triethyl phosphite (16.6 g, 0.1 mol) at 15–20°. After the complete addition of the phosphite, the reaction mixture was fractionated to give ethyl chloride (5.0 g), 2,3-dichloropropionitrile (10.5 g, 80%), bp 63–65° (35 mm), and triethyl phosphate (15.5 g), bp 85–87° (20 mm).

Reaction of 2,2,3-Trichloropropionitrile and Triphenylphosphine.—Triphenylphosphine (26.2 g, 0.1 mol) in dry ether (100 ml) was added dropwise to the 2,2,3-trichloropropionitrile (16.0 g, 0.1 mol) in dry ether (70 ml) under constant stirring. An exothermic reaction resulted and a white precipitate was formed. After complete addition of the triphenylphosphine, the white precipitate (31.0 g) was filtered. The filtrate on fractionation yielded 2-chloroacrylonitrile (6.0 g, 70%), bp 88–89°. The white precipitate, on treatment with water, gave triphenylphosphine oxide, mp 151–153° (lit.[7] mp 152–153°).

Reaction of 2,2,3-Tribromopropionitrile and Triphenylphosphine.—This reaction was carried out in the manner described above, using 2,2,3-tribromopropionitrile (29.2 g, 0.1 mol) in dry ether (30 ml) and triphenylphosphine (26.2 g, 0.1 mol) in dry ether (100 ml). The reaction gave 2-bromoacrylonitrile (77 g, 60%) and triphenylphosphine dibromide, which was then converted into triphenylphosphine oxide (20.0 g), mp 152–153°.

Registry No.—2,2,3-Trichloropropionitrile, 813-74-1; triethyl phosphite, 122-52-1; 2,2,3-tribromopropionitrile, 22929-17-5; 2,3-dibromo-2-chloropropionitrile, 22929-18-6; trimethyl phosphite, 121-45-9; triphenylphosphine, 603-35-0; triphenylphosphine oxide, 791-28-6; 2-chloroacrylonitrile, 920-37-6; diethyl chlorophosphate, 814-49-3.

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Amino Acid Insertions in Solid-Phase Peptide Synthesis[1a,b]

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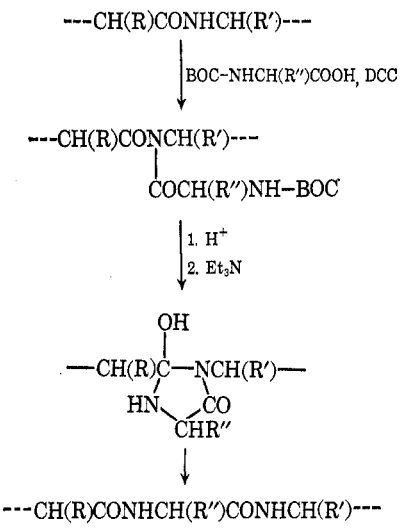
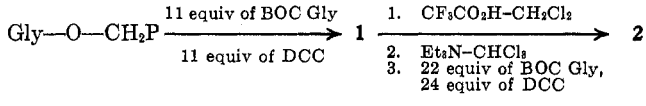
In solid-phase peptide synthesis,[2-4] peptide bonds are most frequently formed through the reaction of excess N-protected amino acid and N,N′-dicyclohexylcarbodiimide[5] with amino acid or peptide derivatives of polystyrene. Brenner[6] has stated that acylation of peptide bonds, followed by aminoacyl insertion,[7] may be possible under such conditions (Scheme I). The occurrence of insertion reactions would yield side products closely resembling the desired product. Since the use of solid-phase peptide synthesis is increasing,[8-12] it is of interest to establish whether or not amino acid insertions can occur in this method of peptide synthesis.

SCHEME I



As insertion reactions should be most favored in the absence of bulky side chains (R = R′ = R″ = H) a model system utilizing glycine was devised (Scheme II).

SCHEME II



Large excesses of N-*t*-butoxycarbonylglycine[13] and N,N′-dicyclohexylcarbodiimide were used in both coupling reactions in an attempt to promote acylation of the peptide bond.

When a portion of product **1** was treated with trifluoroacetic acid in methylene chloride, then with triethylamine in chloroform, and finally with hydrogen bromide in trifluoroacetic acid, the products were glycine (54.8%) and glycylglycine (45.2%), as determined with an amino acid analyzer calibrated with glycine, diglycine, triglycine, and tetraglycine. Had aminoacyl insertion occurred, some triglycine would have been formed.

Product **2** was treated in the manner described for product **1** yielding glycine, diglycine, and triglycine upon paper chromatography of the cleavage products. The chromatogram from the amino acid analyzer indicated the presence of glycine (6.0%), diglycine (46.1%), triglycine (47.7%), and two trace peaks. One of these peaks emerged at the position of tetraglycine and represented 0.2% of the total products. The presence of tetraglycine would indicate that amino acid insertions can occur during solid-phase peptide synthesis when very large excesses of acylating agents are used. On the basis of these experiments it appears

(1) (a) This work was supported in part by grants from the U. S. Public Health Service (GM-10591), the American Heart Association, the National Science Foundation, and by a Heart Research Center Grant (HE-06308). (b) The following abbreviations are used in this paper: BOC = *t*-butyloxycarbonyl; DCC = N,N′-dicyclohexylcarbodiimide; P = polystyrene-2% divinylbenzene copolymer. (c) Research Career Development Awardee of the U. S. Public Health Service.

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(3) R. B. Merrifield, *Biochemistry*, **3**, 1385 (1964).

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(7) M. Brenner, *J. Cell Comp. Physiol.*, **54**, 221 (1959).

(8) R. B. Merrifield in "The Handbook of Biochemistry with Selected Data for Molecular Biology," H. A. Sober Ed., The Chemical Rubber Co., Sandusky, Ohio, 1968, pp C83–C90.

(9) H. Takashima, V. du Vigneaud, and R. B. Merrifield, *J. Amer. Chem. Soc.*, **90**, 1323 (1968).

(10) J. Meienhofer and Y. Sano, *ibid.*, **90**, 2996 (1968).

(11) J. Blake and C. H. Li, *ibid.*, **90**, 5882 (1968).

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(13) E. Schnabel, *Ann. Chem.*, **702**, 188 (1967).

that amino acid insertions, though possible, are not significant side reactions under the usual conditions of solid phase peptide synthesis.

Experimental Section[14]

Glycyl Resin.—A solution of N-*t*-butyloxycarbonylglycine[13] (1.31 g, 7.48 mmol) and triethylamine (1.04 ml, 7.48 mmol) in 60 ml of ethanol was added to 10.00 g of chloromethylated polystyrene–2% divinylbenzene copolymer, 200–400 mesh[2] (0.374 mmol of Cl/g). The mixture was stirred under reflux for 46 hr and filtered. The resin was washed with ethanol and acetic acid followed by treatment with trifluoroacetic acid for 15 min. The resin was washed with chloroform, ethanol, and methylene chloride. The trifluoroacetate was neutralized by treatment with triethylamine (10%) in chloroform followed by washes of chloroform, ethanol, and methylene chloride. A sample was hydrolyzed in 1:1 dioxane–12 *N* HCl for 24 hr at 110°. Amino acid analysis gave a glycine content of 0.128 mmol/g.

Coupling Reaction I.—Glycyl resin (3.00 g, 0.384 mmol) was placed in a reaction vessel and treated in the following manner: (1) washed (three 30-ml portions) with methylene chloride, (2) introduced N-*t*-butyloxycarbonylglycine (0.740 g, 4.22 mmol) in 30 ml of methylene chloride and mixed for 10 min, (3) introduced N,N′-dicyclohexylcarbodiimide (0.910 g, 4.42 mmol) and allowed to react for 24 hr, (4) washed (three 30-ml portions) with methylene chloride, (5) washed (three 30-ml portions) with dimethylformamide, (6) washed (three 30-ml portions) with acetic acid, (7) washed (three 30-ml portions) with ethanol, (8) washed (three 30-ml portions) with methylene chloride, and (9) dried *in vacuo* to yield product **1**.

Coupling Reaction II.—A portion (1.00 g) of product **1** was treated in the manner described above for the conversion of N-*t*-butyloxycarbonylglycyl resin to glycyl resin. The resulting resin was then treated according to the procedure used for coupling reaction I, with the exception that a 22-fold excess of N-*t*-butyloxycarbonylglycine and a 24-fold excess of N,N′-dicyclohexylcarbodiimide were used to prepare product **2**.

Characterization of Products 1 and 2.—Portions (0.500 g) of **1** and **2** were treated in the following manner: (1) washed (three 15-ml portions) with methylene chloride, (2) mixed with trifluoroacetic acid–methylene chloride (2:3) for 15 min, (3) washed (three 15-ml portions) with methylene chloride, (4) washed (three 15-ml portions) with ethanol, (5) washed (three 15-ml portions) with chloroform, (6) mixed with triethylamine (10%) in chloroform, (7) washed (three 15-ml portions) with chloroform, (8) washed (three 15-ml portions) with methylene chloride, (9) suspended in trifluoroacetic acid (20 ml) and treated with a stream of hydrogen bromide for 30 min, (10) washed (two 15-ml portions) with trifluoroacetic acid, (11) washed (two 15-ml portions) with trifluoroacetic acid–methylene chloride (1:1), and (12) washed (two 15-ml portions) with methylene chloride. The filtrates from steps 9–12 were pooled and evaporated *in vacuo* at 20°. The resultant residues were dissolved in 1% HCl and subjected to paper chromatography and ion exchange chromatography using an amino acid analyzer.

The presence of only glycine (R_f 0.26) and diglycine (R_f 0.38) was indicated by paper chromatography of the cleavage products from **1**. The amino acid analyzer indicated the presence of 38.4 μmol glycine/g (54.8%) and 31.6 μmol diglycine/g (45.2%) in **1**. The cleavage products from **2** were glycine (R_f 0.27), diglycine (R_f 0.37), and triglycine (R_f 0.50), as determined by paper chromatography. The amino acid analyzer indicated the presence of 5.3 μmol glycine/g (6.0%), 40.4 μmol diglycine/g (46.1%), 41.8 μmol triglycine/g (47.7%), 0.2 μmol tetraglycine (0.2%), and an unidentifiable trace peak equal in area to that of the presumed tetraglycine.

(14) Reference 5 gives detailed instructions on the methodology used for solid phase peptide synthesis. Paper chromatography was performed using the ascending method on Whatman No. 1 filter paper developed with phenol–water in a ratio of 75:25. Ninhydrin was used for revealing the chromatograms. Amino acids and peptides were quantitatively determined with a Technicon amino acid autoanalyzer as described by A. R. Mitchell and R. W. Roeske, *J. Chromatogr.*, **43**, 266 (1969). A buffer gradient from pH 3.10 to pH 3.80 allowed the resolution of glycine, diglycine, triglycine, and tetraglycine at elution times of 95, 189, 199, and 172 min, respectively. Glycine, diglycine, triglycine, and tetraglycine were purchased from Mann Research Laboratories, New York, N. Y.

Products of the Action of Peracetic Acid on Isolongifolene

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Treatment of isolongifolene[1] with perbenzoic acid has been reported to give the corresponding epoxide.[2] We have attempted to carry out this reaction using peracetic acid. Commercial peracetic acid brought to pH 3.6 with sodium acetate reacted sluggishly with isolongifolene. A 65% yield of the isolongifolene was recovered, and the remainder consisted of a mixture of a ketone (VI),[2] a lactone (V),[2] and an alcohol (VII) in a ratio of 14:1:4 along with a trace of an epoxide (III).[2] The alcohol, $C_{15}H_{24}O$, was assigned the structure VII on the basis of the following considerations.

(a) The infrared spectrum exhibits a hydroxy stretching vibration at 2.94 μ (Nujol), indicating the presence of alcohol.

(b) The nuclear magnetic resonance spectrum of VII is in accord with the assigned structure. It exhibits the following resonances: δ 5.51 (d, 1 H, corresponding to one olefinic proton, >=CH—C, J = 3 cps), 3.7 (t, 1 H, HCOH, J = 7.5 cps), 0.89, 0.86, 0.80, and 0.70 (4 s, 12 H, four methyl groups), 1.0–1.58 (m, 8 H, $>CH_2$), and 2.1 (m, 1 H, corresponding to the group C=C—CH—C).

(c) The mass spectrum shows a parent peak at *m/e* 220, 177 (M − 43), 164, etc.

The structure is further supported by the chemical evidence. Oxidation by the Jones reagent gave a ketone to which the structure VIII is assigned on the basis of the spectral data.

(a) The infrared spectrum (film) exhibits a carbonyl stretching band at 5.87 μ, indicating a six-membered unconjugated ketone.

(b) The ultraviolet spectrum exhibits λ_{max} 221 (ϵ_{max} 300), ruling out the possibility of a conjugated ketone.

(c) The nuclear magnetic resonance spectrum of VIII supports the above-assigned structure, indicating signals at δ 5.6 (d, 1 H, assigned to olefinic proton, >C=C—C, J = 3 cps), 0.68, 0.80, 0.82, and 0.86 (4 s, 12 H, *gem*-dimethyls), and 1.0–1.57 (m, 8 H, $>CH_2$).

(d) The mass spectrum shows ions at *m/e* 218 (parent peak), 175 (M − 43), etc.

It was of interest to see if epoxide III, when treated with peracetic acid under the same conditions, would give the same products. Isolongifolene epoxide (III) was treated with peracetic acid buffered at pH 3.6 and gave at 60° three major products. They were shown by spectral analysis to be VI, V, and VII in a ratio of 15:1:5. These products suggest that the epoxide III could be an intermediate to give all the products shown in Scheme I.

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(2) S. Dev, J. Prahlad, R. Ranganathan, U. Ramdas Nayak, and T. S. Santhanakrishnan, *Tetrahedron Lett.*, No. 8, 417 (1964).