for 4 hr. After cooling, the crystals were filtered and recrystallized from methanol to give white needles, m.p. > 280°, 440 mg. Spectral properties were identical with XVIII.

Anal. Calcd. for $C_{10}H_{18}IN_3O_2$: C, 35.41; H, 5.35; I, 37.41; N, 12.39. Found: C, 35.29; H, 5.29; I, 37.30; N, 12.26.

Paper electrophoresis at pH 10.58 (0.05 *N* sodium carbonate), 700 v., 100 min., gave the following migrations: XVIII obtained from VIa, VIb, and VII showed −16.4 cm.; VII, VIa, and VIb showed −3.5 cm.

Alkaline Hydrolysis of VIa.—A small amount of VIa hydrochloride was dissolved in *N* sodium hydroxide and heated at ~90° for 20 hr. After neutralization to pH 7, the solution was analyzed by paper electrophoresis (pH 10.58, 700 v., 70 min.). Two ultraviolet-absorbing spots were obtained migrating at −2.3 and +5.5 cm. (VIa migrates at −2.3 cm. in this system). The +5.5 cm. spot was excised and eluted with water; ultraviolet absorption properties: at pH 1, maximum at 260 mμ; at pH 14, λ_{max} at 285 mμ; ratio of absorbancies $A_{260\ m\mu,\ pH\ 1}/A_{285\ m\mu,\ pH\ 14} = 0.69$ (3-methyluracil gives 0.68).[14]

The excised 5.5-cm. spot was run in paper electrophoresis at pH 5.2 (0.1 *N* ammonium acetate) (70 min., 700 v.) where it showed a migration of −10.6 cm.; VIa which also has a basic amine showed a migration of −14.0. Uracil, 1-methyluracil, and 3-methyluracil showed migrations of −0.5 to −2 cm. These data attest to the presence of a basic amino function in the alkaline hydrolysate product consistent with 3-(β-aminoethyl)uracil.

Alkaline Hydrolysis of VIb.—A small sample of VIb was treated with *N* alkali for 20 hr. at 60°. The results were similar; ultraviolet properties: λ_{max} 260 mμ at pH 1; λ_{max} 285 mμ at pH 14; $A_{260\ m\mu,\ pH\ 1}/A_{285\ m\mu,\ pH\ 14} = 0.68$.

Alkaline Hydrolysis of XVI.—After 10-hr. reflux in alkali, XVI was converted to the 3-(aminopropyl)uracil as shown by the ultraviolet properties: λ_{max} at 260 mμ (pH 1); λ_{max} at 284 mμ (pH 14); ratio of $A_{max\ pH\ 1}/A_{max\ pH\ 14} = 0.66$.

Alkaline Hydrolysis of VII.—A dilute solution of VII in 0.01 *N* sodium hydroxide was heated at 85° for 48 hr. The ultraviolet absorption maximum of the hydrolysate was 268 mμ at pH 1–14. (Appreciable loss of absorption was noted during the hydrolysis characteristic of 1,3-dialkylated uracils.[14]) Another sample of VII was refluxed with 0.1 *N* sodium hydroxide for 2 hr. and applied to paper electrophoresis (pH 10.58, 800 v., 1 hr.). The ultraviolet-absorbing spots were obtained with migrations of −2.5 and +1.0 cm. (Starting material migrates at −2.5 cm. and uracil gives +6.7 cm.) The 1.0-cm. spot was excised and eluted with water and showed a maximum at 268 mμ, minimum at 237 mμ between pH 1–14. (1,3-Dimethyluracil gives a maximum at 266 and a minimum at 234 mμ in the same pH range.[14]) Paper electrophoresis at pH 3.75 (0.1 *N* NH_4OAc, 800 v. 1 hr.) also gave two spots (−11.5 and −8.0 cm.). The −11.5-cm. spot was starting material VII. These data attest to the formation of 1-methyl-3-(β-aminoethyl)uracil.

Synthesis of 3-Methyl-4-thio-2-pyrimidinone.—A suspension of 5.0 g. of 3-methyluracil[21] and 7.0 g. of phosphorus pentasulfide in 100 ml. of pyridine was heated to reflux with efficient stirring. A few drops of water was added so that the reaction mixture assumed an orange-turbid appearance. After 5 hr. the stirred, refluxing solution was cooled and allowed to remain at room temperature overnight. The mixture was decanted from a dark oil and the decantate concentrated *in vacuo* to dryness. The residue was treated with benzene and the benzene removed *in vacuo*. This process was repeated several times. The residual sirup was triturated five times with 50-ml. portions of absolute ethanol. The combined triturates were concentrated under vacuum to a solid mass which was dissolved in ~80 ml. of boiling water, treated with charcoal, and filtered hot. The filtrate was allowed to cool slowly. Yellow feathered clusters were obtained; 2.34 g., m.p. 181–182°. Recrystallization from water gave m.p. 183–184°. Ultraviolet properties agree with those for 4-thio-2-pyrimidinones[22]: pH 2–7, λ_{max} at 322 mμ and 260 mμ; ratio: max. at 322 mμ/max. at 260 mμ = 5.0. At pH 14: max. at 333 mμ, shoulder at 250 mμ, min. at 280 mμ; ratio: max./min. = 15.0.

Anal. Calcd. for $C_5H_6N_2OS$: N, 19.72; S, 22.54. Found: N, 19.42; S, 22.43.

3-Methylcytosine (II) and 3-methylcytidine were prepared according to Brookes and Lawley.[7]

Spectrophotometric Studies.—Ultraviolet absorption data were determined with a Cary recording spectrophotometer, Model 15, using buffer and techniques previously described.[14] The apparent pK_a values are accurate to ±0.05 pH unit and were determined spectrophotometrically by methods previously employed.[14,23]

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Insulin Peptides. VIII. A Synthetic Heptadecapeptide Derivative Corresponding to the C-Terminal Sequence of the B-Chain of Insulin[1,2]

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A synthesis is described of the protected heptadecapeptide N-carbobenzoxy-L-alanyl-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycyl-γ-benzyl-L-glutamyl-N^{ω}-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-alanine methyl ester and the partially protected octapeptide N-carbobenzoxy-γ-benzyl-L-glutamyl-L-alanyl-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine. The former peptide derivative corresponds to the arrangement of the carboxyl terminal 17 amino acid residues, positions 14 to 30, of the B-chain of insulin, and the latter to positions 13 to 20 of the same chain.

The amino acid sequence glutamyl-alanyl-leucyl-tyrosyl-leucyl-valyl-cysteinyl-glycyl-glutamyl-arginyl-glycyl-phenylalanyl-phenylalanyl-tyrosyl-threonyl-prolyl-lysyl-alanine represents the carboxyl terminal portion of the B-chain of insulin from several species.[3] In connection with our studies[4] directed toward the synthesis of the insulin molecule we have reported, in previous communications, the preparation of a protected nonapeptide and of two decapeptide derivatives containing the C-terminal portion of the aforementioned sequence.[5] In the present communication we report detailed experimental procedures for the preparation of N-carbobenzoxy-γ-benzyl-L-glutamyl-L-alanyl-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine (VII) and N-carbobenzoxy-L-alanyl-L-leuycl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycyl-γ-benzyl-L-glutamyl-N^{ω}-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-alanine methyl ester (VIII). The partially protected octapeptide VII occupies the N-terminal position of the aforementioned segment of the B-chain of insulin. The fully protected heptadecapeptide VIII contains the C-terminal sequence of that fragment.

The synthesis of the octapeptide derivative VII was accomplished by the stepwise elongation approach which we now use routinely in our studies. Starting

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(2) A preliminary report of portions of the work described in this paper 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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with the C-terminal residue, glycine ethyl ester, and using the *p*-nitrophenyl esters[6,7] of appropriate carbobenzoxy amino acids as the activated "carboxyl component" at each synthetic step, the protected heptapeptide N-carbobenzoxy-L-alanyl-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine ethyl ester (V) was first synthesized.[8] Saponification of this ester afforded the carboxyl-free heptapeptide VI; this in turn was decarbobenzoxylated and subsequently condensed with N-carbobenzoxy-γ-benzyl-L-glutamic acid *p*-nitrophenyl ester[9] to yield the desired protected octapeptide VII. The yields of the individual synthetic steps ranged from 80 to 95% of theory with the exception of the last step which proceeded with a 78% yield.

The chemical homogeneity of the various intermediates and of the protected octapeptide VII was ascertained by elemental analysis and paper chromatography of the decarbobenzoxylated derivatives. Chromatograms in two solvent systems exhibited single ninhydrin-positive spots indicative of the presence of homogeneous components.

The chemical purity of the partially protected heptapeptide VI and octapeptide VII was further established by amino acid analysis of the respective acid hydrolysates. In both instances the constituent amino acids were obtained in the theoretically expected ratios.

Exposure of N-carbobenzoxy-S-benzyl-L-cysteinylglycine ethyl ester[10] to HBr in acetic acid and coupling of the ensuing product with N-carbobenzoxy-L-valine *p*-nitrophenyl ester[11] afforded the crystalline tripeptide N-carbobenzoxy-L-valyl-S-benzyl-L-cysteinylglycine ethyl ester[12] (I) in 85% yield. The protected tetrapeptide N-carbobenzoxy-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine ethyl ester (II) was prepared in crystalline form (yield 95%) by treating N-carbobenzoxy-L-leucine *p*-nitrophenyl ester[7] with the product obtained by HBr in acetic acid decarbobenzoxylation of I. A tetrapeptide of the same structure was synthesized by Tritsch and Woolley[13] from N-carbobenzoxy-L-leucyl-L-valine plus S-benzyl-L-cysteinylglycine ethyl ester using N,N′-dicyclohexylcarbodiimide[14] as condensing reagent. The physical characteristics, however, of their product [m.p. 208°, becoming birefringent at 170° and $[\alpha]^{23}D$ −26° (*c* 1.74, methylene chloride)] differed considerably from those of our preparation [m.p. 170°, $[\alpha]^{23}D$ −44° (*c* 1.97, methylene chloride)].

Exposure of II to HBr in acetic acid resulted in removal of its amino protecting group, and reaction of the deacylated product with N-carbobenzoxy-O-benzyl-L-tyrosine *p*-nitrophenyl ester[7] gave the pentapeptide N-carbobenzoxy-O-benzyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine ethyl ester (III) in 97% yield.

The protected hexapeptide N-carbobenzoxy-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine ethyl ester (IV) was prepared in 80% yield by the reaction of N-carbobenzoxy-L-leucine *p*-nitrophenyl ester with the product obtained by decarbobenzoxylation and debenzylation of III on treatment with HBr in acetic acid. Removal of the carbobenzoxy group from IV on exposure to HBr in acetic acid and coupling of the ensuing product with N-carbobenzoxy-L-alanine *p*-nitrophenyl ester[9] afforded the protected heptapeptide N-carbobenzoxy-L-alanyl-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine ethyl ester (V) in 80.5% yield. Treatment of V with 1 *N* NaOH yielded the partially protected derivative N-carbobenzoxy-L-alanyl-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine (VI) in 88% yield. The chemical purity of this peptide derivative was discussed previously. For evaluation of stereochemical homogeneity, the protected peptide VI was decarbobenzoxylated by exposure to HBr in acetic acid and the ensuing product was incubated with leucine aminopeptidase (LAP). The deblocked peptide was highly insoluble in the incubating medium and only a small proportion underwent digestion. Consequently, unequivocal proof of the stereochemical homogeneity of the heptapeptide derivative could not be obtained. However, paper chromatography of the digest strongly suggested that the solubilized heptapeptide was completely hydrolyzed by LAP to the constituent amino acids. As indicated in the Experimental section, the chromatogram of the digest in the Partridge system[15] exhibited only ninhydrin-positive spots with R_f values corresponding to the constituent amino acids. The R_f values of all the intermediate amino-free peptide derivatives are considerably different from those of the constituent amino acids (Experimental section). Hence, peptide derivatives that might have remained undigested would be readily differentiated on the paper chromatogram. Since the chromatogram exhibited no other ninhydrin-positive spots besides those corresponding to the constituent amino acids, it is concluded that the LAP digestion was complete. This implies that no racemization of the constituent amino acids had occurred during the synthetic processes leading to the heptapeptide VI.

For the synthesis of the protected heptadecapeptide VIII, the heptapeptide derivative VI was activated by the use of 2-ethyl-5-phenyloxazolium-3′-sulfonate[16] and then treated with the product obtained by decarbobenzoxylation of N-carbobenzoxy-γ-benzyl-L-glutamyl-N^{ω}-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-alanine methyl ester (IX).

The synthesis of the later decapeptide derivative was reported in a previous communication.[5] For the purification of the crude heptadecapeptide VIII we took advantage of the fact that the subunits used for its preparation, namely, the partially protected heptapeptide VI and the decarbobenzoxylated derivative of the decapeptide IX, are soluble in a mixture of $KHCO_3$–water–dimethylformamide–methanol and aqueous acetic acid, respectively. Consequently, by washing the crude reaction product with the aforementioned solvent systems, as described in the Experimental section, we were able to obtain the protected heptadecapeptide VIII in a homogeneous form and in 38% yield. This heptadecapeptide derivative was prepared in 56% yield by using N,N′-carbonyldiimidazole[17] as the activating reagent in the condensation of the heptapeptide and decapeptide subunits.

The chemical purity of the protected heptadecapeptide was ascertained by elemental analysis, paper chromatography of the decarbobenzoxylated derivative, and amino acid analysis of an acid hydrolysate.

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The elementary composition was in good agreement with that expected for a trihydrate of the protected polypeptide, and paper chromatograms of the decarbobenzoxylated derivative developed in two solvent systems exhibited sharp single ninhydrin-positive spots. An acid hydrolysate of the homogeneous product was shown by quantitative amino acid analysis to contain the constituent amino acids in the theoretically expected molar ratios with an average amino acid recovery 96% of theory.

The optical homogeneity of the decarbobenzoxylated decapeptide IX has been established previously[5] and the optical purity of the partially protected heptapeptide VI was discussed earlier in this communication. The former was coupled to a C-terminal glycine heptapeptide VI, a step wherein racemization of the glycine residue could not occur, in order to help maintain the stereochemical homogeneity of the constituent amino acids in the protected heptadecapeptide during the synthetic processes.

Experimental

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

For paper chromatography the protected peptides were deblocked on exposure to 2 *N* HBr in acetic acid and the ensuing hydrobromides were chromatographed on Whatman No. 1 filter paper at room temperature. R_f^1 values refer to the Partridge system; R_f^2 values refer to the system[18] 1-butanol–pyridine–acetic acid–water, 30:20:6:24, and are expressed as a multiple of the distance traveled by a histidine marker; R_f^3 values refer to the system[19] 2-butanol–formic acid–water, 5:1:4. The enzymatic analysis (LAP) was performed according to the procedure described by Hofmann, *et al.*[20,21] The amino acid analyses of acid hydrolysates were carried out with a Beckman–Spinco amino acid analyzer, Model 120, according to the method of Spackman, Stein, and Moore.[22] Optical rotations were determined with a Rudolph precision polarimeter, Model 80.

N-Carbobenzoxy-L-valyl-S-benzyl-L-cysteinylglycine Ethyl Ester (I).—N-Carbobenzoxy-S-benzyl-L-cysteinylglycine ethyl ester (35.9 g.) was dissolved in acetic acid (40 ml.) and treated with 4 *N* HBr in acetic acid (100 ml.). After 1 hr. at room temperature, the solvent was removed by evaporation *in vacuo*. The remaining oily product was poured into anhydrous ether (800 ml.). The separated semisolid material was isolated by decantation and on reprecipitation from ethanol–ether was obtained in a solid form. To a solution of this solid in dimethylformamide (150 ml.) containing triethylamine (10.9 ml.), N-carbobenzoxy-L-valine-*p*-nitrophenyl ester (30.5 g.) was added. After 16 hr. at room temperature, triethylamine (1 ml.) was added and the yellow solution stored at 0° for 24 hr. Addition of the reaction mixture into cold 1 *N* NH_4OH (800 ml.) resulted in the precipitation of the product which was isolated by filtration and washed successively with water, 1 *N* HCl, and water again. On reprecipitation from ethyl acetate–ether, 35.7 g. (85%) of crystalline product was obtained, m.p. 173°, $[\alpha]^{28}D$ −26.5° (*c* 2.19, dimethylformamide) [lit.[12] m.p. 178–180°, $[\alpha]^{20}D$ −27.2 (*c* 2, dimethylformamide)].

Anal. Calcd. for $C_{27}H_{35}N_3O_5S$: C, 61.2; H, 6.65; N, 7.9. Found: C, 61.5; H, 6.75; N, 8.1.

For paper chromatography a sample was decarbobenzoxylated with 2 *N* HBr in acetic acid; R_f^1 0.84, R_f^2 4.69 × his, single ninhydrin-positive spot.

N-Carbobenzoxy-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine Ethyl Ester (II).—N-Carbobenzoxy-L-valyl-S-benzyl-L-cysteinylglycine ethyl ester (21.2 g.) was dissolved in acetic acid (40 ml.) and treated with 4 *N* HBr in acetic acid (60 ml.). After 1 hr. at room temperature, the solvent was evaporated *in vacuo*. The remaining peptide ester hydrobromide was washed thoroughly with anhydrous ether and reprecipitated from ethanol–ether. To a solution of this solid in dimethylformamide (150 ml.), triethylamine (5.2 ml.) was added followed by N-carbobenzoxy-L-leucine *p*-nitrophenyl ester (15.6 g.). After 16 hr., triethylamine (0.8 ml.) was added and the reaction mixture was stirred at 0° for another 16 hr. The product was then isolated by a procedure similar to that used in the isolation of the tripeptide and crystallized from methylene chloride–ether; wt. 23.5 g. (91%), m.p. 170°, $[\alpha]^{27}D$ −44° (*c* 1.97, methylene chloride) [lit.[13] m.p. 208°, $[\alpha]^{23}D$ −26° (*c* 1.75, methylene chloride)].

Anal. Calcd. for $C_{33}H_{46}N_4O_7S$: C, 61.7; H, 7.21; N, 8.7. Found: C, 61.8; H, 7.12; N, 8.8.

For paper chromatography a sample was decarbobenzoxylated on exposure to 2 *N* HBr in acetic acid; R_f^1 0.93, R_f^2 5.01 × his, single ninhydrin-positive spot.

N-Carbobenzoxy-O-benzyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine Ethyl Ester (III).—A solution of N-carbobenzoxy-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine ethyl ester (20.6 g.) in acetic acid (40 ml.) was treated with 4 *N* HBr in acetic acid (60 ml.). After 2 hr. at room temperature, the solvent was removed *in vacuo* and the remaining product was washed with ether, dried, and dissolved in dimethylformamide (150 ml.). To this solution triethylamine (4.3 ml.) was added followed by N-carbobenzoxy-O-benzyl-L-tyrosine *p*-nitrophenyl ester (16.6 g.). After 48 hr. at room temperature, the reaction mixture was diluted with cold 1 *N* NH_4OH (800 ml.). The precipitated product was isolated by filtration and washed successively with water, 1 *N* HCl, water, ethanol, and ether. For further purification a suspension of the product in methylene chloride (200 ml.) was refluxed for 2 hr., then cooled, and mixed with ether; wt. 27.3 g. (97%), m.p. 208°. A sample for analysis was reprecipitated from methylene chloride–ether; m.p. unchanged, $[\alpha]^{26}D$ −24.8° (*c* 1.3, acetic acid).

Anal. Calcd. for $C_{49}H_{61}N_5O_9S$: C, 65.7; H, 6.85; N, 7.8. Found: C, 65.7; H, 6.74; N, 7.5.

For paper chromatography a sample was deblocked with 2 *N* HBr in acetic acid; R_f^1 0.91, R_f^2 4.91 × his, single ninhydrin-positive spot.

N-Carbobenzoxy-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine Ethyl Ester (IV).—N-Carbobenzoxy-O-benzyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine ethyl ester (26 g.) was dissolved in acetic acid (40 ml.), and 4 *N* HBr in acetic acid (80 ml.) was added to the solution. After 2 hr. at room temperature, the solution was evaporated to dryness *in vacuo* and the remaining peptide ester hydrobromide was triturated with ether and dried over KOH. To a solution of this product in dimethylformamide (150 ml.), triethylamine (4.8 ml.) was added followed by N-carbobenzoxy-L-leucine *p*-nitrophenyl ester (10.9 g.). After 48 hr., the reaction mixture was diluted with 1 *N* NH_4OH (5 ml.), stirred for 10 min., and poured into ice-cold 1 *N* NH_4OH (800 ml.). The precipitated product was filtered off and washed successively with water, 1 *N* HCl, and water again. On reprecipitation from ethanol–ether, 21.2 g. (80%) of product was obtained, m.p. 236–238°, $[\alpha]^{28}D$ −39.4° (*c* 2.4, acetic acid).

Anal. Calcd. for $C_{48}H_{66}N_6O_{10}S$: C, 62.7; H, 7.23; N, 9.1. Found: C, 62.7; H, 7.36; N, 9.1.

For paper chromatography a sample was decarbobenzoxylated with HBr in acetic acid; R_f^1 0.94, R_f^2 4.96 × his, single ninhydrin-positive spot.

N-Carbobenzoxy-L-alanyl-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine Ethyl Ester (V).—N-Carbobenzoxy-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine ethyl ester (19.7 g.) was dissolved in acetic acid (35 ml.) and treated with 4 *N* HBr in acetic acid (70 ml.). After 2 hr., the solvent was removed *in vacuo* and the remaining product was triturated with ether, dried over KOH, and dissolved in dimethylformamide (150 ml.). To this solution triethylamine (3.1 ml.) was added followed by N-carbobenzoxy-L-alanine *p*-nitrophenyl ester (7.5 g.). After 48 hr. at 5°, the reaction mixture was diluted with 1 *N* NH_4OH (5 ml.) and poured into ice-cold 1 *N* NH_4OH (800 ml.). The precipitated material was isolated by filtration, washed successively with water, 1 *N* HCl, and water, and dried. For purification the product was suspended in 95% aqueous ethanol and the mixture was refluxed for 1 hr., cooled, and mixed with ether; wt. 17.1 g. (80.5%), m.p. 248–250°. A sample for analysis was dissolved in 95% ethanol and precipitated with ether; m.p. 254°, $[\alpha]^{26}D$ −42.3° (*c* 0.96, acetic acid).

Anal. Calcd. for $C_{51}H_{71}N_7O_{11}S$: C, 61.9; H, 7.22; N, 9.9. Found: C, 62.5; H, 7.50; N, 9.8.

For paper chromatography the protected peptide was decarbobenzoxylated on exposure to HBr in acetic acid; R_f^1 0.87, R_f^2 4.6 × his, single ninhydrin-positive spot.

N-Carbobenzoxy-L-alanyl-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine (VI).—To a solution of N-carbobenzoxy-L-alanyl-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine ethyl ester (2 g.) in dimethylformamide (30 ml.), 1 *N* NaOH (1.0 ml.) was added. After 2 hr. at room temperature, the solution was acidified with concentrated HCl and diluted with water (200 ml.). The precipitated product was filtered off, washed with water, dried, and reprecipitated from dimethylformamide–ether; wt. 1.7 g. (88%), m.p. 244°, $[\alpha]^{28}D$ −43.5° (*c* 1.04, acetic acid).

Anal. Calcd. for $C_{49}H_{67}N_7O_{11}S$: C, 61.1; H, 7.01; N, 10.2. Found: C, 60.6; H, 7.09; N, 9.8.

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The protected heptapeptide was decarbobenzoxylated on exposure to HBr in acetic acid and subjected to paper chromatography; R_f^1 0.89, R_f^2 4.85 × his, single ninhydrin-positive spot. A sample of the decarbobenzoxylated heptapeptide was digested with LAP. Paper chromatography of the digest in the Partridge system showed the presence of only six ninhydrin-positive spots with R_f's 0.23, 0.28, 0.46, 0.50, 0.64, and 0.71, identical with the R_f's of authentic sample of glycine, alanine, tyrosine, valine, leucine, and S-benzylcysteine, respectively.

Amino acid analysis of an acid hydrolysate of the deblocked heptapeptide showed the expected composition expressed in molar ratios: $gly_{0.95}ala_{0.91}val_{1.05}leu_{2.0}tyr_{0.95}$. S-Benzylcysteine present in a paper chromatogram of the hydrolysate (R_f^1 0.71) was not determined.

N-Carbobenzoxy-γ-benzyl-L-glutamyl-L-alanyl-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine (VII).—N-Carbobenzoxy-L-alanyl-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine (1.42 g.) was dissolved in acetic acid (15 ml.) and treated with 4 *N* HBr in acetic acid (15 ml.). After 1 hr. at room temperature, the solvent was removed *in vacuo* and the remaining peptide hydrobromide was triturated with ether and dried over KOH. To a solution of this product in dimethylformamide (20 ml.) was added N-carbobenzoxy-γ-benzyl-L-glutamic acid *p*-nitrophenyl ester (0.76 g.) dissolved in dimethylformamide (10 ml.) and triethylamine (0.4 ml.). The solution was stirred for a few minutes and the excess of base was neutralized with acetic acid (*ca.* 0.2 ml.). The reaction mixture was stirred for 24 hr. at 0° and for 48 hr. at room temperature. Some insoluble material was removed by filtration and the filtrate was poured into 1 *N* HCl (500 ml.). The precipitated product was isolated by filtration, washed with water, and dried; wt. 1.39 g. (78%), m.p. 248–250°. A sample for analysis was reprecipitated from dimethylformamide–ether; m.p. 251–252°, $[\alpha]^{28}D$ −38° (*c* 1.12, acetic acid).

Anal. Calcd. for $C_{61}H_{80}N_8O_{14}S$: C, 62.0; H, 6.82; N, 9.5. Found: C, 61.5; H, 6.71; N, 9.4.

For paper chromatography a sample was decarbobenzoxylated on exposure to HBr in acetic acid; R_f^1 0.86, R_f^2 3.91 × his, single ninhydrin-positive spot. Amino acid analysis of an acid hydrolysate showed the expected composition expressed in molar ratios: $gly_{0.98}glu_{0.80}ala_{0.98}val_{1.1}leu_{2.0}tyr_{0.60}$. S-Benzylcysteine present in a paper chromatogram of the hydrolysate (R_f^1 0.71) was not determined.

N-Carbobenzoxy-L-alanyl-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycyl-γ-benzyl-L-glutamyl-Nω-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-Nε-tosyl-L-lysyl-L-alanine Methyl Ester (VIII). A.—A solution of N-carbobenzoxy-γ-benzyl-L-glutamyl-Nω-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-Nε-tosyl-L-lysyl-L-alanine methyl ester[5] (1.76 g.) in acetic acid (15 ml.) was treated with 4 *N* HBr in acetic acid (12 ml.). After 45 min. at room temperature, the reaction mixture was poured into anhydrous ether (100 ml.). The precipitated decapeptide ester hydrobromide was isolated by filtration, washed with ether, and reprecipitated from methanol–ether. This product was subsequently used for condensation with the heptapeptide VI. To a cooled (0°) solution of compound VI (0.99 g.) in dimethylformamide (15 ml.) containing triethylamine (0.15 ml.) was added 2-ethyl-5-phenyloxazolium-3′-sulfonate (0.26 g.). The reaction mixture was stirred at 0° for 1 hr. and then diluted with a solution of the decapeptide ester in dimethylformamide prepared as noted: the hydrobromide salt of the decapeptide ester which was made as described above was dissolved in dimethylformamide (12 ml.) containing triethylamine (0.18 ml.) and then added to the activated carboxyl component prepared as described previously. After 24 hr. at room temperature, the reaction mixture was cooled and diluted with a mixture consisting of saturated $KHCO_3$ (10 ml.), water (150 ml.) and methanol (40 ml.). The precipitated product was isolated by centrifugation and washed on the centrifuge successively with a mixture of dimethylformamide–methanol–water (1:1:7), and 20% aqueous methanol. On reprecipitation from 50% aqueous acetic acid, 1 g. (38%) of product was obtained, m.p. 265–268°, $[\alpha]^{27}D$ −33.6° (*c* 0.36, dimethylformamide).

Anal. Calcd. for $C_{129}H_{167}N_{21}O_{29}S_3 \cdot 3H_2O$: C, 59.0; H, 6.52; N, 11.20. Found: C, 58.6; H, 6.80; N, 11.40.

The protected heptadecapeptide was decarbobenzoxylated on exposure to 2 *N* HBr in acetic acid and chromatographed on paper; R_f^1 0.95, R_f^3 0.82, single sharp ninhydrin-positive spots. Amino acid analysis of an acid hydrolysate of the protected heptadecapeptide by the automatic analyzer showed the following composition expressed in molar ratios: $lys_{0.92}arg_{1.00}$ S-benzylcysteine$_{0.84}thr_{0.96}glu_{1.12}pro_{1.04}gly_{2.08}ala_{1.84}val_{1.08}leu_{2.16}tyr_{1.88}phe_{2.00}$. Average amino acid recovery was 96% of theory.

B.—To a precooled (0°) solution of VI (0.5 g.) in dimethylformamide (15 ml.), N,N′-carbonyldiimidazole (0.12 g.) was added. The reaction mixture was stirred at 0° for 1.5 hr. and then diluted with a solution of the decapeptide ester hydrobromide in dimethylformamide (10 ml.) containing triethylamine (0.12 ml.). The decapeptide ester hydrobromide was prepared, as described in A, from 1.1 g. of the carbobenzoxy derivative and 20 ml. of 2 *N* HBr in acetic acid. After 2 hr. at 0° and 20 hr. at room temperature, the reaction mixture was treated as in A and yielded 0.74 g. (56%) of product, m.p. 265–268°.

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COMMUNICATIONS TO THE EDITOR

cis- and *trans*-1,2-Diphenylnaphtho[*b*]cyclobutenes. A Novel Synthesis of a Naphthalene Nucleus

Sir:

In order to effect the synthesis of a stabilized aromatic-fused cyclobutadiene,[1] it was necessary to develop practical routes to the 1,2-diphenylnaphtho[*b*]cyclobutene system. Two entirely unrelated approaches are described below.

Reaction of 3-benzyl-2-naphthoic acid (I)[2] with thionyl chloride in methylene chloride–benzene gives the corresponding acid chloride which, without isolation, is converted directly by gaseous ammonia into 3-benzyl-2-naphthamide (II),[3] m.p. 197.5–198.5°, in 93% yield. Phosphorous oxychloride dehydration of amide II affords 3-benzyl-2-cyanonaphthalene (III), m.p. 111–112°, in 78% yield. Addition of phenylmagnesium bromide to nitrile III gives, after acid hydrolysis of the intermediary imine, 3-benzyl-2-benzoylnaphthalene (IV), m.p. 82–83°, in 69% yield. Sodium borohydride reduction of ketone IV gives the alcohol V which, without purification, is converted by thionyl chloride into the corresponding chloride VI; reaction of crude VI with potassium *t*-butoxide affords, in 78% yield (based on IV), *trans*-1,2-diphenylnaphtho[*b*]cyclobutene (VII), m.p. 158–159°.[4] Although VII is more stable thermally than the related *trans*-1,2-diphenylbenzocyclobutene,[5] it undergoes rearrangement in good yield (83%) in boiling dimethylformamide (*ca.* 150°) to give 5-phenyl-5,12-dihydronaphthacene (VIII), m.p. 149–150°; fusion of a mixture of VII and N-phenylmaleimide at 150° gives a Diels–Alder adduct (IX), m.p. 276–278°, in 50% yield. Free-radical

(1) M. P. Cava, B. Hwang, and J. P. Van Meter, *J. Am. Chem. Soc.*, **85**, 4032 (1963).

(2) E. de B. Barnett and R. A. Lowry, *Ber.*, **65**, 1649 (1932).

(3) Melting points are uncorrected. Satisfactory analyses were obtained for all new compounds, the spectra of which were also consistent with the assigned structures.

(4) The analogous dehydrohalogenation of an α-halo-α,α′-diphenyl-*o*-xylene to *trans*-1,2-diphenylbenzocyclobutene was first reported in 1958 (see ref. 5). We are grateful to Dr. A. J. Berlin for valuable suggestions concerning improvements in this type of reaction.

(5) F. R. Jensen and W. E. Coleman, *J. Am. Chem. Soc.*, **80**, 6149 (1958).