The Use of a Macroreticular Ion-exchange Resin in the Isolation Stage of the Picolyl Ester Method of Peptide Synthesis

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Summary Amberlyst-15 cation-exchange resin has been used advantageously for the isolation of coupling product in the picolyl ester method of peptide synthesis.

THE picolyl ester method of peptide synthesis provides a basic 'handle' (the 4-picolyl ester of the *C*-terminal amino-acid) by which, after each coupling reaction, the growing peptide is separated from the co-products and by-products.[1] The separation has been effected by adsorption on Sulphoethyl–Sephadex and by extraction into aqueous citric acid, and simplified syntheses of bradykinin[2] and Val[5]-angiotensin-II[3] have used these methods. We now find that the macroreticular sulphonic acid resin Amberlyst-15 is advantageous for this purpose, because it can be used in anhydrous organic solvents.

In a typical case, the reaction mixture from the condensation of t-butoxycarbonylglycine (0·6 mmol) with the pentapeptide 4-picolyl ester (I) (0·5 mmol, liberated from the trifluoroacetate by triethylamine) in dimethylformamide

Cha-Ser(Bzl)-Pro-Cha-Arg(NO_2)-OPic

(I)

Cha = β-cyclohexylalanine; Pic = 4-picolyl

(3·3 ml) with dicyclohexylcarbodi-imide (0·6 mmol) and 1-hydroxybenzotriazole[4] (0·6 mmol) was diluted with ethyl acetate (15 ml); the filtered solution was evaporated and the residue was taken up in ethyl acetate (40 ml); the filtrate was washed with water and brine and then dried. The solution was passed down a column (20 ml) of Amberlyst-15 (saturated with 3-bromopyridine[1] and equilibrated with ethyl acetate) and the eluate was recycled until no product could be detected in the eluate (10 cycles). Non-basic co-products and by-products were washed off by ethyl acetate (500 ml) and the product was eluted with a

mixture of pyridine and dimethylformamide (1 : 3 v/v; 500 ml). (In general we prefer pyridine as the eluting base in place of the triethylamine used earlier). Evaporation of the eluate and trituration with ether gave protected hexapeptide 4-picolyl ester (0·44 mmol, 89%) having, without further purification, a satisfactory elemental analysis and showing no contaminants on t.l.c. in three different solvents.

This isolation procedure has been used satisfactorily in many cases, including several of the stages of the synthesis of [5-β-cyclohexylalanine]-bradykinin and every stage of the syntheses of [8-β-cyclohexylalanine]- and [5,8-β-cyclohexylalanine]-bradykinins, with overall yields of protected nonapeptide of 36, 42, and 33% respectively.[5] The protected octadecapeptide 4-picolyl ester (II) has also been

Z-[Arg(NO_2)-Pro-Pro-Gly-Phe-Ser(Bzl)-Pro-Phe-Arg-
(II)
(NO_2)]$_2$-OPic

isolated in this way from a solution in dichloromethane; non-basic contaminants were washed off the column by a mixture of dichloromethane and dimethylformamide (3 : 1 v/v), and the product was eluted as usual. We are now examining the effectiveness of the method for other large peptides.

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