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HYDROGENATION IN SOLID PHASE PEPTIDE SYNTHESIS. I. REMOVAL OF PRODUCT FROM THE RESIN

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Although it is well known that catalytic hydrogenation is an extraordinarily mild and desirable method for converting protected peptides into final products, no reports of the successful application of hydrogenation to solid phase peptide synthesis[1] have appeared. In the first[2] of two excellent recent reviews of the subject, catalytic hydrogenation was not even mentioned as a speculative possibility for eliminating protecting groups from resin-bound peptides or for cleaving a peptide from the resin after completion of synthesis. In the second review[3], the authors referred to unpublished unsuccessful attempts to hydrogenolyze the resin benzyl ester bond using a variety of catalysts.

We have found hydrogenation conditions which permit removal of a peptide bound through an ester link to a conventional cross-linked polystyrene resin. One example is described here and a second in the accompanying communication.[4] In addition to the gentle conditions of the reaction, the product may be obtained with intact blocking groups derived from t-butyl alcohol thus giving further opportunity for purification.

We reasoned that if a catalyst could be made to penetrate the resin beads, the chances of hydrogenolysis would be greatly enhanced. To this end, palladium (II) acetate was dissolved in DMF, an excellent swelling solvent for resins to which a peptide chain has been attached, and the resin added. The mixture was allowed to equilibrate. Upon shaking with hydrogen, the resin instantly turned black and hydrogen uptake continued as cleavage occurred. The catalyst and resin were removed by filtration and the DMF distilled off under high vacuum. The crude peptide could then be purified by any suitable procedure.

A number of variables have been investigated. The pressure was changed from atmospheric to 500 psi with no apparent advantage to high pressure. Reactions are currently carried out at 60 psi. Temperatures from ambient to 60° were explored; we have settled on 40°. This seems to increase the rate of hydrogenation and is unlikely to cause decomposition of the product. Hydrogenations were run for times of 5 to 72 h. We have adopted 24 h. If the yield of crude peptide is low, the resin can be rehydrogenated after the addition of

more catalyst. About 10 ml of DMF is required for each gram of resin. The first 5 ml is completely absorbed while the second 5 ml permits adequate agitation. Under these conditions only half the catalyst actually diffuses into the resin. We have tried molar ratios of palladium (II) acetate to peptide substrate in the range of one to four; a ratio of two or three seems to work well. Other catalysts failed completely; e.g.: palladium black, $[(C_6H_5)_3P]_2PdCl_2$, $[(C_6H_5)_3P]_3RhCl$. The latter two are soluble in DMF but still liberated no peptide at all from the resin.

Experimental

Boc-Tyr-Gly-Gly-Phe-Val-Ⓟ was prepared starting from Boc-Val-Ⓟ (11.8 g, 0.86 mmole/g). The resin was chloromethylated 1% cross-linked polystyrene in which about 90% of the chloromethyl groups were caused to react with Boc-Val.[5] The coupling cycle was as described in the next paper[4] except that acetylation was omitted. A ninhydrin test[6] was negative after each coupling. Boc-Tyr was attached via the pentachlorophenyl ester.

Peptide resin (5.5 g, 0.0035 mole based on weight gain) was added to a solution of 2.37 g (0.0105 mole) of $Pd(OAc)_2$ in 55 ml of DMF at 40°. The mixture was shaken 15 min. to swell the resin and diffuse the catalyst into it. The container was pressurized to 60 psi with hydrogen and shaken 24 h at 40°. The solids were returned to a solution of 0.79 g of $Pd(OAc)_2$ in DMF and hydrogenation repeated. The first run gave 1.99 g of crude Boc-Tyr-Gly-Gly-Phe-Val and the second 0.39 g. The combined material was purified by partition chromatography on Sephadex LH-20 using the system $CHCl_3$-CH_3OH-CH_3CO_2H-H_2O 7:3:2:4 with a 4 x 55 cm column and a flow rate of 60 ml per h; 20 ml fractions were collected. The desired product was eluted in fractions 96-146 to give 2.09 g of Boc-Tyr-Gly-Gly-Phe-Val as the monoacetate. The peptide was dissolved in 15 ml CH_3OH, 50 ml of H_2O added, and the mixture taken to dryness at 40°. Two repetitions gave the compound free of acetic acid. The yield was 1.60 g (71%), homogeneous on tlc (96 parts CH_3OH-$CHCl_3$ 1:4 plus 4 parts CH_3CO_2H-H_2O 1:2). $[\alpha]_D$ + 5.9° (c 1, CH_3OH). Amino acid analysis: Tyr 0.95, Gly 2.00, Phe 1.08, Val 1.00. Anal. Calcd. for $C_{32}H_{43}N_5O_9$: C,59.89; H,6.76; N,10.92. Found: C,59.76; H,6.79; N,10.77.

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