

PREPARATION OF 7-AMINOCEPHALOSPORANIC ACID AND 6-AMINOPENICILLANIC ACID

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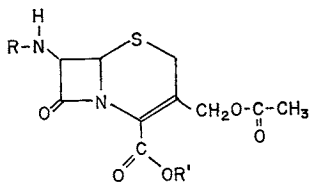
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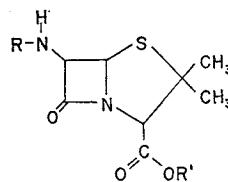
The utility of acetyl mixed anhydride as a carboxylic acid blocking group during acylamido cleavage of cephalosporins and penicillins is illustrated. The formation of N-chloroacetyl cephalosporin C in the isolation of cephalosporin C is described.

7-Aminocephalosporanic acid and 6-aminopenicillanic acid are starting materials for numerous commercially important semi-synthetic cephalosporins and penicillins. The N-acyl side chain of cephalosporin C (I) and of benzylpenicillin (II) are efficiently cleaved to their respective nuclei in a series of reactions proceeding with phosphorus pentachloride and pyridine to form an imino-chloride intermediate, followed by an alcohol to convert this to an imino-ether, then water to effect hydrolysis¹⁻³).

An early report¹⁾ of this chemical deacylation of cephalosporin C described procedures for blocking the amino and carboxylic acid functions in preceding, separate steps. The product was an ester of 7-aminocephalosporanic acid that required an additional hydrolytic or reductive de-esterification step to afford the cephalosporin nucleus in its zwitter-ionic form (III, 7-ACA). A subsequent application of this PCl₅ cleavage reaction sequence to a penicillin silyl ester²⁾ represented a significant innovation in the overall cleavage scheme. Silylation of the carboxylic acid and deacylation could then be effected in the same reaction vessel without isolation of intermediates. The spontaneous decomposition of the silyl block concomitant with side chain cleavage afforded 6-aminopenicillanic acid (IV, 6-APA) directly. A number of publications³⁾ ensued disclosing the successful use of silylating agents to simultaneously protect the amino and carboxylic acid groups in cephalosporin C during side chain cleavage, leading to high yields of 7-ACA.



- I R=HO₂C-CH(NH₂)-(CH₂)₃-CO, R'=H
 III R=R'=H
 VI R=CH₃CO₂CO-CH(NHCOCH₃)-(CH₂)₃-CO, R'=COCH₃
 VII R=HO₂C-CH(NHCOCH₂Cl)-(CH₂)₃-CO, R'=H
 VIII R=CH₃CO₂-CO-CH(NHCOCH₂Cl)-(CH₂)₃CO, R'=COCH₃



- II R=C₆H₅CH₂CO, R'=Na
 IV R=R'=H
 V R=C₆H₅CH₂CO, R'=COCH₃

Meanwhile, work directed at finding new carboxylic acid protecting groups in our laboratories lead to the effective use of the mixed anhydride block during side chain cleavage of both cephalosporins and penicillins. In a modification of the literature method⁴, benzyl penicillin, sodium salt, was converted to its acetic mixed anhydride (V) which was not isolated. The reaction mixture was subjected to the PCl_5 reaction sequence. 6-APA was isolated from water at its iso-electric pH, in 60 % yield. Similarly, cephalosporin C, free amino acid, was solubilized in chloroform by saturating the solvent at room temperature with ketene. The product, presumed to be the N-acyl di-acetic mixed anhydride (VI), was not isolated. The CHCl_3 solution was subjected to the PCl_5 cleavage reaction conditions resulting in the isolation of 7-ACA in 56 % yield. No effort was made to optimize the reaction conditions and yields in the preceding examples.

We were further interested in finding means to improve the efficiency in isolation of cephalosporin C. Traditionally, cephalosporin C has been isolated by a combination of carbon and resin chromatography and crystallization of its mono sodium salt, dihydrate. We have found it expedient to N-acylate cephalosporin C directly in either filtered fermentation broth or in eluates from ion-exchange resin treatment of the broth. This allows ready separation of the N-acyl derivative by solvent extractions at low pH and a convenient precipitation of the product as an insoluble salt. Our most satisfactory isolation proceeds through the N-chloroacetyl derivative (VII) which crystallizes as a monoquinoline salt, monohydrate in 70 % yield. This product is solubilized in methylene chloride by reaction with acetyl chloride and diethylaniline in the formation of N-chloroacetyl cephalosporin C diacetic mixed anhydride (VIII). This mixture undergoes the PCl_5 cleavage reaction in the usual way, giving 7-ACA in yields of 75~85 %.

A combination of these steps describes a simple and efficient process for the preparation of 7-ACA and demonstrates the practical utility of the mixed anhydride block during side chain cleavage of both cephalosporins and penicillins.

Experimental Section

N-Chloroacetylcephalosporin C (VII)

Cephalosporin C fermentation eluate was adjusted to pH 8.5 with NaHCO_3 , diluted with acetone to the extent of 20 % (v/v) and acylated at room temperature by slow and simultaneous addition of four equivalents of chloroacetyl chloride and NaOH to maintain the pH constant. The product was separated by cold extractions with EtOAc at pH 2. Four moles of quinoline added to this EtOAc solution caused precipitation of a crystalline monoquinoline salt, monohydrate. Isolation yields were generally about 70 % of theory, based on the cephalosporin C content of the beer. m.p. 131°C (dec.); IR (mull): 2.84 (amide NH), 5.61 (β -lactam carbonyl), 5.78 (ester carbonyl), 6.02 and 6.05 (amide carbonyls) and 6.52 μ (amide II). UV (pH 7 buffer): λ_{max} 265 m μ ($\epsilon=11,750$). NMR (DMSO d_6): 8.5~8.2 (m, 4H, C_3' and C_4' CH_2), 7.93 (s, 3H, C_3CH_3 of acetoxymethyl), 7.9~7.6 (m, 2H, C_2' CH_2), 6.40 (2d, 2H, C_2H_2), 5.82 (s, 2H, C_5' CH_2 of chloroacetamidomethylene), 5.08 (d, 2H, C_3CH_2), 4.87 (d, 1H, C_6H), 4.27 (q, 1H, C_7H) and 3.0~0.9 τ (m, 9H, aromatic H and amide NH's).

Anal. calcd for $\text{C}_{27}\text{H}_{31}\text{ClN}_4\text{O}_{10}\text{S}$: C 50.74, H 4.89, N 8.77, Cl 5.55.

Found: C 50.48, H 4.99, N 8.98, Cl 5.73.

KARL FISHER Anal. for H_2O : 2.8~2.9 % (calcd. 2.82 %).

7-Aminocephalosporanic Acid (III).

Method A: Cephalosporin C, free amino acid, (5 mM), was suspended in 40 ml of freshly distilled $CHCl_3$ containing 10 drops of DMF. Ketene was passed through the mixture at room temperature for 1 hour at which time the starting material was completely in solution. Dry nitrogen was then passed through the solution to drive out excess ketene. The reaction solution was chilled to $-22^\circ C$ for addition of dimethyl aniline, 2.1 g (17.3 mM) and PCl_5 , 2.4 g (11.5 mM). After 2 hours, 12 ml of *n*-propanol were added. Cooling and stirring were maintained for an additional 2 hours. The cooling bath was removed for addition of 20 ml of water. The mixture was stirred vigorously for 10 minutes. The aqueous portion was separated and immediately adjusted to pH 3.5 with saturated NH_4HCO_3 . The precipitated product was washed with cold methanol and was vacuum dried for several hours at $40^\circ C$. Weight: 760 mg. IR, UV, and NMR compared exactly with that of an authentic sample of 7-ACA.

Method B: N-Chloroacetyl cephalosporin C, monoquinoline salt, monohydrate (20 mM), was suspended in 150 ml of $CHCl_3$ containing 1 ml of DMF. The mixture was stirred and cooled to about $15^\circ C$ and then treated with diethylaniline, 10.2 g (68.2 mM) and acetylchloride, 8.9 g (112 mM). The starting material was in solution within 30 minutes. The mixture was cooled in a CCl_4 dry ice bath. Diethylaniline, 10.2 g (68.2 mM) and PCl_5 , 9.8 g (47 mM) were added. Cooling and stirring were maintained for 1 hour. Fifty ml of cold MeOH was added, followed after 1 hour with addition of 100 ml of cold water. The cooling bath was removed and the two-phase system stirred vigorously for 15 minutes. The aqueous phase was separated and immediately adjusted to pH 3.5. The precipitated product was filtered, washed first with cold 50 % aqueous MeOH, then with cold acetone and vacuum dried. Weight: 4.5 g. IR, UV, and NMR were consistent with that of 7-ACA.

6-Aminopenicillanic Acid (IV).

Benzylpenicillin, sodium salt, (60 mM), was suspended in 150 ml of CH_2Cl_2 . Diethylaniline, 20 g (140 mM) and acetyl chloride, 15.3 g (195 mM) were added. The mixture was stirred at room temperature for 2.5 hours. The reaction mixture was chilled to $-60^\circ C$ and then treated first with diethylaniline, 15 g (100 mM) and then with PCl_5 , 20 g (96 mM) in 300 ml of CH_2Cl_2 . After 1.5 hours, 150 ml of MeOH was added.

Stirring and cooling were maintained for 1.5 hours. The cooling bath was removed for addition of 300 ml of water. The two-phase system was stirred vigorously for 5 minutes. The aqueous layer was separated and immediately adjusted to pH 3.5 with conc. NH_4OH . The precipitated product was filtered, washed with cold acetone and vacuum dried for 18 hours at $40^\circ C$. Weight: 7.8 g. IR and NMR, compared exactly with that of an authentic sample of 6-APA.

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