pental was bound when a sixteen-fold molar excess of TCA (a similar excess to that used in the *in vivo* experiments) was incorporated into the buffer. This suggests a competitive protein binding phenomenon that may account for the above differences, and pharmacologically inert but highly protein bound materials such as TCA may have a role in pharmaceutical formulations, particularly those parenterally administered.

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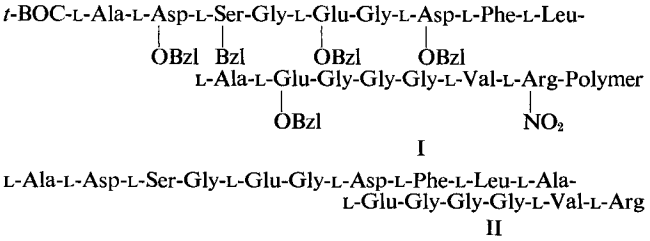
Rapid Peptide Synthesis: Synthesis of Human Fibrinopeptide A

Keyphrases ☐ Fibrinopeptide A, human—synthesis ☐ Column chromatography—separation ☐ Optical rotation—identity ☐ Paper chromatography—purity testing ☐ Electrophoresis—purity testing

Sir:

Recently the *N-t*-butyloxycarbonyl-L-amino acid pentachlorophenyl esters have been described (1) and shown to be useful intermediates for extremely rapid peptide synthesis (2). We wish to report a further application of these intermediates for use with the solid phase or Merrifield method (3) of peptide synthesis. For this purpose the first synthesis of the naturally occurring human peptide, fibrinopeptide A, (4, 5) L-alanyl-L-aspartyl-L-serylglycyl-L-glutamyl-glycyl-L-aspartyl-L-phenylalanyl-L-leucyl-L-alanyl-L-glutamyl-glycylglycylglycyl-L-valyl-L-arginine II, is described The synthesis is shown in Scheme I, its facility being due to the ease of purification of the starting amino acid pentachlorophenyl esters (1) and their high activity towards aminolysis (6).

The fully protected, resin bound, hexadecapeptide, fibrinopeptide A (I), was synthesized by the stepwise addition of the appropriate *N-t*-butyloxycarbonyl-L-amino acid pentachlorophenyl ester to an insoluble polystyrene resin (7) substituted with 0.43 mmoles/g.



Scheme I

of *N-t*-butyloxycarbonyl-nitro-L-arginine. The cycle for the addition of each amino acid residue consisted of the following steps; removal of the *N-t*-butyloxycarbonyl protecting group by treatment with excess 1 *N* hydrogen chloride in glacial acetic acid for 30 min.; neutralization of the resulting hydrochloride salt with excess triethylamine in dimethylformamide, and then coupling the resulting free amino residue with three equivalents of the appropriate *N-t*-butyloxycarbonyl-L-amino acid pentachlorophenyl ester in dimethylformamide for 12 hr. At the end of this chain-lengthening sequence, the protected peptide was cleaved from its polymer support by hydrogen bromide in trifluoroacetic acid, under which conditions the side-chain protecting groups, except that on arginine, were also cleaved. The partially protected peptide was then catalytically hydrogenated over 10% palladium on charcoal to give the crude hexadecapeptide, fibrinopeptide A. The peptide was purified by passage through a column of synthetic polysaccharide (Sephadex G-25) (100 × 2.5 cm.), using water as eluent at a flow rate of 6 ml./hr. The pure fibrinopeptide A, (II) $[\alpha]_D^{28}$ −44.8° (c 2.30 in water), was eluted as the first major fraction (13% overall yield) and the amino acid ratios[1] were: Ala, 2.0; Arg, 0.9; Asp, 1.9; Glu, 2.2; Gly, 5.3; Leu, 1.1; Phe, 1.0; Ser, 0.9; Val, 0.8. It was found to be homogeneous by paper chromatography; R_f 0.75 (butanol–acetic acid–water, 2:2:1); R_f 0.86 (phenol saturated with water). Electrophoresis on paper gave a single spot in phosphate buffer pH 6.9, which migrated 3.5 cm. towards the anode at 10 v/cm. for 2 hr.[2].

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[1] Amino acid analyses were performed by Arrow Labs., Joliet, Ill.