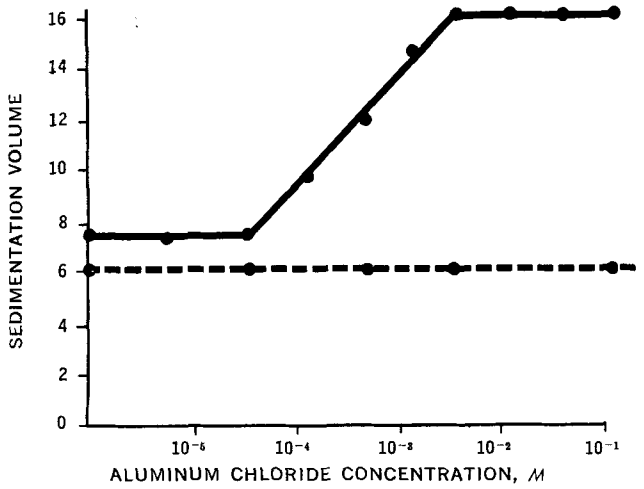

Figure 1—*Aggregation of sulfaguanidine in a suspension containing polysorbate 80 and aluminum chloride. Key:* ●—●, *from Jones* et al. (7); *and* ●- - -●, *this report.*

doubtful that in this experiment aggregation by adsorption bridging would occur.

We do not believe that the theory of long-range forces of attraction, classically believed to be responsible for aggregation in the secondary minimum, applies to the aggregation of hydrophilic particles (5). Since the sulfaguanidine covered with surfactant is a hydrophilic system, no flocculation will occur by mechanism *a*.

What we believed likely to happen is that the dispersed sulfa particles settle as individual entities and/or aggregate by surfactant–water film to surfactant–water film interactions to settle as close packed coagula (1). In either or both cases, the final height could not vary in the manner described in the report.

Since the properties of the system used did not appear to us to be capable of producing a flocculated structure on the basis of any of the three mechanisms enumerated, we studied these same systems for clarification.

Sulfaguanidine NF suspensions in water with polysorbate 80 and aluminum chloride were prepared in the exact manner described in the report (7), with the exception that mixing was done by a magnetic stirrer. The results of our experiments were always a sedimentation to a small volume (Fig. 1). The height did not change over the range of aluminum chloride concentrations shown. The suspensions sedimented in about 3 hr. and the final (*Hu*/*Ho*) was measured after 24 hr. The experimental results reported here indicate that the particles are either in the dispersed state and/or the coagulated state (1).

The system reported to be flocculated, *i.e.*, to increase to approximately twice its minimum (*Hu*/*Ho*) height was found in this laboratory to give only a contant height under all conditions stated in the report. These results support our *a priori* assumption that flocculation cannot take place in this system.

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Carboxyl Protection Using Salt Formation for the Synthesis of Linear Sequential Polypeptides: Synthesis of Poly-(L-tyrosyl-L-glutamyl-L-alanylglycyl)glycine-1-^{14}C Ethyl Ester

Keyphrases □ Polypeptides, linear sequential—synthesis □ Poly-(L-tyrosyl-L-glutamyl-L-alanylglycyl)glycine-1-^{14}C ethyl ester— synthesis □ Carboxyl protection—peptide synthesis □ Immunochemical properties—linear sequential polypeptide

Sir:

The least elaborate approach to carboxyl protection in peptide synthesis is the use of salt formation with such bases as triethylamine, tributylamine, or dicyclohexylamine. This method of protection seems to work best when an activated ester of an *N*-protected amino acid is used for the coupling reaction to amino acids and peptides which were carboxyl protected by salt formation (1, 2).

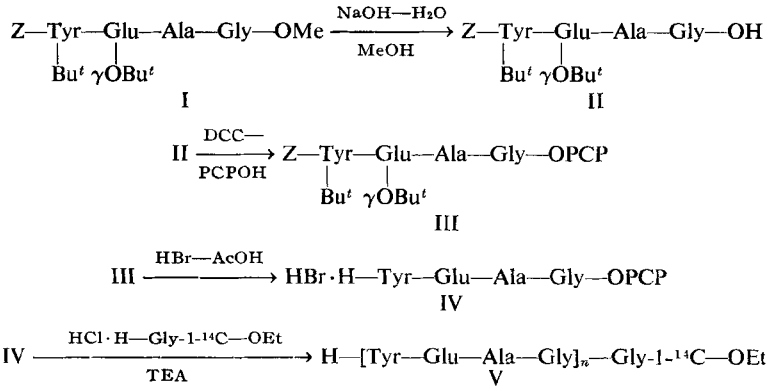
We have extended this method of protection to the synthesis of high molecular weight linear polypeptides. This is illustrated by a new synthesis of the antigenic polymer poly-(L-tyrosyl-L-glutamyl-L-alanylglycyl)glycine-1-^{14}C ethyl ester (Scheme I).

The previously reported tetrapeptide (3), *N*-carbobenzoxy-*O*-*tert*-butyl-L-tyrosyl-γ-*tert*-butyl-L-glutamyl-L-alanylglycine methyl ester (I), was saponified with 1 equivalent of *N* NaOH to yield the tetrapeptide free acid, *N*-carbobenzoxy-*O*-*tert*-butyl-L-tyrosyl-γ-*tert*-butyl-L-glutamyl-L-alanylglycine (II), m.p. 159–160°, $[\alpha]_D^{25}$ − 10.3° (c 4.23 in dimethylformamide).

Anal.—Calcd. for $C_{35}H_{48}N_4O_{10}$: C, 61.4; H, 7.1; N, 8.2. Found: C, 61.5; H, 7.15; N, 8.1.

Coupling II with pentachlorophenol, using dicyclohexylcarbodiimide, yielded the tetrapeptide activated ester, *N*-carbobenzoxy-*O*-*tert*-butyl-L-tyrosyl-γ-*tert*-butyl-L-glutamyl-L-alanylglycine pentachlorophenyl ester (III), m.p. 185°, $[\alpha]_D^{28}$ − 17.0° (c 1.06 in dimethylformamide).

Anal.—Calcd. for $C_{41}H_{47}Cl_5N_4O_{10}$: C, 52.8; H, 5.1; N, 6.0. Found: C, 52.6; H, 4.9; N, 6.0.



where DCC = dicyclohexylcarbodiimide, PCP = pentachlorophenyl, and TEA = triethylamine

Scheme I

Treatment of III with anhydrous hydrogen bromide in glacial acetic acid removed the *N*-carbobenzoxy and the *tert*-butyl protecting groups to yield the polymerizing unit, L-tyrosyl-L-glutamyl-L-alanylglycine pentachlorophenyl ester hydrobromide (IV), m.p. 180°, $[\alpha]_D^{28}$ − 3.3° (c 1.83 in dimethylformamide).

Anal.—Calcd. for $C_{25}H_{26}BrCl_5N_4O_8$: C, 39.1; H, 3.4; N, 7.3 Found: C, 39.1; H, 3.6; N, 7.6.

The polymerization of IV was conducted under dilute conditions in the presence of a preformed monomer glycine-1-^{14}C ethyl ester hydrochloride. This established polymerizing procedure has been shown to yield linear high molecular weight polypeptides (3–6) when the side groups are protected. In this case, the polymerizing unit (IV), dissolved in dimethyl sulfoxide, was added dropwise to a solution of glycine-1-^{14}C ethyl ester hydrochloride containing the total amount (3.5 equivalents) of triethylamine such that the final concentration of reactants was never more than 70 mmole/l. The polymerization was allowed to proceed for a week, after which the mixture was acidified and dialyzed extensively for 3 days. The precipitated polypeptide, poly-(L-tyrosyl-L-glutamyl-L-alanylglycyl)glycine-1-^{14}C ethyl ester (V), was collected by centrifugation, converted to its sodium salt, and dialyzed extensively for a week to remove all low molecular weight materials. This dialyzed polymer was lyophilized, converted to the free acid form by acidification, and again dialyzed to remove all traces of salt. Radioactive assay indicated 85% incorporation of the starting preformed monomer.

Anal.—Calcd. for $C_{19}H_{24}N_4O_7 \cdot \frac{1}{2}\ H_2O$: C, 53.15; H, 5.85; N, 13.05. Found: C, 52.8; H, 5.9; N, 13.0.

Filtration of the polymer through a calibrated column (7) of synthetic polysaccharide[1] (2.5 × 45 cm.), using a solution of 0.1 *M* NaCl–0.05 *M* $NaHCO_3$ buffer as eluent, indicated a molecular weight of at least 1 × 10^5.

To evaluate this method of preparing polypeptides with one that uses conventional protecting groups (3–6), a comparison was made of this polypeptide with that prepared using the *tert*-butyl ester for carboxyl protection (3). It was found that both polymers eluted from a column of the polysaccharide (2.5 × 45 cm.) in the same fractionation pattern, using a 0.1 *M* NaCl–0.05 *M* $NaHCO_3$ buffer as eluent. The two polymers were considered to be structurally identical since both materials were similarly antigenic in rabbits; each polypeptide crossreacted with the antibodies produced by the other, giving the same precipitin curve which was similar to that previously reported (8). From this evidence it was concluded that this method of carboxyl protection is compatible with the synthesis of linear high molecular weight polypeptides.

[1] Sephadex G-100.

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Aporphines V: Total Synthesis of (±)-Apomorphine

Keyphrases □ (±)-Apomorphine—total synthesis □ IR spectrophotometry—structure □ UV spectrophotometry—structure

Sir:

(−)-Apomorphine [(−)-I], the semisynthetic alkaloid obtained by vigorous treatment of morphine with strong mineral acids, has found medicinal application