, however, we have examined the products of decomposition of 6-heptenoyl peroxide in 1,2-dimethoxyethane at 50° in the presence and absence of a scavenger (Koelsch's radical). Methylcyclopentane was the only scavengeable C_6 product (0.44 mole/mole of peroxide in the absence of scavenger). The yield of open-chain C_6 hydrocarbons was 0.05 mole/mole of peroxide, and this was not detectably affected by the presence of a scavenger. Thus, all of the detectable noncage C_6 hydrocarbon product was methylcyclopentane. A reduction of 5-hexenyl bromide with sodium naphthalene at 50° in 1,2-dimethoxyethane gave results similar to those reported above.

(4) R. C. Lamb, P. W. Ayers, and M. K. Toney, *J. Am. Chem. Soc.*, **85**, 3483 (1963).

(5) C. Walling and M. S. Pearson, *ibid.*, **86**, 2226 (1964).

(6) A tacit assumption here is that the cyclopentylmethyl radical does not react with solvent before it is reduced. Since the 5-hexenyl radical does not, and since both radicals are primary alkyl radicals, this assumption is justified by the consideration that both radicals should have similar reactivities.