Polymer-Bound Carbonic Anhydrides in *N*-Acylation of 7-Aminocephalosporanic Acid

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Abstract □ 7-Aminocephalosporanic acid *tert*-butyl ester was reacted with polystyrene-bound mixed carbonic–carboxylic anhydrides to give the corresponding *N*-acylated derivatives. Cleavage of the *tert*-butyl protecting group with trifluoroacetic acid gave the corresponding cephalosporanic acid.

Keyphrases □ Cefaloram—synthesized by *N*-acylation of *tert*-butyl 7-aminocephalosporanate using polystyrene-bound anhydrides □ Polymer-bound anhydrides—used in synthesis of cefaloram by *N*-acylation of *tert*-butyl 7-aminocephalosporanate □ Cephalosporins—cefaloram synthesized by *N*-acylation of *tert*-butyl 7-aminocephalosporanate using polystyrene-bound anhydrides □ Solid phase syntheses—cefaloram, *N*-acylation of *tert*-butyl 7-aminocephalosporanate using polystyrene-bound anhydrides □ Antibiotics—cefaloram, synthesized by *N*-acylation of *tert*-butyl 7-aminocephalosporanate using polystyrene-bound anhydrides

The advantages of using insoluble polymer supports in the syntheses of polypeptides (1) and polynucleotides (2) have been well documented. The success of this solid phase technique prompted various syntheses with polymer-bound reagents (3, 4). One dominant reason for using the insoluble support is that the excess reagent and the products arising from it can be removed by simple filtration. Thus, excess reagent can be added to ensure complete conversion of the substrate and the product can be isolated by filtration and solvent evaporation. The ease of this workup procedure is especially important in preparing sensitive molecules such as antibiotics. This report presents initial studies on the use of insoluble reagents in the *N*-acylation of 7-aminocephalosporanic acid.

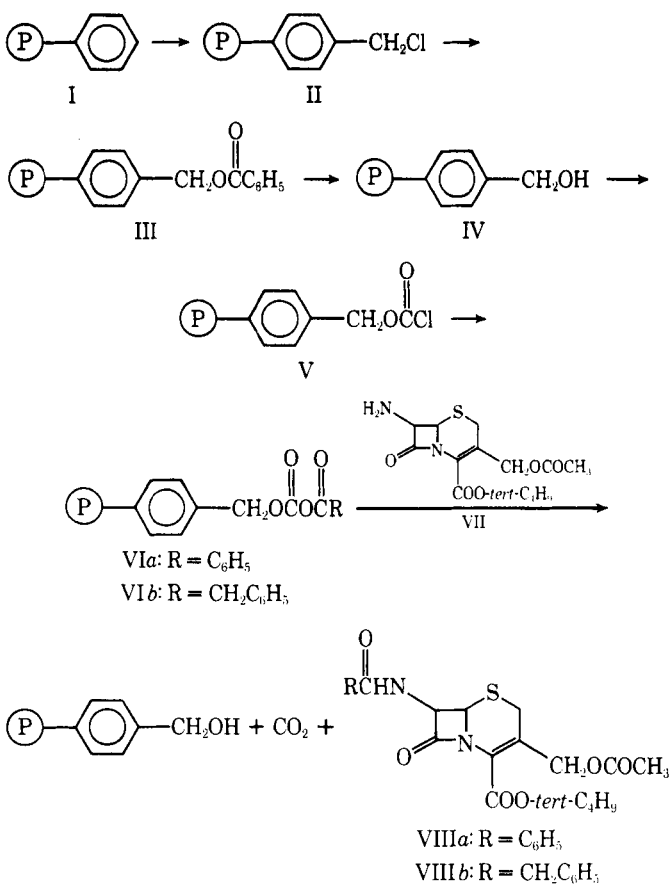
Mixed carbonic–carboxylic anhydrides have been utilized in the *N*-acylation of both 7-aminocephalosporanic and 6-aminopenicillanic acids (5). Due to their instability, these reagents are prepared *in situ*. These highly reactive anhydride functions can be created on insoluble polystyrene (6), resulting in a polymer capable of benzoylation of simple aliphatic amines. Since *N*-acylation of 7-aminocephalosporanic acid by phenylacetic acid is required to produce an active antibiotic (cefaloram), a study of the formation and utilization of polystyrene with carbonic–phenylacetic anhydride functions was undertaken.

RESULTS AND DISCUSSION

The preparation of polymeric mixed anhydrides is illustrated in Scheme I. "Popcorn polystyrene" (I) was chloromethylated according to the general procedure of Merrifield (1), resulting in the production of resins bearing approximately 1.8 mEq/g of chloromethyl residues. Subsequent reaction of the chloromethyl polystyrene (II) with sodium benzoate and triethylamine gave the benzoate ester (III), which, on alkaline hydrolysis, gave quantitative conversion to the benzyl alcohol-bearing resin (IV).

Treatment of IV with a 12.5% solution of phosgene in benzene converted the alcohol to the corresponding chloroformate (V). The conversion of V to the carbonic–benzoic anhydride (VI*a*) was achieved using triethylamine as the catalyst at 0° in 30 min (6). Attempts to prepare the corresponding phenylacetic anhydride (VI*b*) under the same conditions resulted in poor yields, because of the rapid decomposition of the anhydride functions caused by the presence of triethylamine. Stability studies on such mixed anhydrides in solution have shown that some tertiary amines catalyze their conversion to the corresponding esters, accompanied by the loss of carbon dioxide (7). The use of another tertiary amine, quinoline, as the catalyst at 25° gave the desired resin (VI*b*) in fair yield (0.8 mEq/g of acid).

Prior to the reaction with the resin anhydrides, 7-aminocephalosporanic acid was converted to its *tert*-butyl ester (VII) by the use of 2-methylpropene and sulfuric acid (8) for two reasons. One was the necessity of carrying out the reactions of polystyrene-based reagents in solvents, such as benzene, that swell the resin bead. The solubility of



Scheme I

7-aminocephalosporanic acid in benzene is negligible. The second was the blocking of the carboxylic acid function, which left only the amine function to react with the resin-bound reagents (6).

Acylation was carried out by addition of the ester to the benzene suspensions of resins VI*a* and VI*b*. The corresponding *N*-acyl derivatives were obtained after the evaporation of the filtrates in 75 and 50% yields, respectively. Cleavage of the *tert*-butyl ester from *tert*-butyl 7-(*N*-phenylacetamido)cephalosporanate (VIII*b*) resulted in the production of 7-(*N*-phenylacetamido)cephalosporanic acid (cefaloram), a biologically active cephalosporin (5, 8). Subsequent biological activity testing of cefaloram, produced by *N*-acylation with the polymeric mixed anhydride method, by the paper disk method of Bauer *et al.* (9) gave antibiotic activity parallel with that obtained following conventional synthetic preparation (5, 9). Against *Staphylococcus aureus* (3074), a minimum inhibitory concentration of 2.5 μg/ml was obtained.

The reason for the relatively low yields in the acylation reactions was the incomplete conversion of the chloroformate functions of V to the mixed anhydrides. Thus, the polymers VI*a* and VI*b* possessed some chloroformate functions. A portion of the substrate amine was lost because of the reaction with the chloroformate moieties, resulting in binding to the resin as the carbamates. This conclusion was supported by the presence of sulfur in VI*a* and VI*b* after reaction with VII. Attempts are being made to reduce the number of the unreacted chloroformate functions on VI*b*.

Although yields obtained in the *N*-acylation of the cephalosporin nucleus at this time are only comparable to other synthetic procedures, the solid phase method is of potential interest in the preparation of semisynthetic cephalosporins because of its simplicity.

EXPERIMENTAL[1]

Compound I—The resin was prepared according to the procedure described by Letsinger *et al.* (10) and was reduced to 40–100 mesh using a blender[2].

Compound II—To 70 g of I in 500 ml of chloroform at 0° was added, over 30 min, 135 ml of a solution containing 100 ml of chloromethyl methyl ether and 35 ml of anhydrous stannic chloride. The mixture was stirred for 1 additional hr at 0° and then for 2 hr at 25°. The resin was then washed with 500-ml aliquots of each of the following: dioxane, dioxane–water (1:1), 10% HCl (hot), water, dioxane, benzene, and anhydrous ether. The pale-yellow resin was dried *in vacuo* to yield 80.5 g.

Polystyrene Benzyl Alcohol (IV)—To 12.0 g of II in 100 ml of 2-methoxyethanol were added 5 g of potassium benzoate and 2 ml of triethylamine, and the mixture was refluxed for 4 hr. The polymer was washed by decantation with 200 ml of 2-methoxyethanol while still hot. Then the resin was transferred to a second flask containing 4.0 g of potassium hydroxide in 100 ml of 2-methoxyethanol, and the mixture was refluxed for an additional 4 hr. The resin was washed as previously described and dried *in vacuo* to give 11.2 g of IV; IR (4% KBr pellet): 3300 cm^{-1}.

Polystyrene Benzylchloroformate (V)—To a flask containing 75 ml of a 12.5% solution of phosgene in benzene was added 10.0 g of IV. The mixture was shaken at 25° for 5 hr. Then the resin was filtered, washed with benzene (2 × 100 ml) and anhydrous ether (2 × 100 ml), and dried *in vacuo* to give 12.9 g of V; IR (4% KBr pellet): 1765 cm^{-1} (no absorption at 3300 cm^{-1}).

Polystyrene Mixed Carbonic–Carboxylic Anhydrides (VI*a* and VI*b*)—To 8.0 g of V in 50 ml of dry benzene was added 30 m*M* of the desired carboxylic acid and 30 m*M* of the necessary tertiary amine. For benzoic acid, triethylamine was added and the reaction was allowed to proceed at 0° for 30 min; for phenylacetic acid, quinoline was added and the reaction was conducted at 25° for 2 hr. The resin was washed with dioxane–water (1:1) (2 × 100 ml), dioxane (2 × 100 ml), benzene (2 × 100 ml), and anhydrous ether (2 × 100 ml) and dried *in vacuo;* IR (4% KBr pellet): 1740 and 1795 cm^{-1}.

Compound VIII*b*—To 3.0 g of VI*b* in 50 ml of dry benzene at 50° was added 0.85 g of *tert*-butylaminocephalosporanate (VII). The mixture was allowed to react for 2 hr, and then the resin was removed by filtration. After evaporation of the solvent, the residue was recrystallized from methylene chloride–ether to give 0.65 g (50%) of VIII*b*, mp 159–160° [lit. (11) mp 148–150°].

Cefaloram—Cleavage of the *tert*-butyl ester-protecting group was conducted by dissolving 1.0 g of VIII*b* in 10 ml of anhydrous ice-cold trifluoroacetic acid and allowing it to stand at 25° for 30 min (8). Then the solution was concentrated under reduced pressure to give a gummy residue, which was dissolved in a minimum of methylene chloride. The product precipitated with ether to give 0.54 g (62%) of cefaloram (5), mp 168–171° dec.; IR (chloroform): 1780, 1720, 1670, and 1640 cm^{-1}; NMR (acetone-d_6): δ 2.02 (s, 3H, $COCH_3$), 3.45–3.55 (d, 2H, C-2 CH_2), 3.6 (s, 2H, $C_6H_5CH_2$), 4.6–5.2 (m, 3H, C-6 H and CH_2O), 5.75 (quartet, 1H, C-7 H), 7.25 (s, 5H, C_6H_5), and 7.85–8.0 (broad doublet, 1H, NH) ppm.

[1] IR spectra were recorded on a Perkin-Elmer 564 or 700 instrument. NMR spectra were taken on a Varian EM-360 or A-60-A instrument. Mass spectra were obtained on a Perkin-Elmer RMU-67 instrument at 70 ev. Melting points were taken with a Thomas-Hoover capillary melting-point apparatus and are uncorrected. Elemental analyses were performed by Microanalysis Inc., Wilmington, Del., and were within 0.4% of theory.

[2] Waring.

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