Chemistry of Cephalosporin Antibiotics. XIV.[1] The Reaction of Cephalosporin C with Nitrosyl Chloride

Robert B. Morin, Bill G. Jackson, Edwin H. Flynn, Roger W. Roeske, and Sandra L. Andrews

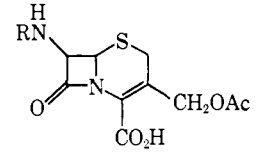
Contribution from The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46206. *Received October 23, 1968*

Abstract: The interaction of cephalosporin C with nitrosyl chloride was examined in detail. In addition to 7-aminocephalosporanic acid (7-ACA, **1b**), the 7-chlorocephalosporanic acid was isolated as its methyl ester (**5**). Three substances resulting from intermolecular reactions of the intermediate **3** were isolated and characterized (**7a**, **7b**, and **7c**). α-Hydroxyadipic acid and its lactone were obtained in yields corresponding to those products resulting from intramolecular reactions.

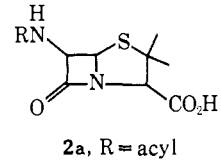
Cephalosporin C, an antibiotic produced by a strain of *Cephalosporium sp.*, has been shown to have structure **1a**.[2] The antibacterial activity of this antibiotic is too low for practical therapeutic use; however, the structural similarity between the penicillins (**2a**) and cephalosporin C is readily apparent; and, *a priori*, it may be argued that modification of the latter substance would lead to a useful antibiotic.

While the natural penicillins possess the advantages of high activity against many pathogenic bacteria and low toxicity toward the host, they suffer the disadvantages of being unstable in acid and sensitive to cleavage of the β-lactam (thus rendering them biologically inactive) by penicillinase, an enzyme produced by certain strains of bacteria. Cephalosporin C, on the other hand, is relatively stable in acid solution and is resistant to penicillinase. It has been shown[3] that variation of the R group in **2a** [by acylation of 6-aminopenicillanic acid (**2b**)] leads to new penicillins possessing acid stability, penicillinase resistance, or improved antibacterial spectrum; however, no single new penicillin had all three of these improved features. It was hoped that analogous variations in the cephalosporin series would lead to a highly active, acid stable, penicillinase resistant, nontoxic antibiotic with increased potency vs. a wide range of bacteria.[4]

In order to carry out such a modification program it was necessary to remove the α-aminoadipyl side chain from cephalosporin C and to replace it by other acyl radicals. The conversion of cephalosporin C to 7-aminocephalosporanic acid (7-ACA, **1b**) constituted the first objective of this program.



1a, R = $H_3\overset{+}{N}C(CO_2^-)H(CH_2)_3C(=O)-$
b, R = H
c, R = acyl



2a, R = acyl
b, R = H

Microbiological methods which were useful in the synthesis of 6-aminopenicillanic acid[5] were unsuccessful in the cephalosporins.

Loder, Newton, and Abraham[6] removed the side chain with dilute acid and obtained a low (<1%) yield of 7-ACA, which was then reacylated to give a variety of 7-acylamidocephalosporanic acids (**1c**). These latter substances possessed greater antibacterial activity than the parent cephalosporin C and were penicillinase and acid resistant.

The low yield of 7-ACA resulting from acid hydrolysis of cephalosporin C, coupled with the failure of microbiological methods for achieving this conversion, necessitated seeking an alternate procedure[7] for removal of the α-aminoadipyl radical. This paper reports the full experimental details of our study.

Acid hydrolysis would not be expected to cleave the adipyl amide bond in preference to the labile amide linkage in the β-lactam; however, the fortuitous position of the amino function at C-5′ in the side chain does suggest that an intramolecular reaction,[8] as indicated in Scheme I, might lead to the desired preferential cleavage. This hypothesis was supported by the

(1) R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon, and S. L. Andrews, *J. Amer. Chem. Soc.*, **91**, 1401 (1969).

(2) E. P. Abraham and G. G. F. Newton, *Biochem. J.*, **79**, 377 (1961); D. C. Hodgkin and E. N. Maslen, *ibid.*, **79**, 393 (1961).

(3) *Inter alia* Y. G. Perron, W. F. Minor, C. T. Holdrege, W. J. Gottstein, J. C. Godfrey, L. B. Crast, R. B. Babel, and L. C. Cheney, *J. Amer. Chem. Soc.*, **82**, 3934 (1960).

(4) (a) For a summary of the biological properties of selected semisynthetic cephalosporins prepared in these laboratories, see R. R. Chauvette, E. H. Flynn, B. G. Jackson, E. R. Lavagnino, R. B. Morin, R. A. Mueller, R. P. Pioch, R. W. Roeske, C. W. Ryan, J. L. Spencer, and E. Van Heyningen, "Antimicrobial Agents and Chemotherapy," American Society for Microbiology, Ann Arbor, Mich., 1962, pp 687–694. (b) Subsequent to the completion of this work, two parenteral cephalosporin antibiotics, Keflin (cephalothin, Lilly) (1c, R = 2-thienylacetyl) and Loridine (cephaloridine, Lilly) having low toxicity, penicillinase resistance, and excellent activity against both Gram-positive and Gram-negative bacteria, as well as penicillin-resistant *Staphylococci*, have been marketed by Eli Lilly and Company.

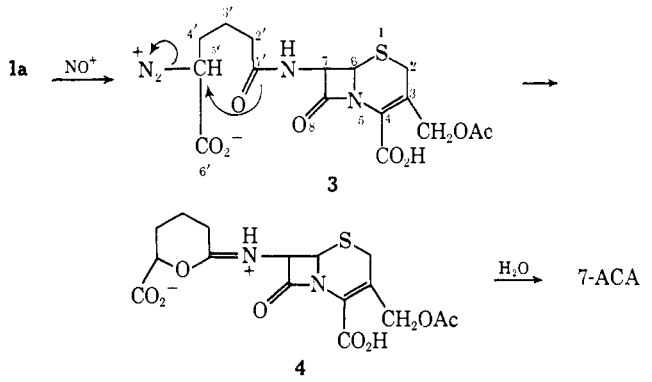
(5) These methods utilized two approaches: direct fermentation of 6-aminopenicillanic acid, F. R. Batchelor, F. P. Doyle, J. H. C. Nayler, and G. N. Rolinson, *Nature*, **183**, 257 (1959); and enzymatic cleavage of the C-6 acylamido function by amidases, G. N. Rolinson, F. R. Batchelor, D. Butterworth, J. Cameron-Wood, M. Cole, G. C. Eustace, M. V. Hart, M. Richards, and E. B. Chain, *Nature*, **187**, 236 (1960).

(6) B. Loder, G. G. F. Newton, and E. P. Abraham, *Biochem. J.*, **79**, 408 (1961).

(7) (a) A preliminary report of the present work has been published: R. B. Morin, B. G. Jackson, E. H. Flynn, and R. W. Roeske, *J. Amer. Chem. Soc.*, **84**, 3400 (1962); (b) B. Fechtig, H. Peter, H. Bickel, and E. Vischer, *Helv. Chim. Acta*, **51**, 1108 (1968).

(8) For a review of facile cleavages of amide bonds by analogous intramolecular reactions, see L. A. Cohen and B. Witkop, *Angew. Chem.*, **73**, 253 (1961).

Scheme I



fact that cephalosporin C upon treatment with aqueous nitrous acid gave 2 molar equiv of nitrogen,[9] 1 mol arising from the side-chain amino group and the other presumably from the amino group of 7-ACA resulting from the hydrolysis of the intermediate iminolactone **4**. Hydrolysis of the iminolactone in the presence of nitrosating agent was avoided by using nonaqueous solvents; for when acetic acid was used with nitrosyl chloride and the nitrosating agent removed before contact with water, a 7% yield of 7-ACA resulted.

A study of reaction conditions resulted in adoption of formic acid as solvent and nitrosyl chloride as nitrosating agent. Use of this combination gave 25–40% yields of 7-ACA. The yield of 7-ACA is influenced by the temperature of the reaction mixture and the time of contact with NOCl. Optimum results are obtained when the temperature does not exceed 30° and the reaction period is short (*ca.* 5 min). Common polar organic solvents other than formic and acetic acid possess limited solubility for cephalosporin C. Yields of 7-ACA utilizing these solvents were usually lower. Mixtures of formic acid and other solvents gave yields of 7-ACA comparable to those obtained with formic acid alone.

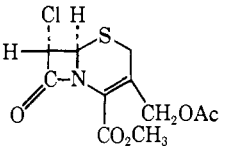
Isolation of 7-ACA was accomplished by evaporating the reaction mixture *in vacuo*, dissolving the residue in water, and adjusting the pH to 3.5 (isoelectric point) by addition of mineral base, whereupon 7-ACA precipitated as a pale yellow, crystalline solid. This substance, after washing with water and acetone, could be used directly for further reaction without additional purification. Various assay procedures indicated that the crude 7-ACA so prepared was 80–95% pure, the usual contaminants being inorganic salts.

As a corollary study, investigation of the remainder of the reaction mixture was undertaken. Initially, it was of interest to determine if there was any cephalosporin C remaining after treatment with nitrosyl chloride. Paper chromatography of the mother liquors from 7-ACA showed five biologically active (*vs. B. subtilis*) substances, *viz.*, cephalosporin C, 7-ACA, and three less polar materials. The three less polar substances could be extracted into ethyl acetate at pH 2.0, and the amount of cephalosporin C in the aqueous portion was estimated by microbiological assay, using *Salmonella gallinarum* as the assay organism. Analysis of five different lots of 7-ACA mother liquors revealed that 3–5% of the cephalosporin C survived the nitrosating conditions.

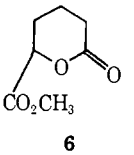
For analysis of the complete reaction mixture, an alternate work-up was utilized. After removal of solvent and excess nitrosyl chloride, the crude residue was lixiviated with acetone, giving an acetone-soluble and an acetone-insoluble fraction. The insoluble portion was analyzed by infrared spectroscopy and paper chromatography and was found to contain 7-ACA, 7-ACA hydrochloride, and cephalosporin C. The acetone-soluble component, a mixture of carboxylic acids, was treated with excess ethereal diazomethane, and the solvents were evaporated under reduced pressure.

Thin layer chromatography (silica, ethyl acetate) of the mixture of methyl esters showed the presence of at least five components. Separation of these substances was effected by column chromatography, using 1:1 silicic acid–Hyflo Super Cel as adsorbent. Elution with benzene, benzene–ether mixtures, and ether resulted in isolation of six substances. One of these was readily identified as dimethyl α-hydroxyadipate by comparison with an independently synthesized sample of that substance. Evidence for the structures of the remaining five (designated A through E, in order of their elution) will be discussed. Four of the five substances contained the cephalosporin ring system, as indicated by ir and nmr spectra.[10]

Compound A was obtained as a chromatographically homogeneous oil which could not be crystallized. Elemental composition of this substance corresponded to $C_{11}H_{12}ClNO_5S$, and its ultraviolet spectrum had a maximum at 266 mμ (ϵ 6600), characteristic of the cephalosporins.[6] The infrared spectrum showed absorption characteristics of a fused β-lactam (1800 cm^{-1}), ester (1745, 1738, and 1245 cm^{-1}), and carbon–carbon double bond (1648 cm^{-1}). The nmr spectrum of A indicated presence of a cephalosporin ring system. In view of these data, structure **5** is proposed for A. Such a molecule could be formed by solvolysis of the iminolactone **4**, giving 7-ACA, which subsequently reacts with nitrosyl chloride to produce **5**.



5



6

Interaction of 7-ACA and nitrosyl chloride in formic acid or acetonitrile, followed by esterification with diazomethane, gave a methyl ester whose properties were identical with those of compound A.

The stereochemistry of the C–Cl bond was not proved, although circumstantial evidence suggests that the bond is α oriented (Cl *cis* to the C-6 hydrogen). The amino group in the parent 7-ACA is β oriented, and the mechanistic route would most likely involve

(9) This experiment was performed by E. E. Logsdon of these laboratories.

(10) For a detailed discussion of ir and nmr spectra of cephalosporins and penicillins, see G. F. H. Green, J. E. Page, and S. E. Staniforth, *J. Chem. Soc.*, 1595 (1965).

nucleophilic displacement of the diazotized amino function at C-7 with inversion.

It is known[11] that α-halo carbonyl compounds undergo facile displacements; however, **6** was inert to a variety of nucleophilic agents (methylamine, azide ion, acetate ion, and potassium phthalimide) with respect to displacement of the halogen. Prolonged treatment of the chloro ester with these nucleophiles, accompanied by periodic testing for chloride ion, gave negative results.

The nonreactivity of the C-7 halogen, in spite of its being adjacent to a carbonyl moiety, suggests that approach to C-7 is hindered on the side opposite the chlorine atom. This fact, coupled with the ready formation of **5** from 7-ACA, strongly suggests an α orientation for the halogen, since approach of a nucleophile to the front side of C-7 would be hindered by the six-membered ring and its substituents.

The nmr spectrum of **5** shows an AB pattern for the two protons on the four-membered ring (J = 1.7 Hz). In other cephalosporin methyl esters, this coupling constant has a value of 3–5 Hz. From the studies of others on the coupling constants in the nmr of β-lactam compounds,[12] the *trans* relationship of the hydrogens in **5** can be assigned with some degree of certainty.

The infrared spectrum of B ($C_7H_{10}O_4$) was similar to, but not identical with, the infrared spectrum of dimethyl α-hydroxyadipate. Analysis of the nmr spectra of dimethyl α-hydroxyadipate and the $C_7H_{10}O_4$ compound suggested that the new compound is the lactone **6**. Wiley[13] has reported the synthesis of **6** and has noted that the substance is hygroscopic. This latter fact is most likely responsible for hydroxyl absorption in the infrared spectrum of **6**.

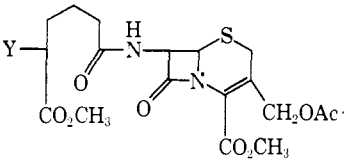
The relative proportion of lactone to hydroxy diester was *ca.* 5:1, and the total yield of these two materials was 25–38%. This yield of α-hydroxyadipic type products corresponds reasonably well to the combined yields of 7-ACA (*ca.* 30% on this scale) and 7-chlorocephalosporanic acid methyl ester (4–7%). Isolation of only 25–38% of the adipic side chain suggests that the remainder of the products still have the adipic side chain attached. This hypothesis was borne out by the characterization of substances C, D, and E.

Substance C was eluted with benzene–ether (95:5). Its ultraviolet spectrum indicated a cephalosporin nucleus (λ_{max} 262 mμ (ε 7940)); presence of an ester (1745 and 1230 cm^{-1}) and a secondary amide (3450, 1695, and 1505 cm^{-1}), as well as the β-lactam (1800 cm^{-1}), were inferred from its infrared spectrum. The compound was analyzed for $C_{18}H_{23}ClN_2O_8S$.

Compound D, which was analyzed for $C_{19}H_{24}N_2O_{10}S$, was eluted following C. The ultraviolet and infrared spectra of this substance were nearly identical with those of C, except that D possessed a strong band in the infrared at 1160 cm^{-1}.

Compound E, $C_{18}H_{24}N_2O_9S$, was obtained by elution of the column with ether. Its infrared spectrum was remarkably like that of C, except for the presence of a hydroxyl band at 3530 cm^{-1}.

The nmr spectra of C, D, and E showed the typical cephalosporin pattern and, in addition, bands characteristic of an adipyl side chain substituted at C-5′. Thus, these three compounds may be formulated as of the type illustrated by **7**. The formulation of C as **7a** was derived from elemental analysis and was consistent with all available data for that compound.

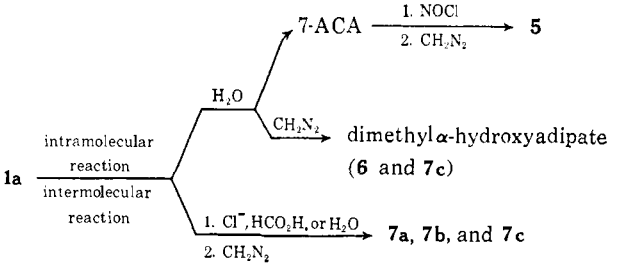


7a, Y = Cl
b, Y = OCHO
c, Y = OH

Infrared absorption at 1160 cm^{-1} is typical of formate esters,[14] and the presence of such a moiety in D was borne out by the presence in its nmr spectrum of a signal at δ 8.04, consistent with this formulation. In addition to the infrared evidence for the presence of hydroxyl, there was a signal at δ 3.03 in the nmr of compound E which disappeared after shaking the sample with D_2O. Thus structure **7c** was indicated for compound E. It was concluded from the erratic melting point behavior and from the doubling of the C-5′ proton signals in the nmr spectra of compounds C, D, and E that the substances were mixtures of C-5′ epimers.[15]

The yields of **7a, 7b,** and **7c** were roughly equal, being 15, 10, and 10%, respectively.

Formation of the various products obtained has been rationalized in the following fashion. Initially, nitrosyl chloride attacks the amino group of cephalosporin C, giving the diazonium intermediate **3**; this in turn reacts either intramolecularly to give the iminolactone **4** or intermolecularly by nucleophilic attack of chloride ion, formic acid (or formate), or water [present as a contaminant in formic acid and cephalosporin C (dihydrate)] to produce **7a, 7b,** or **7c,** respectively. Hydrolysis of the iminolactone would give 7-ACA and (after diazomethane treatment) the lactone **6** or the dimethyl α-hydroxyadipate. Alternatively, hydrolysis of iminolactone could lead to **7c** as well as 7-ACA.



(11) J. Hine, "Physical Organic Chemistry," 2nd ed, McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p 157.

(12) H. B. Kagen, J. J. Basselier, and J. L. Juche, *Tetrahedron Letters*, 941 (1964); K. D. Barrow and T. M. Spotswood, *ibid.*, 3325 (1965); I. McMillan and R. J. Stoodley, *ibid.*, 1205 (1966).

(13) R. H. Wiley and A. J. Hart, *J. Amer. Chem. Soc.*, 77, 2340 (1955).

(14) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed, Methuen and Co., Ltd., London, 1958, p 189.

(15) The authors are indebted to Dr. H. Boaz of these laboratories for assistance in the interpretation of the nmr spectra.

Experimental Section

All melting points are uncorrected. Ultraviolet spectra were recorded on a Carey spectrophotometer; infrared spectra on a Beckman IR-7 or Perkin-Elmer Model 21 spectrophotometer; and nmr spectra with a Varian Associates HR-60 spectrophotometer

Preparation of 7-ACA (1b) from Cephalosporin C (1a). Cephalosporin C [sodium salt, dihydrate; 20 g (0.042 mol)] was dissolved in 98–100% formic acid (79 ml) and the solution stirred and cooled in an ice bath. To this was added a cold solution of 5.5 g (0.084 mol) of nitrosyl chloride (Matheson) in formic acid in one portion.[16] An immediate evolution of gas took place, and the solution temperature rose to about 30°. After a 5-min reaction period the mixture was evaporated *in vacuo* and the residue dissolved in 75 ml of H_2O. The solution was stirred and cooled in an ice bath; the pH was adjusted to 3.5 by the addition of 1 *N* NaOH solution. The precipitated 7-ACA was filtered, washed well with deionized water and acetone, and dried, giving 3.5 g (31%). The 7-ACA so prepared was assayed by ultraviolet spectrometry and found to be 90% pure.[17] X-Ray diffraction of the material showed the presence of sodium chloride as a contaminant.

When larger batches of cephalosporin C were employed, yields of 7-ACA were improved. For example, 80 g of cephalosporin C, when treated in a corresponding fashion, gave 7-ACA in 38% yield.

Determination of Amount of Unreacted Cephalosporin C from Cleavage Reaction. Cephalosporin C (sodium salt dihydrate) was treated with nitrosyl chloride in the above fashion. The mother liquors from the 7-ACA filtration were layered with *ca.* 75 ml of ethyl acetate, and the pH was adjusted to 2.0 with concentrated hydrochloric acid. The aqueous portion was extracted twice more with 50-ml portions of ethyl acetate, and the remaining solution brought to pH 5.5 with 5 *N* NaOH. The volume was then measured, and an aliquot was withdrawn for paper chromatography and bioassay. Paper chromatography (70% propanol, bioautograph) revealed that the only biologically active materials present were cephalosporin C and 7-ACA. Since the activity of 7-ACA is only 1/100th the activity of cephalosporin C, bioassay of the solution would be a direct measure of the amount of cephalosporin C therein. Assay *vs. Salmonella gallinarum* gave the concentration of cephalosporin C in micrograms/milliliters. Table I gives the results of five such determinations.

Table I

Batch	Amount of cephalosporin C, g	Vol after extrctn, ml	Bioassay of extrctd soln, μg/ml	% cephalosporin C remaining
1	30	226	6600	5.0
2	30	204	5200	3.5
3	30	249	3900	3.2
4	30	168	8600	4.8
5	60	335	7000	4.1

Alternate Work-up Procedure Used for Isolation of Side Products. Purified cephalosporin C [sodium salt dihydrate, 4.73 g (10 mmol)] was dissolved in 98–100% formic acid (10 ml), stirred, and cooled in an ice bath. Nitrosyl chloride (1.5 ml, 2.2 g, 33 mmol) was condensed in a centrifuge tube immersed in Dry Ice–ethanol mixture. The liquid NOCl was diluted with 1 ml of chloroform and then added in one portion to the solution of cephalosporin C in formic acid. The reaction was allowed to proceed for 5 min, after which the solvents were evaporated *in vacuo*. The residue was lixiviated with acetone, and the portion insoluble in that solvent was filtered and washed well with acetone. The amorphous solid remaining after drying *in vacuo* weighed 1.28 g. The material soluble in acetone was evaporated *in vacuo* and stored overnight in a vacuum desiccator containing KOH pellets to remove residual formic acid. This crude acidic material (4.04 g) was redissolved in 50 ml of acetone; the solution was decanted from some sludge and then treated with excess ethereal diazomethane. After a 5-min reaction period the solvents were removed under reduced pressure, and the residue was dissolved in 100 ml of ethyl acetate, washed with 100 ml of 5% sodium bicarbonate solution, dried ($MgSO_4$) and again stripped of solvent. The residue (3.36 g) was chromatographed as described in a later section.

The infrared spectrum of the acetone-insoluble solid obtained from this procedure was very similar to that of 7-ACA hydrochloride,[18] and paper chromatography indicated the presence of 7-ACA and cephalosporin C. No further characterization of this solid was performed; however, in addition it doubtless contained sodium chloride and sodium formate.

Chromatography of the Mixture of Methyl Esters Obtained from the Cleavage Reaction. Thin layer chromatography (silicic acid; ethyl acetate–benzene 10:90) of the mixture indicated the presence of five components, reasonably well separated. For chromatography, 50 g of a 1:1 mixture of silicic acid (Mallinckrodt 100 mesh) and Hyflo Super-Cel was employed. Fractions were collected in 25-ml portions. Fractionation was followed by thin layer chromatography and infrared spectroscopy. The results of this chromatography are shown in Table II. Table III lists pertinent data for the new compounds isolated.

Table II

Fraction	Eluting solvent	Wt, mg	Composition
A	100% benzene	200	Essentially pure **5**
B	100% benzene	154	**5**–**6** 20:80
C	Benzene–ether (98:2)	200	Essentially pure **6**
D	Benzene–ether (95:5)	95	Essentially pure dimethyl α-hydroxyadipate
E	Benzene–ether (95:5)	33	Dimethyl α-hydroxyadipate and unknown material
F	Benzene–ether (95:5)	49	Unknown material and **7a**
G	Benzene–ether (95:5)	644	**7a**
H	Benzene–ether (1:1)	321	**7b**
I	Benzene–ether (1:1) and 100% ether	157	Mixture of **7b** and **7c**
J	Ether–acetone (95:5)	424	**7c**
K	Ether–acetone (90:10)	137	Noncrystalline unknown containing β-lactam, ester, and amide
L	Ether–acetone (1:1)	553	Noncrystalline unknown containing β-lactam, ester, and amide
M	Acetone	167	Noncrystalline unknown containing β-lactam, ester, and amide

Preparation of 5 from 7-ACA. 7-ACA (4.13 g, 0.015 mol) which had been purified by repeated isoelectric precipitation was stirred and suspended in 60 ml of acetonitrile. To this suspension was added, in one portion, a solution of 2 g (0.031 mol) of nitrosyl chloride in 60 ml of the same solvent. After a reaction period of *ca.* 45 sec, the solvent was stripped, and the residue taken up in 100 ml each of water and ethyl acetate and filtered to remove traces of unreacted 7-ACA. The phases were separated, and the aqueous portion was extracted twice with 50-ml portions of ethyl acetate. The combined organic solutions were dried (anhydrous $MgSO_4$) and treated with excess ethereal diazomethane. The residue (3.7 g) obtained after solvent removal *in vacuo* was chromatographed over 75 g of silica (Merck, Darmstadt), eluting with benzene–ethyl acetate (19:1). Fractionation was followed by thin layer chromatography (silicic acid–benzene). The chloroester (1.53 g) was obtained. Infrared and nmr spectra of this

(16) Oxidation of formic acid by nitrosyl chloride is quite rapid, especially above 30°. Even at 5° the NOCl in HCO_2H rapidly decreased in titer (iodometric). For this reason the solution must be freshly prepared and kept cold.

(17) The identity of this compound was established by comparison of physical and chromatographic data supplied by Dr. E. P. Abraham in a personal communication. See also ref 6.

(18) E. R. Lavagnino, Lilly Research Laboratories, personal communication.

Table III

Compd	Mp, °C	Calcd, % C	H	N	S	Cl	Found, % C	H	N	S	Cl
5	Gum	43.21	3.95	4.58	10.49	11.60	43.43	4.20	4.82	10.26	11.52
6[a]	Liquid	53.16	6.37				52.23	6.63			
7a	122.5–123.5	46.70	5.00	6.05	6.93	7.66	46.97	5.27	6.22	7.23	7.74
7b	142.5–143.5	48.30	5.12	5.93	6.79		47.99	5.14	6.08	6.86	
7c	141–142	48.90	5.42	6.48	7.48		48.64	5.44	6.30	7.21	

[a] This compound has been reported previously,[13] but spectral data were not given.

product were identical with those of the chloro ester obtained from the treatment of cephalosporin C with nitrosyl chloride.

Experiments Testing Reactivity of Chlorine in 5. A. With Potassium Phthalimide. **5** (100 mg, 0.33 mmol) and 61 mg (0.33 mmol) of potassium phthalimide were each dissolved in 15 ml of methanol, cooled, and mixed. The mixture was allowed to stand in an ice bath for 2 hr. Solvent was removed *in vacuo* and the residue taken up in *ca.* 20 ml each of water and ethyl acetate. The aqueous portion was discarded, and the organic phase dried ($MgSO_4$) and evaporated *in vacuo*. Thin layer chromatography revealed six to seven components in the resulting residue. The crude product (94 mg) was chromatographed over 10 g of silica (Merck, Darmstadt), using ethyl acetate–benzene (1:9) as eluent. This procedure gave phthalimide (15 mg) and starting material (6 mg) as the only identifiable products. The infrared spectrum of the isolated material was not quite identical with that of pure **5**, and the ultraviolet spectrum of this fraction revealed some isomerization to the Δ^2 isomer.

B. With Methylamine. **5** (103 mg, 0.33 mol) and 24 mg (0.77 mmol) of methylamine were dissolved in 10 ml of acetonitrile; the solution was allowed to stand in an ice bath for 135 min. Solvent was removed under reduced pressure. and the residue chromatographed over 3 g of silica (Merck, Darmstadt). Elution with benzene–ethyl acetate (19:1) gave recovered starting material (23 mg) as the only β-lactam containing substance.

C. With Sodium Acetate. Sodium acetate (65 mg) was dissolved in deionized water (5 ml) and added to a solution of 120 mg (0.39 mmol) of **5** in 10 ml of acetonitrile. The resulting solution was allowed to stand at room temperature, and aliquots were withdrawn at reaction periods of 1, 4, 24, 96, and 174 hr. The aliquots were tested for chloride ion, using saturated alcoholic silver nitrate. All tests were negative. At the end of 174 hr the solution was pured into ~50 ml of saturated brine and extracted twice with 20-ml portions of ethyl acetate. The extracts were dried ($MgSO_4$) and stripped of solvent. The residue (32 mg) was not characterized fully, but infrared and ultraviolet spectra show that it contains a considerable amount of the Δ^2 isomer of **5**.

The above experiment was repeated at reflux temperature of the mixed solvents. An aliquot removed after 15 min gave a positive chloride ion test with alcoholic silver nitrate. After refluxing 1.5 hr, the solvent was stripped; the residue was taken up in ethyl acetate, washed with water, and dried ($MgSO_4$), and again solvent was evaporated *in vacuo*. The residue weighed 76 mg and had infrared and ultraviolet abosorbing properties virtually identical with the product from the room temperature experiment. The positive chloride test was rationalized in the following manner. Rupture of the β-lactam occurred, giving a substance with a reactive chloride atom which subsequently was soluble in water. Only the unreacted **5** and its Δ^2 isomer were found in the organic phase.

Preparation of Dimethyl α-Hydroxyadipate. This compound was prepared in four steps, starting with monoethyl adipate. The half ester was converted to diethyl α-bromoacetate according to the procedure of Schwenk and Papa.[19] Although hydrolysis of the bromo diester to α-hydroxyadipic acid has been reported,[20] we were unable to repeat these procedures. Instead it was converted to diethyl α-acetoxyadipate, using the method of Staudinger and Ruzicka,[21] and it subsequently hydrolyzed. The acetoxy diester (16.5 g, 0.064 mol) was stirred under reflux with 50 ml of concentrated hydrochloric acid for 16 hr. The mixture was concentrated to a semicrystalline paste using a rotary evaporator and 100 ml of 50% sodium hydroxide added. The resulting mixture was allowed to stand at room temperature for 16 hr. It was then acidified, using concentrated hydrochloric acid, was saturated with ammonium sulfate and extracted continuously with ether for 72 hr. The ethereal extract after drying ($MgSO_4$) was evaporated to dryness. The crude α-hydroxyadipic acid weighed 7.5 g, mp 97–101°. Recrystallization from ethyl acetate–benzene gave a solid having mp 101–104°. The reported melting point for this substance is 162°;[21] however, satisfactory analytical and spectral data indicate that our synthetic acid is the desired material. Titration in 66% DMF showed two titratable groups with $pK_a' = 5.70$ and 7.40 and an apparent molecular weight of 166 (theory 162).

Anal. Calcd. for $C_6H_{10}O_5$: C, 44.44; H, 6.22. Found: C, 44.67; H, 6.33.

A solution of 5.0 g of the above sample of α-hydroxyadipic acid in 50 ml of tetrahydrofuran and 50 ml of ether was treated with excess ethereal diazomethane. Solvents were stripped, and the residue distilled, bp 99–100° (0.5 mm), giving 2.9 g of diester. The infrared and nuclear magnetic resonance spectra of this substance were identical with those of the dimethyl α-hydroxyadipate obtained from the 7-ACA mother liquors.

Anal. Calcd for $C_8H_{14}O_5$: C, 50.52; H, 7.42. Found: C, 50.33; H, 7.65.

Acknowledgments. The authors wish to thank the members of the Lilly molecular structure research group for physical chemical measurements and microanalyses.

(19) E. Schwenk and D. Papa, *J. Amer. Chem. Soc.*, **70**, 3626 (1948).
(20) W. Treibs and O. Holbe, *Chem. Ber.*, **85**, 608 (1952); C. K. Ingold, *J. Chem. Soc.*, **119**, 951 (1921).
(21) H. Staudinger and L. Ruzicka, *Helv. Chim. Acta*, **7**, 442 (1924)