was removed by filtration, and the filtrate was adjusted to pH 7.0. The neutral solution was steam distilled, and seven 100-ml. fractions were collected. The first fraction ($\lambda_{max}$ 227 mμ, $a$ 394) was mixed with 1.5 l. of Brady reagent and allowed to stand at room temperature for 12 hr. The precipitate was isolated by filtration and recrystallized once from acetic acid and a second time from benzene–petroleum ether (60–70°); m.p. 184–186° (lit. for crotonaldehyde 2,4-dinitrophenylhydrazone, 187–188°). The infrared spectrum was identical with that of an authentic sample of crotonaldehyde 2,4-dinitrophenylhydrazone.

The fourth fraction from the steam distillation was combined with 500 ml. of Brady reagent and allowed to stand at room temperature for 12 hr. The precipitate was isolated by filtration and recrystallized from ethanol, m.p. 164–166° (lit. for formaldehyde 2,4-dinitrophenylhydrazone, 164–166°). The infrared spectrum was identical with that of an authentic sample of formaldehyde 2,4-dinitrophenylhydrazone.

**Methyl Actinospectoic Acid Methyl Ester (XXII).**—Ten grams of actinospectinoic acid was added to a mixture of 95 ml. of acetyl chloride and 600 ml. of methanol, and the solution was allowed to stand at room temperature for 48 hr. The precipitated actinamine dihydrochloride (wt. 8.05 g.) was removed by filtration. The filtrate was mixed with an equal volume of ether and allowed to stand at room temperature for 24 hr. A second precipitate had formed and was removed by filtration. The filtrate was concentrated to a volume of 340 ml. under reduced pressure and adjusted to a pH of 6.4 with methanolic sodium hydroxide prepared by dissolving 12 g. of sodium hydroxide in 400 ml. of absolute methanol. After the precipitate had been removed by filtration, the filtrate was concentrated to 450 ml. and again filtered. This filtrate was concentrated to 150 ml., and 500 ml. of ether and 300 ml. of acetone were added. The mixture was allowed to stand at room temperature for 12 hr. and filtered. The organic solvents were removed from the filtrate by evaporation under reduced pressure, leaving 6.0 g. of viscous liquid. The liquid was fractionated, collecting the material boiling at 51–54° at 0.05 mm. The infrared spectrum of the distillate had absorption bands at 3450 and 1750–1740 cm.$^{-1}$ and $[\alpha]^{25}D$ −65.5° ($c$ 0.998, 95% ethanol).

*Anal.* Calcd. for $C_8H_{14}O_5$: C, 50.57; H, 7.43; O, 42.11; $CH_3C(3)$, 7.85; $CH_3O(2)$, 32.6. Found: C, 49.49; H, 7.75; O, 43.31; $CH_3C$, 6.8; $CH_3O$, 26.62.

**N,N′-Bis-(ethylcarbamoyl)-actinospectinoic Acid (XIX).**—A solution of 5.0 g. of N,N′-bis-(ethylcarbamoyl)-actinospectacin in 25 ml. of 0.1 $N$ barium hydroxide was allowed to stand at room temperature for 5 hr. Sufficient 0.1 $N$ sulfuric acid was added to remove barium ion, and the precipitate was removed by centrifugation. The supernatant was filtered and freeze-dried. The residue was distributed through 500 transfers in a countercurrent distribution apparatus using a 1-butanol–water system. Tubes 180–260 were combined and evaporated to dryness under reduced pressure. The residue was dissolved in 50 ml. of water, and the solution was freeze-dried giving 1.35 g. of amorphous solid, m.p. 165–171° dec., $[\alpha]^{25}D$ −63° ($c$ 1, $H_2O$). The $pK_a'$ of this material was 3.57. The infrared spectrum had bands at 3390, 1720, 1606, 1535, 1245, 1190, 1055, and 1023 cm.$^{-1}$.

*Anal.* Calcd. for $C_{20}H_{36}N_4O_{10}$: C, 48.77; H, 7.37; N, 11.40; mol. wt., 474. Found: C, 49.22; H, 7.34; N, 11.06; mol. wt. (elect. titr.), 481.

**Periodate Titration of N,N′-Bis-(ethylcarbamoyl)-actinospectinoic Acid.**—This was run by the Fleury–Lange procedure[16] using 492 mg. (1.04 mmoles) dissolved in 30 ml. of 0.1 $M$ sodium periodate solution. There was no periodate consumption in 3.5 hr.

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# Insulin Peptides. VII. The Synthesis of Two Decapeptide Derivatives Containing the C-Terminal Sequence of the B-Chain of Insulin[1]

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The partially protected decapeptide γ-methyl-L-glutamyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester dihydrochloride and the fully protected decapeptide N-carbobenzoxy-γ-benzyl-L-glutamyl-N$^{\omega}$-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester have been synthesized and their chemical and stereochemical homogeneity has been established. These decapeptide derivatives contain amino acid sequences found in the C-terminal portion of the B-chain of insulin.

Studies are under way in this laboratory directed toward the synthesis of the A- and B-chains of insulin and eventually to the total synthesis of this protein.[2] To this end several peptide derivatives embodying within their structures amino acid sequences found in the insulin chains have been prepared and the detailed synthesis of a number of these derivatives has been already reported.[3–8]

As a step toward the synthesis of the B-chain of insulin the preparation of certain derivatives of the decapeptide L-glutamyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-L-lysyl-L-alanine was desired. This decapeptide contains the C-terminal sequence of the B-chain of insulin. Peptide derivatives containing some of the amino acid sequences of the above decapeptide have been prepared by other investigators.[9–11] In the present communication the synthesis of the partially protected decapeptide γ-methyl-L-glutamyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester dihydrochloride (V) and of the fully protected decapeptide N-carbobenzoxy-γ-benzyl-L-glutamyl-N$^{\omega}$-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester (VII) is reported.

The key intermediate in the synthesis of these derivatives was the protected heptapeptide N-carbobenzoxy-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester, whose preparation has been reported previously.[5] Removal of the carbobenzoxy group from the protected heptapeptide by catalytic hydrogenation and coupling of the resulting product with the N-carbobenzoxyglycine $p$-nitrophenyl ester[12] afforded the protected octapeptide N-carbobenzoxyglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester (I) in 80% yield. The

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(2) A preliminary report of the work described in this paper and of the work carried out thus far in our laboratory which is directed toward the synthesis of insulin has been presented (P. G. K.) in the Eighth National Medicinal Chemistry Symposium of the American Chemical Society held in Boulder, Colo., June 18–20, 1962.

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same peptide derivative was prepared by Kunde and Zahn[11] in 31% yield by the condensation of N-carbobenzoxyglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosine with L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester by the carbodiimide[13] method and by Ke *et al.*,[10] by coupling L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester with N-carbobenzoxyglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonine by the carbodiimide or the azide method in 42 and 53% yield, respectively.

The chemical homogeneity of the protected octapeptide I was ascertained by elemental analysis and paper chromatography of the decarbobenzoxylated derivative in two solvent systems. In both systems the chromatograms exhibited sharp single spots indicative of the presence of a single component.

Catalytic hydrogenation of the protected octapeptide resulted in the removal of the carbobenzoxy group. The resulting product was condensed with tricarbobenzoxy-L-arginine[14,15] by the carbodiimide method to give tricarbobenzoxy-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester (II) in 44% yield. Elemental analysis and paper chromatography of the decarbobenzoxylated derivative in two solvent systems established the chemical purity of this peptide derivative. Decarbobenzoxylation of the protected nonapeptide by catalytic hydrogenation in the presence of HCl resulted in the formation of L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester dihydrochloride (III) in 98% yield. The chemical purity of this partially protected nonapeptide was established by elemental analysis, paper chromatography in two solvent systems, and amino acid analysis of an acid hydrolysate. In the latter case the constituent amino acids were obtained in the expected ratio and in an average recovery of 88% of theory. For ascertaining its stereochemical homogeneity the partially protected nonapeptide III was digested with leucine aminopeptidase (LAP) and the digest was analyzed by an automatic analyzer. The containing amino acids were obtained in the expected ratios and in 72% average recovery, indicating that the digestion was complete. This suggests[16] that no racemization of the constituent amino acids had occurred during the synthetic processes leading to the preparation of the nonapeptide derivative.

Interaction of the partially protected nonapeptide III with N-carbobenzoxy-$\gamma$-methyl-L-glutamic acid *p*-nitrophenyl ester[17,18] afforded the decapeptide hydrochloride N-carbobenzoxy-$\gamma$-methyl-L-glutamyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester (IV) in 60.5% yield. The chemical homogeneity of this decapeptide was established by elemental analysis and paper chromatography of the decarbobenzoxylated derivative. Chromatograms obtained in two solvent systems exhibited single ninhydrin and Sakaguchi positive spots. Catalytic hydrogenation of the protected decapeptide in aqueous acetic acid containing HCl, followed by lyophilization, yielded almost quantitatively the partially protected decapeptide $\gamma$-methyl-L-glutamyl-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester dihydrochloride (V). This peptide derivative gave the correct elemental analysis and produced a single spot, ninhydrin and Sakaguchi positive, on paper chromatography in two solvent systems. Amino acid analysis of an acid hydrolysate showed the expected composition with an average amino acid recovery of 93% of theory. The decapeptide derivative was completely digestible by LAP. This was ascertained by amino acid analysis of the digest by an automatic analyzer. The constituent amino acids, with the exception of glutamic acid, were obtained in the stoichiometrically correct proportions with an average recovery of amino acids of 89%. As can be seen, from the amino acid ratios reported (Experimental section) only traces of glutamic acid were obtained. Hofmann, *et al.*,[19] observed a similar situation with glutamine during LAP digestion of glutamine-containing peptides. In that case quantitative determination of glutamine by the ninhydrin technique was not feasible because of pyrrolidonecarboxylic acid formation. It then appears possible that during LAP digestion of the partially protected decapeptide and the subsequent amino acid analysis of the digest by the automatic analyzer the $\gamma$-methylglutamic acid residue was converted to a large extent to pyrrolidonecarboxylic acid and hence did not give a ninhydrin reaction. This interpretation is supported by the finding that acid hydrolysis of the decapeptide yields glutamic acid in the expected amounts.

For the synthesis of the fully protected nonapeptide $N^{\alpha}$-carbobenzoxy-$N^{\omega}$-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester (VI) the product resulting by hydrogenolysis of N-carbobenzoxyglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester (I) was condensed with $N^{\alpha}$-carbobenzoxy-$N^{\omega}$-tosyl-L-arginine.[20] Activation of the latter compound was accomplished by the use of 2-ethyl-5-phenyloxazolium-3′-sulfonate.[21] The protected nonapeptide VI was thus obtained in 87% yield and in analytically pure form. Elemental analysis, paper chromatography of the decarbobenzoxylated derivative in two solvent systems, and amino acid analysis of an acid hydrolysate served as criteria of the chemical homogeneity of this peptide derivative.

From the results obtained by paper chromatography and quantitative amino acid analysis, with the use of an automatic analyzer, of the acid hydrolysate of the protected nonapeptide (Experimental section) it is apparent that the tosyl group was split almost quantitatively from lysine[5] and arginine during acid hydrolysis.

Exposure of the fully protected nonapeptide VI to HBr in acetic acid and coupling of the ensuing decarbobenzoxylated derivative with N-carbobenzoxy-$\gamma$-benzyl-L-glutamic acid *p*-nitrophenyl ester[22] afforded the protected decapeptide N-carbobenzoxy-$\gamma$-benzyl-L-glutamyl-$N^{\omega}$-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester (VII) in 70% yield. The chemical purity of this product was established by elemental analysis, paper chromatography of the decarbobenzoxylated derivative in two solvent systems, and amino acid analysis of an acid hydrolysate. For evaluation of stereochemical homogeneity, the protected decapeptide was decarbobenzoxylated on exposure to HBr in

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acetic acid and the product incubated with LAP. The high insolubility of the deblocked peptide derivative in the incubation medium made impossible the digestion of the entire incubated sample and hence it was impractical to express the results of the digestion in quantitative terms. However, amino acid analysis of the digest by the automatic analyzer and by paper chromatography provided convincing evidence regarding the optical purity of the decapeptide. The chromatogram from the analyzer exhibited peaks[23] corresponding to $N^{\epsilon}$-tosyllysine, $N^{\omega}$-tosylarginine, threonine, glutamic acid, glycine, alanine, tyrosine, phenylalanine, and proline only.

Similarly, the paper chromatogram of the digest in the Partridge system[24] exhibited only ninhydrin positive spots with $R_f$'s corresponding to the same amino acid residues. It is therefore concluded that the digestion of the decapeptide derivative by LAP is complete and this is indicative of its optical purity.

It may be noted that whereas LAP digestion of the γ-methyl derivative of the decapeptide V followed by amino acid analysis gave only traces of glutamic acid, the γ-benzyl derivative of the decapeptide under similar conditions produced glutamic acid in amounts corresponding to that of the other constituent amino acids. The possibility then appeared that γ-benzyl-L-glutamic acid is hydrolyzed during incubation with LAP. This was confirmed by incubating γ-benzyl-L-glutamic acid with LAP, followed by paper chromatography of the digest. The chromatogram developed in the Partridge system exhibited only a single ninhydrin positive spot with $R_f$ identical with that of glutamic acid.

## Experimental

Capillary melting points were determined for all compounds and are uncorrected.

For paper chromatography the protected peptides were deblocked either with HBr in acetic acid or by catalytic hydrogenation and chromatographed on Whatman No. 1 filter paper at room temperature. $R_f^1$ values refer to the Partridge system[24]; $R_f^2$ values refer to the system[25] 1-butanol–pyridine–acetic acid–water, 30:20:6:24, and are expressed as a multiple of the distance traveled by a histidine marker. The enzymatic analyses (LAP) were performed according the procedure described by Hofmann *et al.*[26,27] The amino acid analyses of acid and LAP hydrolysates were carried out with a Beckman–Spinco amino acid analyzer, Model 120, according to the method of Spackman, Stein, and Moore.[28]

**N-Carbobenzoxyglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine Methyl Ester Hydrate (I).**—N-Carbobenzoxy-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester (5 g.) was hydrogenated for 8 hr. over 10% palladium–charcoal catalyst (1.5 g.) in methanol (80 ml.) containing 1 *N* HCl (7 ml.). The catalyst was filtered off and the filtrate evaporated to dryness *in vacuo*. The remaining product was dried by the addition of methanol followed by evaporation under reduced pressure. The residue was dissolved in dimethylformamide (40 ml.) containing triethylamine (1 ml.). To this solution N-carbobenzoxyglycine *p*-nitrophenyl ester (1.8 g.) was added. After 24 hr. the reaction mixture was diluted with 1 *N* $NH_4OH$ (3 ml.), stirred 1 hr., and subsequently mixed with ethyl acetate (500 ml.) and water (200 ml.). The organic layer was washed with 1 *N* $NH_4OH$, water, 1 *N* HCl, and water again. (In order to prevent precipitation of the product during the washing, methanol was added to the ethyl acetate solution.) Concentration of the solvent to a small volume resulted in the precipitation of the product, which was isolated by filtration and was further purified by reprecipitation from aqueous methanol; wt. 4.2 g. (80%), m.p. 182–185° (the reported melting point for the dihydrate[10] is 190–193° and for the anhydrous[11] compound is 195–199°); $[\alpha]^{28}D$ −25.8° (*c* 0.21, dimethylformamide). For paper chromatography a sample was decarbobenzoxylated by catalytic hydrogenation in the presence of HCl. $R_f^1$ 0.88, $R_f^2$ 6.1 × his, single sharp ninhydrin positive spot.

*Anal.* Calcd. for $C_{63}H_{77}N_9O_{15}S \cdot H_2O$: C, 60.8; H, 6.32; N, 10.0. Found: C, 60.6; H, 6.29; N, 9.7.

**Tricarbobenzoxy-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine Methyl Ester 0.5 Hydrate (II).**—N-Carbobenzoxyglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester (2 g.) was dissolved in methanol (25 ml.) containing 1 *N* HCl (2 ml.) and hydrogenated for 6 hr. in the presence of 10% palladium–charcoal catalyst (0.8 g.). The catalyst was filtered off and the filtrate was concentrated to dryness *in vacuo*. Traces of HCl were eliminated from the remaining product by the addition of methanol followed by evaporation. The residue was dissolved in dimethylformamide (15 ml.) containing triethylamine (0.24 ml.) and cooled at 0°; tricarbobenzoxy-L-arginine (0.95 g.) was added to this solution followed by N,N′-dicyclohexylcarbodiimide (0.45 g.). The reaction mixture was stirred at 5° for 24 hr. and acidified with drops of acetic acid. The precipitated N,N′-dicyclohexylurea was filtered off and the filtrate mixed with cold water (150 ml.) containing HCl (1 ml.). The precipitated product was collected by filtration, washed with water, and dried. For purification this crude product was dissolved by slight warming in dimethylformamide (12 ml.) and the solution cooled to 0°. A second crop of N,N′-dicyclohexylurea was precipitated and removed by filtration. The filtrate was mixed with tetrahydrofuran (10 ml.) and ether (80 ml.). The precipitated white solid was isolated by filtration and was further purified by reprecipitation from aqueous acetic acid; wt. 1.2 g. (44%), m.p. 188–190°, $[\alpha]^{28}D$ −37.5° (*c* 0.22, dimethylformamide). For paper chromatography a sample was decarbobenzoxylated by catalytic hydrogenation in the presence of HCl; $R_f^1$ 0.80, $R_f^2$ 8.35 × his, single ninhydrin positive spot.

*Anal.* Calcd. for $C_{85}H_{101}N_{13}O_{20}S \cdot 0.5\ H_2O$: C, 61.3; H, 6.16; N, 10.9. Found: C, 60.7; H, 6.03; N, 11.3.

**L-Arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine Methyl Ester Dihydrochloride Hydrate (III).**—Tricarbobenzoxy-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester (1.45 g.) was dissolved in a mixture of acetic acid (32 ml.), water (18 ml.), and 2 *N* HCl (1.1 ml.) and hydrogenated for 6 hr. in the presence of 10% palladium–charcoal catalyst (0.8 g.). The catalyst was removed by filtration and the filtrate was lyophilized. The white solid thus obtained was further dried over $P_2O_5$ *in vacuo;* wt. 1.14 g. (98%); $[\alpha]^{28}D$ −25.6° (*c* 0.23, 50% aqueous acetic acid); $R_f^1$ 0.80, $R_f^2$ 8.35 × his, single spot ninhydrin and Sakaguchi positive.

*Anal.* Calcd. for $C_{61}H_{85}N_{13}O_{14}SCl_2 \cdot H_2O$: C, 54.4; H, 6.51; N, 13.5. Found: C, 53.9; H, 6.46; N, 13.4.

Amino acid analysis of an acid hydrolysate showed the expected composition expressed in molar ratios: $arg_{1.02}gly_{1.12}phe_{2.04}tyr_{0.98}thr_{0.98}pro_{1.05}lys_{0.94}ala_{0.92}$. The average amino acid recovery was 88% of theory.

A sample of the product was digested with LAP and the digest was analyzed with the automatic amino acid analyzer. The following ratios were obtained[29]: $arg_{1.00}gly_{0.98}phe_{2.05}tyr_{1.08}thr_{1.10}pro_{1.00}$ $N^{\epsilon}$-$tosyllys_{0.93}ala_{0.93}$. Average recovery 72%.

**N-Carbobenzoxy-γ-methyl-L-glutamyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine Methyl Ester Hydrochloride 0.5 Hydrate (IV).**—To a solution of L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester dihydrochloride (1 g.) in dimethylformamide (12 ml.), triethylamine (0.11 ml.) was added followed by N-carbobenzoxy-γ-methyl-L-glutamic acid *p*-nitrophenyl ester (0.32 g.). The reaction mixture was allowed to stand at room temperature for 36 hr., diluted with 1 *N* $KHCO_3$ (1 ml.), stirred for 1 hr., and poured into cold 1 *N* $KHCO_3$ (60 ml.). The precipitated product was isolated by filtration and washed successively with 1 *N* $NH_4OH$, water, 1 *N* HCl, water, and dried. On reprecipitation from methanol–ether, 0.71 g. (60.5%) of product was obtained, m.p. 161–163°, $[\alpha]^{28}D$ −33.8° (*c* 0.2, dimethylformamide).

*Anal.* Calcd. for $C_{75}H_{99}N_{14}O_{19}SCl \cdot 0.5H_2O$: C, 57.1; H, 6.38; N, 12.4. Found: C, 56.6; H, 6.41; N, 12.7.

For paper chromatography a sample was decarbobenzoxylated by catalytic hydrogenation in the presence of HCl; $R_f^1$ 0.78, $R_f^2$ 8.53 × his, single spot ninhydrin and Sakaguchi positive.

**γ-Methyl-L-glutamyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine Methyl Ester 3 Hydrate (V).**—Carbobenzoxy-γ-methyl-L-glutam-

(23) $N^{\omega}$-Tosylarginine and $N^{\epsilon}$-tosyllysine are eluted from the short column (15 cm.) of the Beckman–Spinco analyzer, which is routinely used for the determination of the basic amino acids [S. Moore, D. H. Spackman, and W. H. Stein, *Anal. Chem.*, **30**, 1185 (1958)] after 40 ml. and 60 ml. of effluent, respectively.

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(28) See reference in footnote 23.

(29) The reported value for arginine is in reality the sum of arginine and ornithine which was produced from arginine during the LAP digestion of the peptide [K. Hofmann, *et al.*, *J. Am. Chem. Soc.*, **83**, 2294 (1961)].

yl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester hydrochloride 0.5 hydrate (92 mg.) was dissolved in a mixture of acetic acid (6 ml.) water (2 ml.), and 1 *N* HCl (0.2 ml.) and hydrogenated for 6 hr. in the presence of 10% palladium–charcoal catalyst (0.1 g.). The catalyst was filtered off and the filtrate lyophilized. The resulting product was dried over $P_2O_5$ *in vacuo;* wt. 84 mg. (98%); $[\alpha]^{28}D$ −33.6° (*c* 0.19, 50% aqueous acetic acid); $R_f^1$ 0.78, $R_f^2$ 8.53 × his, single spot ninhydrin and Sakaguchi positive.

*Anal.* Calcd. for $C_{67}H_{94}N_{14}O_{17}SCl_2 \cdot 3H_2O$: C, 52.7; H, 6.60; N, 12.9. Found: C, 52.2; H, 6.88; N, 12.9.

Amino analysis of the decapeptide dihydrochloride by an automatic analyzer after acid hydrolysis showed the expected composition expressed in molar ratios: $lys_{0.94}arg_{0.90}thr_{1.04}glu_{1.12}pro_{1.08}gly_{1.04}ala_{1.04}tyr_{0.94}phe_{1.96}$. The average amino acid recovery was 93% of theory.

Amino acid analysis of an LAP digest showed the ratios[29]: $N^{\epsilon}$-tosyl$lys_{0.87}arg_{1.00}thr_{1.04}pro_{0.96}gly_{1.00}ala_{1.00}tyr_{1.06}phe_{2.00}glu_{0.16}$; average recovery 89% of theory.

**$N^{\alpha}$-Carbobenzoxy-$N^{\omega}$-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine Methyl Ester 1.5 Hydrate (VI).**—N-Carbobenzoxyglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester hydrate (4.05 g.) was dissolved in methanol (60 ml.) containing 2 *N* HCl (2 ml.) and hydrogenated for 6 hr. over 10% palladium–charcoal catalyst (1.6 g.). The catalyst was filtered off and the filtrate concentrated to dryness *in vacuo*. The solid residue of octapeptide ester hydrochloride was dried by the addition of methanol followed by evaporation. The residue was then used directly for condensation with $N^{\alpha}$-carbobenzoxy-$N^{\omega}$-tosyl-L-arginine.

A solution of $N^{\alpha}$-carbobenzoxy-$N^{\omega}$-tosyl-L-arginine (1.48 g.) in acetonitrile (40 ml.) was cooled to 0° and triethylamine (0.46 ml.) was added followed by 2-ethyl-5-phenyloxazolium-3′-sulfonate (0.84 g.). After 1 hr. at 0° the reaction mixture was diluted with a solution of the octapeptide ester in dimethylformamide and acetonitrile prepared as noted: the hydrochloride salt, which had been made as described previously, was dissolved in a mixture of dimethylformamide (15 ml.) and acetonitrile (15 ml.) containing triethylamine (0.46 ml.), stirred 5 min., and then added to the reaction mixture prepared as described above. After 24 hr. at room temperature the reaction mixture was diluted with 0.5 *N* $NaHCO_3$ (250 ml.). The precipitated product was collected by filtration, washed successively with water, 1 *N* HCl, and water again. On reprecipitation from methanol–ether, 4.29 g. (87%) of product was obtained, m.p. undetermined; the peptide sinters at 125° and eventually it is converted to a liquid at 160°; $[\alpha]^{27}D$ −23.5° (*c* 0.17, dimethylformamide).

*Anal.* Calcd. for $C_{78}H_{95}N_{13}O_{18}S_2 \cdot 1.5H_2O$: C, 57.5; H, 6.28; N, 11.6. Found: C, 57.4; H, 6.40; N, 11.4.

The protected nonapeptide was decarbobenzoxylated on exposure to HBr in acetic acid and subjected to paper chromatography; $R_f^1$ 0.92, $R_f^2$ 6.8 × his, single ninhydrin positive spot. A sample of the decarbobenzoxylated nonapeptide was subjected to acid hydrolysis. Paper chromatography of the hydrolysate showed the presence of ninhydrin-positive spots with $R_f^1$'s 0.14, 0.16, 0.22, 0.27, 0.33, 0.37, 0.46, and 0.63, identical with the $R_f$'s of authentic samples of lysine, arginine, glycine, threonine, alanine, proline, tyrosine, and phenylalanine, respectively. The chromatogram exhibited also traces of two ninhydrin-positive spots with $R_f^1$ s 0.70 and 0.76 corresponding to the hydrochlorides of $N^{\omega}$-tosylarginine and $N^{\epsilon}$-tosyllysine, respectively.

Amino acid analysis of an acid hydrolysate of the decarbobenzoxylated nonapeptide by an automatic amino acid analyzer showed the ratios: $lys_{0.94}arg_{0.92}thr_{0.98}pro_{1.06}gly_{1.10}ala_{1.08}phe_{2.04}$; average amino acid recovery was 87% of theory.

**N-Carbobenzoxy-γ-benzyl-L-glutamyl-$N^{\omega}$-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine Methyl Ester (VII).**—A suspension of $N^{\alpha}$-carbobenzoxy-$N^{\omega}$-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-$N^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester (4.5 g.) in acetic acid (18 ml.) was treated with 4 *N* HBr in acetic acid (18 ml.). After 1 hr. at room temperature, anhydrous ether (300 ml.) was added and the precipitated product was isolated by filtration, washed with ether, and dried over KOH *in vacuo*. To a solution of this product in dimethylformamide (30 ml.), triethylamine (1.2 ml.) was added followed by N-carbobenzoxy-γ-benzyl-L-glutamic acid *p*-nitrophenyl ester (1.42 g.). After 24 hr. the reaction mixture was diluted with 1 *N* $KHCO_3$ (3 ml.), stirred for 30 min., and poured into ice-cold 0.5 *N* $NH_4OH$ (200 ml.). The precipitated product was filtered off, washed successively with 1 *N* $NH_4OH$, water, 1 *N* HCl, and water, and dried. On reprecipitation from methanol, 3.77 g. (70%) of product was obtained, m.p. 197–200°, $[\alpha]^{27}D$ −38.7° (*c* 0.20, dimethylformamide).

*Anal.* Calcd. for $C_{88}H_{108}N_{14}O_{21}S_2$: C, 60.0; H, 6.17; N, 11.1. Found: C, 59.7; H, 5.99; N, 10.7. For paper chromatography a sample was decarbobenzoxylated on exposure to HBr in acetic acid in the usual manner; $R_f^1$ 0.91, $R_f^2$ 8.93 × his, single ninhydrin positive spot.

Amino acid analysis of an acid hydrolysate of the decarbobenzoxylated peptide showed the expected composition expressed in molar ratios: $lys_{1.08}arg_{0.95}thr_{0.99}glu_{1.2}pro_{1.10}gly_{1.08}ala_{1.05}tyr_{0.50}phe_{2.10}$; average recovery 76% of theory.

The deblocked peptide was digested with LAP. The results of the digestion were discussed previously.

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# COMMUNICATIONS TO THE EDITOR

## The Free Radical-Catalyzed Disproportionation of Arylsilanes. A New Homolytic Aromatic Displacement Reaction

*Sir:*

Although the acid[1]- and base[2]-catalyzed and thermal[3] redistribution of substituents on a silicon are well known, no example involving radical intermediates has been reported. We wish to report that a rapid redistribution of phenyl and hydrogen on silicon occurs in a variety of phenylsilanes in the presence of conventional free radical sources.

$$\overset{*}{\text{PhSi}}\equiv + \text{HSi}\equiv \xrightarrow{R} \text{PhSi}\equiv + \overset{*}{\text{HSi}}\equiv$$

This reaction is a surprisingly efficient homolytic aromatic displacement reaction with silyl groups serving as both the attacking and leaving radicals.

Diphenylsilane undergoes extensive disproportionation on exposure to ultraviolet radiation at 70 to 130° or on warming to 130° with a variety of peroxy or azo initiators.

$$Ph_2SiH_2 \rightleftharpoons Ph_4Si + Ph_3SiH + PhSiH_3 + SiH_4$$

No reaction occurs at these temperatures in the absence of the initiator. The data in Table I give the extent of this reaction with some representative initiators. The dialkylperoxides appear to be the most efficient catalysts[4] with minimum kinetic chain lengths of over 60.[5]

As seen from Table II, a variety of phenylalkylsilanes undergo this radical-catalyzed reaction. No evidence for the redistribution of alkyl groups was observed.

(1) G. A. Russell, *J. Am. Chem. Soc.*, **81**, 4815 (1959).
(2) J. W. Ryan, *ibid.*, **84**, 4730 (1962).
(3) H. Gilman and D. H. Miles, *J. Org. Chem.*, **23**, 326 (1958).
(4) As the data in Table II show increasing chain length with higher temperature, the higher decomposition temperature of the dialkylperoxides is an advantage. These peroxides are also normally less susceptible to induced decomposition which could be a serious side reaction of the electrophilic silyl radical.
(5) The moles of $Ph_2SiH_2$ consumed per possible initiating radical from Table I. This calculation ignores the reverse reaction ($Ph_3SiH + PhSiH_3 \rightarrow 2Ph_2SiH_2$) and is thus a minimum figure.