Chemistry of Cephalosporin Antibiotics. XV. Transformations of Penicillin Sulfoxide. A Synthesis of Cephalosporin Compounds

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Abstract: A procedure for obtaining compounds containing the cephalosporin ring system from penicillins embodying a new general reaction of cyclic sulfoxides, an intramolecular oxidation–reduction in which a carbon β to the sulfur is oxidized, has been developed. Products resulting without rearrangement of the sulfur ring (the substituted penicillin **4**), with ring expansion (cephalosporins **5** and **6**) and with ring contraction (compound **15**) have been observed. Speculations concerning mechanisms and stereochemistry are presented. A correlation of the antibiotic properties of the β-lactam containing substances with the infrared carbonyl frequencies is presented.

The chemistry and structure of penicillin, the subject of tremendous effort during and immediately following World War II, has been summarized in a monograph published in 1949.[1] In the following decade there was little work reported in the field of penicillin chemistry except that of Sheehan and his collaborators, which led to the successful synthesis of penicillin.[2] The availability in 1959 of the penicillin "nucleus," 6-APA, by synthesis, nonprecursed fermentation, and the action of amidases on available penicillins has made possible partial syntheses of new penicillins that have certain therapeutic advantages over natural ones.[3] This work led to a resurgence of research interest in the chemistry of this class of antibiotics.

In general, attempts to modify the β-lactam–thiazolidine ring system of penicillin without loss of antibacterial activity had been unsuccessful. The discovery,[4] structure elucidation,[5] and modification of cephalosporin C,[6] which led to the marketing of important new antibiotics, has provided additional impetus for the study and synthesis of the β-lactam antibiotics.

One interesting facet of this study is the relationship between penicillin and cephalosporin compounds. The structural similarity of the two types of antibiotics and the fact that both are produced in the cephalosporin fermentation suggest that there might be a common intermediate in the biosynthetic pathways. One possibility is that cephalosporin C is a metabolic transformation product of penicillin N. There is no chemical analogy for this transformation in the vast amount of published information on the chemistry of penicillin. Furthermore, there was no indication that the thiazolidine ring in penicillin could be opened, and the desired changes effected, without disruption of the labile β-lactam bond. In order to provide such an analogy and to take advantage of the ready availability of penicillins for the preparation of cephalosporin antibiotics, a program was undertaken to transform chemically a penicillin into a cephalosporin compound.

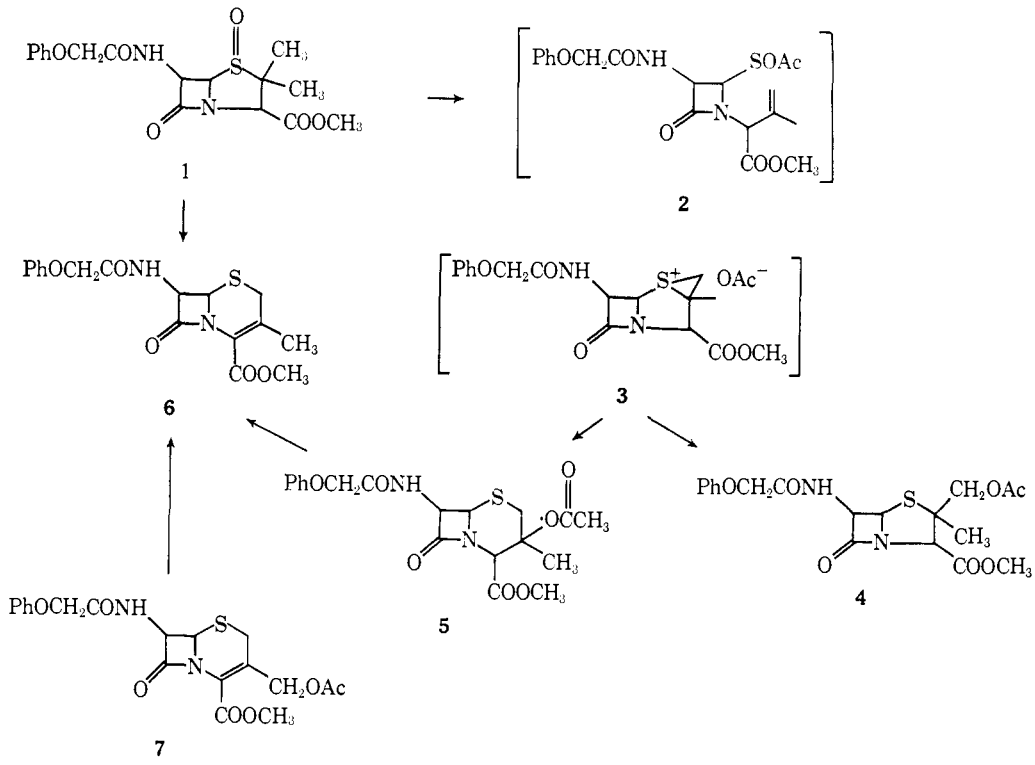
The general approach to the problem was the activation of the sulfur atom by alkylation or oxidation, a facile cleavage of the C_2–sulfur bond with the introduction of a double bond, and then reclosure of the ring in an alternate sense. Our results are reported here.[7]

Since the transformation of a penicillin to a cephalosporin must involve an oxidative step, the chemical reactions of penicillin sulfoxide were initially studied. Sulfoxides that have an α-hydrogen normally undergo the Pummerer reaction on treatment with acetic anhydride under relatively mild conditions, leading to α-acetoxy sulfides. Phenoxymethyl penicillin (pen V) sulfoxide methyl ester was stable to acetic anhydride under these conditions.[8] At reflux temperatures the penicillin sulfoxide derivative was rapidly destroyed; and a new material was isolated, in 60% yield, which retained the strained lactam as indicated by the presence of a 1790 cm^{-1} band in its infrared spectrum. The substance was homogeneous by thin layer chromatography using common solvent systems; however, the nmr spectrum indicated that the product was a 2:1 mixture of two components. After extensive experimentation a thin-layer system utilizing cyclohexane, methylisopropyl ketone (2:1) saturated with water on silica plates showed the presence of two substances. Column chromatography using a scale-up of tlc procedure separated the components; these proved to be isomeric compounds having empirical formula $C_{19}H_{22}N_2O_7S$.

Infrared spectra indicated the presence of a β-lactam and an additional ester function in both compounds. Their uv spectra indicated only the phenoxy grouping of the side chain.

The nmr spectra of the major component and the

(1) H. T. Clarke, J. R. Johnson, and R. Robinson, Ed., "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949.
(2) J. C. Sheehan and K. R. Henery-Logan, *J. Amer. Chem. Soc.*, **81**, 3089 (1959).
(3) G. T. Stewart, "The Penicillin Group of Drugs," Elsevier Publishing Co., New York, N. Y., 1965.
(4) G. G. F. Newton and E. P. Abraham, *Biochem. J.*, **62**, 651 (1956).
(5) E. P. Abraham and G. G. F. Newton, *ibid.*, **79**, 377 (1961); D. C. Hodgkin and E. N. Maslen, *ibid.*, **79**, 393 (1961).
(6) B. Loder, G. G. F. Newton, and E. P. Abraham, *ibid.*, **79**, 408 (1961); 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, *J. Amer. Chem. Soc.*, **84**, 3401 (1962).
(7) R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon, and S. L. Andrews, *ibid.*, **85**, 1896 (1963).
(8) Penicillin sulfoxide may not undergo this reaction because the introduction of a C_5–sulfur double bond as an intermediate would be unfavorable due to the steric requirements of the fused ring system. It is also known that sulfoxide formation in penicillin stabilizes the β-lactam bond toward acidic reagents.



minor component support the assignment of a β-lactam in both materials by groups of bands at δ 5.4–6.0. The 3-proton peaks at δ 1.46 and 1.56 are evidence of a single methyl group attached to a saturated quaternary carbon in each compound. The singlets at δ 4.72 and 4.84 must arise from methine hydrogens attached to carbon adjacent to quaternary centers. A sharp, 3-proton peak ascribable to the methyl of an acetate is found in the spectra of both compounds. Only structures **4** and **5** are consistent with the above data. Structure **4** is assigned to the major component on the basis of the chemical shift of the methylene group (AB pattern, δ 3.90 and 4.37, J = 11 Hz), consistent with an acetoxy methyl function, and the fact that signals from β-lactam protons are identical in position and pattern with those in the spectrum of the starting penicillin methyl ester, but are unlike the corresponding peaks in all cephalosporin compounds that have been examined. The broad 2-proton peak at δ 3.47, which is characteristic[9] of cephalosporin derivatives, indicates that the minor component must be compound **5**.

Formation of **4** and **5** from penicillin sulfoxide methyl ester can arise from the intermediate sulfonium ion **3** and subsequent attack of the acetate ion at the primary or tertiary center. Similar reactions subsequently found to occur with simple dimethyl thiachroman-1-oxides provide ample analogy for the overall reaction.[10]

The structure of **5** was firmly established by the formation on mild base treatment of a substance which has lost the elements of acetic acid. Structure **6** can be unequivocally assigned to this compound on the basis of similarity of the nmr and uv spectra (λ_{max} 268 mμ (ε 7600) with those of the corresponding cephalosporin derivatives. In addition, the methyl 3-methyl-7-(2-phenoxyacetamido)-3-cephem-4-carboxylate[11] (**6**) can be obtained more directly from **1** by heating in an inert solvent with a trace of acid and from phenoxyacetamidocephalosporanic acid ester (**7**) by a palladium-catalyzed hydrogenolysis. Isolation of the same bicyclic derivative **6** from either a penicillin or a cephalosporin provided the first direct chemical correlation between these two series of β-lactam antibiotics.

Additional evidence for structure **4**, in addition to the physical chemical data and mechanistic analogy, rests on the fact that the antibacterial activity produced by the major component is destroyed by penicillinase under conditions in which the activities from **5** and other cephalosporin derivatives are not destroyed. The unlikely possibility, however, that the substance is an alternate stereoisomer of **5** cannot be rigorously excluded on the basis of present chemical evidence.

Compounds **4** and **5** account for nearly all the β-lactam-containing products of the acetic anhydride reaction. Compounds **4** and **5**, although not crystalline, are homogeneous by a variety of chromatographic techniques and by physical chemical data and do not in themselves represent diasteromeric mixtures. Thus, the reaction providing these substances must occur in a stereospecific manner. Evidence for the stereochemistry of **4** can be derived from nmr by consideration of chemical-shift differences of the corresponding protons in phenoxymethyl penicillin methyl ester, the stereochemistry of which is known, and its sulfoxide and compound **4** and its sulfoxide (see Table I). There is little reason to suspect that the stereochemistry at C-5 and C-6 has been changed in the rearrangement.

(9) Sometimes found as a discrete AB pattern, see G. F. H. Green, J. E. Page, and S. E. Staniforth, *J. Chem. Soc.*, 1595 (1965).
(10) R. B. Morin, D. O. Spry, and R. A. Mueller, unpublished results.
(11) For the nomenclature, see footnote 4 in R. B. Morin, B. G. Jackson, E. H. Flynn, and R. W. Roeske, *J. Amer. Chem. Soc.*, **84**, 3400 (1962).

Table I. Chemical-Shift Differences[a] between the Sulfide and Sulfoxide

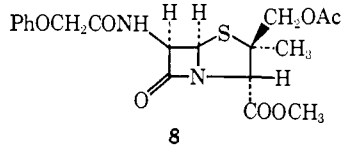
Proton	Phenoxymethyl penicillin	Compound 4
C_2 α	+0.28	+0.23
β	−0.12	−0.45
C_5	+0.53	+0.67
C_6	−0.30	−0.31

[a] In parts per million.

This is substantiated by the close correspondence of the differences indicated in Table I at these carbon atoms in phenoxymethyl penicillin and compound **4**. Furthermore, the sulfoxide oxygen in the two series of compounds has the same stereochemical arrangement relative to the ring system.

In models, the C-6 hydrogen lies above the plane of the five-membered ring, whereas the C-5 hydrogen lies below.

On the assumption that the S=O bond will exert qualitatively the same magnetic influence on hydrogens *cis* to the oxygen relative to the thiazolidine ring and the opposite effect on those *trans*, the data in Table I indicate that the methyl group and C-5 hydrogen in **4** are *cis*. Consequently, the stereochemistry depicted in structure **8** most likely represents that found in the acetoxy penicillin derivative formed in the rearrangement. Published information regarding magnetic anisotropic effects of sulfoxides and sulfides supports this assignment and also indicates that in the sulfoxides of the two penicillins the oxygen is oriented toward the inside of the molecule, *cis* to the phenoxyacetamido side chain.[12] It is most reasonable to explain the stereochemical results by the intramolecular *trans* addition of the sulfenyl anhydride to the double bond[13] in **2**, through the intermediate episulfonium ion **3**, which must have the stereochemistry depicted in **9**. Compound **5** will then have the stereochemistry depicted in structure **10**.



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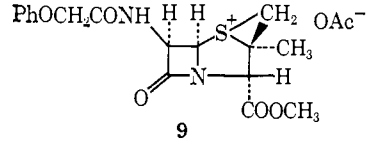

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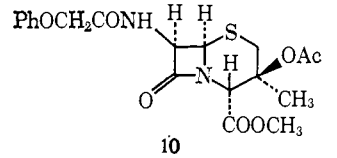

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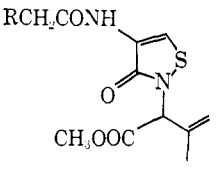
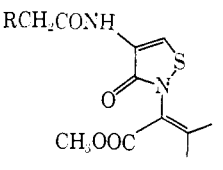
There is no evidence for the formation of the sulfenyl derivative except an analogy to the thermal elimination of sulfoxides to sulfenic acids and olefins.[14] It is conceivable that the sulfur becomes attached to C-2β before the bond to C-2 is broken. We can only speculate whether the carbon of methyl group *cis* to sulfoxide in **1** is the one becoming substituted analogous to the thermal elimination of hydroxylamines from N-oxides.[15] In contrast to these two elimination reactions, the present rearrangement is acid catalyzed. Without the addition of mineral acid or another electrophilic species, rearrangement is not observed. The addition reaction, however, may well be the step that is acid catalyzed.

Three non-β-lactam-containing products of the acetic anhydride reaction of the sulfoxide were isolated as minor components. Examination of the physical data indicated that two of these substances were very closely related to materials independently and simultaneously obtained by Leonard and Wilson from the chlorination of 1,4-thiazepines related to benzylpenicillin (Pen G).[16] Subsequently, the University of Illinois workers have shown that these materials were the isomeric 3-isothiazolones **11a** and **12a**.



11a, b

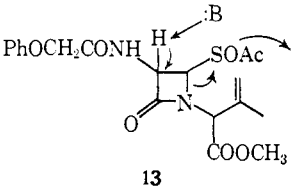


12a, b

a, R = Ph
b, R = PhO

Comparison of nmr, uv, and ir spectra leaves no doubt that the substances obtained in the present work are **11b** and **12b**. As confirmation, a sample of **12a**, identical in all respects with that obtained in the chlorination experiments,[17] was isolated from the acetic anhydride rearrangement of penicillin G sulfoxide methyl ester.[18]

The cyanomethyl ester of phenoxymethyl penicillin sulfoxide underwent rearrangement to an isothiazolone corresponding to **12b** during the course of preparing the ester, presumably due to the presence of triethylamine. Compound **12b** can be obtained most conveniently and in better yield by refluxing sulfoxide **1** in pyridine.



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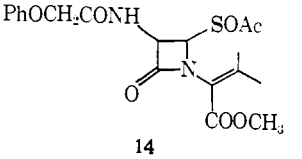

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A plausible mechanism for the formation of **11b** from the sulfenyl derivative **2** is indicated in formula

(12) The effects of the introduction of the sulfoxide oxygen in phenoxymethyl penicillin on the nmr spectra and conformation of the molecule have been examined in great detail; R. D. G. Cooper, P. V. Demarco, J. C. Cheng, and N. D. Jones, *J. Amer. Chem. Soc.*, **91**, 1408 (1969).

(13) A. J. Havlik and N. Kharasch, *ibid.*, **78**, 1207 (1956).

(14) C. A. Kingsbury and D. J. Cram, *ibid.*, **82**, 1810 (1960).

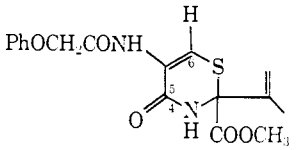
(15) A. C. Cope and E. M. Acton, *ibid.*, **80**, 355 (1958).

(16) N. J. Leonard and G. E. Wilson, Jr., *ibid.*, **86**, 5307 (1964).

(17) We are indebted to Dr. N. J. Leonard for a sample of **12a** and for many valuable discussions of this problem.

(18) The fact that products analogous to **4**, **5**, and **6** were also obtained in this reaction indicates that penicillins other than phenoxymethyl penicillin can be used in the sulfoxide rearrangement, including, interestingly, those that are more acid labile. R. B. Morin, unpublished results.

13. Compound **12b** could arise analogously from **14** or from **11b** by double bond migration. The isomerization of **11a** to **12a** can be effected by the action of triethylamine at room temperature.[16] In the reaction with pyridine, the solvent probably facilitates removal of the C-3 and C-6 hydrogen atoms.



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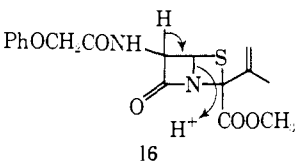

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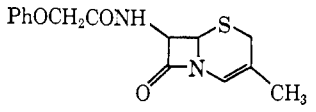
The third minor product was assigned structure **15** on the basis of physical data. The ir peak at 1750 cm^{-1} is indicative that the carbomethoxy group is attached to a saturated carbon. The maximum in the uv spectrum is at 320 mμ (ε 5900), compared with 295 mμ (ε 12,000) for compounds **11a**, **11b**, and **12a**, **12b**. The nmr spectrum affirmed the presence of the phenoxyacetyl side chain (complex pattern at δ 7.2 and 2-proton singlet at δ 4.45), the ester methyl (3-proton singlet at δ 3.88) and NH (proton singlet at δ 9.25), the other NH masked by signals from aromatic hydrogens. The compound contains one C-methyl group with a proton signal at δ 1.92, weakly coupled to the vinylic protons at δ 5.28 and 5.36. The very low-field peak at δ 8.02 can be ascribed to H-6 proton on the dihydrothiazine ring. The corresponding signal in the spectrum of compound **12b** is found at δ 8.70. Although there is no direct analogy, the uv spectrum is not unexpected for a compound with this structure. Compound **15** can be formed from **14** through the intermediacy of **16**.

In the formation of the non-β-lactam products, **11**, **12**, and **15**, the C_5–nitrogen bond cleavage may precede that of the sulfur–C_2 bond. The fact that compound **15** is optically inactive supports this.

Yields of the various products of the acetic anhydride rearrangement are quite dependent upon the exact conditions of the experiment, and rather subtle changes greatly affect the relative ratio of products. The reactions are not unique to the penicillin molecules, as simpler cyclic sulfoxides react analogously.[10] Furthermore, the reactions are formally similar to the oxidative rearrangement of cyclic sulfides with chlorine.[19]

The reactions described in this paper have provided a procedure for obtaining compounds containing the cephalosporin ring system from penicillin. The remaining problem in the synthesis of cephalosporin C derivatives from penicillins is the introduction of the acetoxyl function. Efforts to rearrange the sulfoxide of **4** to obtain **7** have been unsuccessful. If the initial step of the rearrangement is indeed stereospecifically *cis*, this failure might be due to the fact that the oxygen of the sulfoxide of **4** is probably *trans* to the methyl group. Neither the sulfoxide of **4** nor of penicillin methyl ester having the alternate sulfoxide configuration could be prepared. Attempts to introduce a substituent on the methyl carbon in **6** by various allylic oxidative procedures also have been unsuccessful.

In order to determine if the new β-lactam-containing materials produced in this work have antibiotic properties, it became necessary to obtain the products as free acids since most esters of penicillins and cephalosporins exhibit very low *in vitro* antibacterial activity. The only product which could be isolated and characterized from the acetic anhydride and acid-catalyzed rearrangement of penicillin sulfoxide free acids was 3-methyl-7-(2-phenoxyacetamido)-3-cephem (**17**). The infrared spectrum possesses a characteristic peak at 1770 cm^{-1} for the β-lactam.



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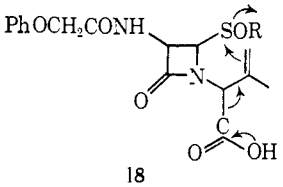
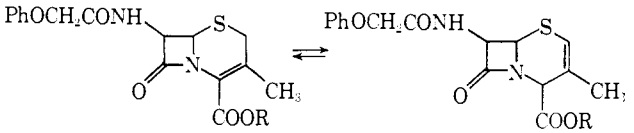
The nmr spectrum contained, in addition to signals that can be unambigously assigned to the side chain and β-lactam protons, peaks at δ 1.78 (C_3-methyl), 3.45, 2.98 (AB pattern, J = 18 Hz, methylene group at C_2), and 6.48 (olefinic proton at C_4).

Interestingly, the uv spectrum is very similar to the corresponding cephalosporin derivative **7** which has a carboxyl group in the 4 position. The sulfoxide rearrangement could not be effected under conditions which did not cause extensive decarboxylation. Compounds **19c** and **20b** were not intermediates in the decarboxylation because they were essentially unaffected by the conditions necessary for rearrangement. The concerted decarboxylative addition reaction depicted in formula **18** is a mechanistic possibility.



18

In our continuing efforts to obtain free acids, compound **6** was subjected to mild alkaline hydrolysis under conditions that had been used successfully in the penicillins.[2] The product formed in excellent yield was not the desired substance, but was the acid **20b**. The structural assignments of the Δ^2-cephalosporin isomers are based on physical data which have recently been discussed by the Glaxo workers.[20] In base a 70:30 equilibrium mixture of the Δ^3 and Δ^2 esters, **19a** and **20a**, is quickly established. The β, γ-unsaturated ester **20a** would be expected to hydrolyze more rapidly, accounting for the isolation of only the Δ^2 acid. Under the same conditions the equilibrium in the corresponding cephalosporin compounds **21a** and **22b** is 70:30 in favor of Δ^2 isomer. The position of equilibrium probably reflects the difference in bulk of the substituent in the 3 position.

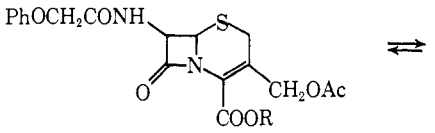


19a, R = CH_3
b, R = $CH(C_6H_5)_2$
c, R = H

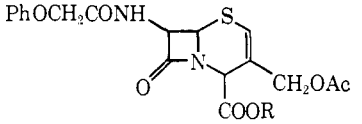
20a, R = CH_3
b, R = H

(19) G. E. Wilson, Jr., *J. Amer. Chem. Soc.*, 87, 3785 (1965).

(20) J. D. Cocker, S. Eardley, G. I. Gregory, M. E. Hall, and A. G. Long, *J. Chem. Soc., C*, 1142 (1966).



21a, R = CH_3
b, R = H



22a, R = CH_3
b, R = H

Mild hydrolysis of **4** and **5** likewise failed to provide the respective acids. The acid **20b** was obtained from **5**.

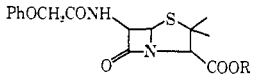
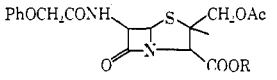
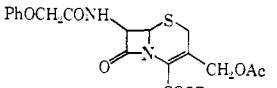
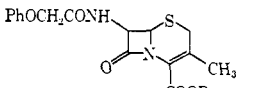
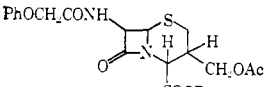
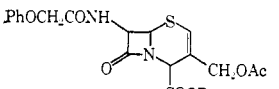
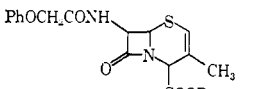
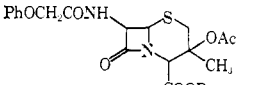
Acid **19c** was ultimately obtained from the penicillin by utilizing an ester protecting group which could be removed reductively. The acid-catalyzed rearrangement of phenoxymethyl penicillin sulfoxide benzhydryl ester gave **19b** which was cleaved catalytically in poor yield to **19c**. An inseparable mixture of benzhydryl esters corresponding to **4** and **5** resulted from acetic anhydride rearrangement of the sulfoxide. Hydrogenolysis of the mixture failed. The acid **19c** has also been obtained by hydrogenolysis of **21b** (as the salt) and by acylation of 7-ADCA.[21,22]

Compounds **4** and **5** did undergo a limited hydrolysis in pH 7 buffer to acids as shown by paper electrophoresis. Products were detected by antibacterial activity; with the penam derivative an antibacterial zone size was produced comparable to that from phenoxymethyl penicillin methyl ester treated analogously.[23]

Activities against a strain of Gram-positive bacteria shown by the various compounds prepared in this work are recorded in Table II. With the invariance of the amide side chain the effects of certain modifications of the nitrogen–sulfur ring on antibacterial activity can be seen. It has been proposed that penicillin acts antibacterially by irreversibly acylating and thereby inactivating a transpeptidase enzyme necessary for cross-linking in the formation of the bacterial cell wall.[24] If we can use the infrared frequency of the β-lactam as an indicator of acylating power (the higher the frequency the better the acylating agent), the data in Table II suggest a rough but positive correlation between acylation ability and biological activity. The correlation of ir frequencies with acylating ability is supported by the fact that excess hydroxylamine, which completely destroys the β-lactam rings and forms hydroxamic acids in the penicillin and cephalosporin C derivatives, leaves the dihydrocephalosporin unaffected and only partially opens the ring in the Δ^2 isomer.[20]

A strained β-lactam, as indicated by high ir frequencies, need not be reactive, notable exceptions being

Table II

Compound	β-Lactam frequency,[a] cm^{-1}	Bioassay[b]
	1790	1800
	1795	High
	1792	300
	1785	25
	1776	4
	1784	6
	1780	15
	1780	Low

[a] Determined in $CHCl_3$ solution on the methyl esters (R = CH_3). [b] Assay on the salts in Oxford units against a penicillin G sensitive *Staphylococcus aureus* strain.

penicillin sulfoxides and anhydropenicillins. It would appear that one necessary condition for good antibacterial activity in this class of antibiotics is a β-lactam reactive to nucleophiles.[25]

Experimental Section

Preparation of Phenoxymethyl Penicillin Sulfoxide.[26] Sodium metaperiodate (8 g, 0.375 mol) was added in one portion with stirring to a solution of 15.5 g of phenoxymethyl penicillin potassium salt in 300 ml of water. After 45 min at room temperature the starch–iodide test became negative, and the solution was diluted with 100 ml of water. The pH of the solution was lowered to 2.3 with dilute HCl. The precipitated product was collected and crystallized from MeOH (200 ml) and H_2O (100 ml) to give 12.3 g, mp 163–4°.

Preparation of Methyl Ester of Phenoxymethyl Penicillin Sulfoxide (1). Three grams of the above acid was suspended in 30 ml of ethyl acetate. Diazomethane in ether was added to the suspension with stirring until the yellow color persisted. The solution was then evaporated to dryness; the residue crystallized from MeOH–H_2O, giving 2.5 g, mp 120–122°. Analytical sample melted at 121.5–122.5°, $[\alpha]D$ + 200°; ir ($CHCl_3$) 1805, 1755, and

(21) E. Van Heyningen and L. K. Ahern, *J. Med. Chem.*, **11**, 933 (1968).
(22) R. J. Stedman, K. Swered, and J. R. E. Hoover, *ibid.*, **7**, 117 (1964).
(23) We are indebted to Dr. E. B. Herr, Jr., for these experiments.
(24) J. L. Strominger in "Antibiotics, Mechanism of Action," Vol. I, D. Gottlieb and P. D. Shaw, Ed., Springer-Verlag, New York, N. Y., 1967.
(25) The effect of substituents at C-3 in cephalosporins on the reactivity of the β-lactam have been calculated from molecular orbital theory. A correlation has been found between both bond strengths of the carbon–nitrogen bond and electron density on the carbonyl carbon and the biological activities, using a different series of compounds and bacterial strains: R. B. Herrmann, personal communication.
(26) We are indebted to Dr. E. H. Flynn of these laboratories for this procedure; *cf.* A. W. Chow, N. M. Hall, and J. R. E. Hoover, *J. Org. Chem.*, **27**, 1381 (1962).

1695 cm^{-1}; nmr δ 1.22 (s, 3), 1.72 (s, 3), 3.82 (s, 3), 4.54 (s, 2), 4.70 (s, 1), 5.07 (d, 1, J = 5 Hz), 6.10 (q, 1, J = 5 and 10 Hz), 7.1 (m, 5), 8.25 (d, 1, J = 10 Hz, NH).

Anal. Calcd for $C_{17}H_{20}N_2O_5S$: C, 53.67; H, 5.29; N, 7.36. Found: C, 53.79; H, 5.32; N, 7.44.

Reaction of Phenoxymethyl Penicillin Sulfoxide Methyl Ester with Ac_2O. A solution of 500 mg of phenoxymethyl penicillin sulfoxide methyl ester in 35 ml of Ac_2O was heated at reflux for 0.5 hr and then evaporated to dryness *in vacuo*. The oily residue was taken up in ethyl acetate, and the solution was washed successively with dilute cold $NaHCO_3$, H_2O, and saturated NaCl solution. The amorphous product (530 mg), obtained after drying (Na_2SO_4) and evaporation, was chromatographed over silicic acid column using a mixture of cyclohexane and methyl isopropyl ketone (4:1) saturated with H_2O as the eluting solvent. Fractions containing 7 ml were collected at 20-min intervals. Fractions 75–88, 195 mg, contained the penam derivative (**4**). This material was rechromatographed for analysis. The product was still an amorphous solid: $[\alpha]D$ + 145.3°; ir at 1795, 1748, 1730, and 1695 cm^{-1}; nmr δ 1.46 (s, 3), 2.05 (s, 3), AB pattern 3.90 and 4.37 (m, 2, J = 11 Hz), 4.57 (s, 2), 4.72 (s, 1), 5.77 (m, 2), 7.2 ppm (m, 6).

Anal. Calcd for $C_{19}H_{22}N_2O_7S$: C, 54.01; H, 5.25; N, 6.63; S, 7.59; acetyl, 10.18; methoxyl, 7.35. Found: C, 53.55; H, 5.47; N, 6.26; S, 7.18; acetyl, 10.41; methoxyl, 6.93.

Fractions 110–115, 15 mg, contained primarily the cepham derivative.

Fractions 89–105, 130 mg, were a mixture of the two products; this material on rechromatography yielded 50 mg of cepham compound. Both this and the 15-mg sample were amorphous solids: ir 1780, 1750, and 1698 cm^{-1}; nmr δ 1.56 (s, 3), 2.00 (s, 3), 3.47 (br s, 2), 3.80 (s, 3), 4.60 (s, 2), 4.84 (s, 1), 5.40 (d, 1, J = 5 Hz), 5.72 (q, 1, J = 5 and 10 Hz), and 7.05 ppm (m, 6).

Anal. Calcd for $C_{19}H_{22}N_2O_7S$: C, 54.01; H, 5.25. Found: C, 54.31; H, 5.71.

In another experiment a solution of 5.032 g of phenoxymethyl penicillin sulfoxide methyl ester and 1.15 g of sodium acetate in 40 ml of Ac_2O was heated at reflux temperature for 10 min, at which time the color was dark brown. The residue, after the removal of the anhydride *in vacuo*, was dissolved in a mixture of ethyl acetate and H_2O. The ethyl acetate solution was washed with dilute $NaHCO_3$, H_2O, and NaCl. The amorphous product (5.1 g), obtained after drying and evaporation of solvent, was chromatographed over 100 g of silica gel (E. Merck) using methyl ethyl ketone–cyclohexane (1:4) as eluent. Fractions (20 ml) were collected. Fraction 80 constituted a pure sample of the dihydrothiazone derivative, **15**; fraction 130, the isothiazolone, **12b**; fractions 165–200, the penam derivative, **4**; the later fractions, a mixture of **4** and **5**.

Compound **15** (fraction 80) was crystallized from CH_2Cl_2–*n*-hexane: mp 174–175°; uv (EtOH) λ_{max} 32.3 mμ (ϵ 5900); ir ($CHCl_3$) 1750, 1680 (sh), and 1660 cm^{-1}; nmr δ 1.92 (s, 3), 3.88 (s, 3), 4.45 (s, 2), 5.28 (br s, 1), 5.36 (s, 1), 7.1 (m, 6, ArH and NH), 8.05 (s, 1), and 9.25 ppm (br s, 1).

Anal. Calcd for $C_{17}H_{18}N_2O_5S$: C, 56.34; H, 5.00; N, 7.73; S, 8.85. Found: C, 56.26; H, 5.41; N, 7.76; S, 8.85.

Compound **12b** was also crystallized from CH_2Cl_2–*n*-hexane: mp 147–148°; uv (EtOH) λ_{max} 294 mμ (ϵ 12,300); ir ($CHCl_3$) 1725, 1695, and 1655 cm^{-1}; nmr δ 1.92 (s, 3), 2.42 (s, 3), 3.72 (s, 3), 4.65 (2 H, singlet), 7.2 (m, 5, ArH), 8.70 (s, 1), and 8.96 ppm (br s, 1, NH).

Anal. Calcd for $C_{17}H_{18}N_2O_5S$: C, 56.34; H, 5.00; N, 7.73; S, 8.85. Found: C, 56.29; H, 5.30; N, 7.93; S, 9.23.

In a similar experiment, but without the addition of sodium acetate, 200 mg of a different non-β-lactam-containing substance, the isothiazolone **11b**, was isolated. This substance was recrystallized from methyl ethyl ketone–*n*-hexane: mp 143–145°; $[\alpha]D$ −17.5°; uv (EtOH) λ_{max} 296 mμ (ϵ 1000); ir ($CHCl_3$) 1750, 1695, and 1650 cm^{-1}; nmr δ 1.86 (br s, 3), 3.78 (s, 3), 4.63 (s, 2), 5.12, 5.27, 5.66 (br s, 3), 7.2 (m, 5, ArH), 8.72 (s, 1), and 8.98 ppm (br s, 1, NH).

Treatment of Phenoxymethyl Penicillin Sulfoxide Methyl Ester with Refluxing Pyridine. The sulfoxide ester (3.66 g) was heated at reflux temperature in 10 ml of pyridine for 0.5 hr. Solvent was removed, and the residue was dissolved in ethyl acetate. The product (3.6 g) obtained after washing, drying, and evaporation was chromatographed over 60 g of silica using 10% ethyl acetate in C_6H_6 (fractions 1–170) and 25% ethyl acetate in C_6H_6 (fractions 171–300) as eluents, collecting 25-ml fractions. Fractions 14–27 provided 0.116 g of **15**; fractions 37–150 gave 1.56 g of pure **12b**. The intermediate fractions were mixtures of the two compounds; later fractions, 151–300, contained mostly starting sulfoxide ester.

Preparation of the Phenylacetyl Derivative 12a. A solution of 1.369 g of penicillin G sulfoxide methyl ester, mp 126–127°, and 0.5 g of sodium acetate in 30 ml of Ac_2O was heated at reflux for 30 min. The dark residue obtained after evaporation of the solvent *in vacuo* was dissolved in ethyl acetate, and the solution was washed and dried. The crude product was chromatographed on 30 g of silica gel (E. Merck). The column was developed with 2% Et_2O–$CHCl_3$; the eluent was collected in 15-ml fractions. From fraction 14 (58 mg) could be obtained, after three recrystallizations from CH_2Cl_2–petroleum ether (bp 30–60°), 13 mg of the isothiazolone **12a**, mp 230.1°, identical by uv and ir with a sample provided by Dr. N. J. Leonard.

Preparation of the Sulfoxide of Acetoxy-Penam Derivative 4. A solution of 136 mg of **4** and 73 mg of periodic acid in Me_2CO–H_2O was allowed to stand at room temperature for 20 hr. After removal of the solvents, the residue was dissolved in ethyl acetate; the solution was washed with $NaHCO_3$, H_2O, and saturated NaCl. The crude product, 96 mg, obtained after drying and evaporation, was chromatographed on 3 g of silica with 10% ethyl acetate in $CHCl_3$ as eluent. The sulfoxide product, 40 mg, was isolated as an amorphous solid: ir 1810, 1750, and 1695 cm^{-1}; nmr δ 1.23 (s, 3), 2.08 (s, 3), 3.79 (s, 3), 4.65 (m, 5), 5.08 (d, 1, J = 4.5 Hz), 6.17 (q, 1, J = 4.5 and 11 Hz), 7.2 (m, 5), 8.2 (d, 1, J = 11 Hz, NH).

Anal. Calcd for $C_{19}H_{22}N_2O_8S$: C, 52.05; H, 5.06. Found: C, 51.77; H, 4.82.

Preparation of Δ^3-Cephem Derivative 6. A solution of 5.0 g of phenoxymethyl penicillin sulfoxide methyl ester and 160 mg of *p*-toluenesulfonic acid in 60 ml of xylene was heated at reflux temperature for 1 hr and then evaporated *in vacuo* to dryness. An ethyl acetate solution of the residue was washed with dilute $NaHCO_3$ and H_2O, dried, and evaporated to yield 4.5 g of dark oil. This was chromatographed on 80 g of silicic acid with a mixture of $CHCl_3$ and hexane (1:1) as eluting solvent. Fractions 115–225, 1.23 g, were combined, and compound **6** was crystallized from MeOH–ether, 306 mg, mp 137–138°. A second crop was obtained, 174 mg, mp 128–36°. Analytical sample melted at 141–142°: $[\alpha]D$ +94°; uv λ_{max} 368 mμ (ϵ 7600); ir 1785, 1721, 1689, and 1637 cm^{-1}; nmr δ 2.16 (s, 3), 3.27 (d, 1, J = 18 Hz), 3.56 (d, 1, J = 18 Hz), 3.88 (s, 3), 4.58 (s, 2), 5.05 (d, 1, J = 4.5 Hz), 5.86 (q, 1, J = 4.5 and 9 Hz), and 7.2 (m, 6).

Hydrolysis of Δ^3-Cephem Derivative 6 to the Δ^2 Acid 20b. The Δ^3-methyl ester (276 mg, 0.76 mmol) was dissolved in 10 ml of pyridine and 15 ml of H_2O, and the solution was cooled in an ice bath. An equivalent (7.6 ml) of 0.100 *N* NaOH was added in one portion, and the solution was stirred in the cold for 3 hr. After evaporation and dissolution in water, the solution was layered with ethyl acetate, and the pH lowered quickly in the cold to 2.0. After the layers separated the organic phase was washed with H_2O and NaCl, dried, and evaporated. The crystalline product, approximately 300 mg, was recrystallized twice from $CHCl_3$–petroleum ether, yielding 83 mg: mp 172–173.5° dec (182–184° dec in preheated block); $[\alpha]D$ +505°; ir (mull) 1764, 1743, and 1672 cm^{-1}.

Anal. Calcd for $C_{16}H_{16}N_2O_5S$: C, 55.17; H, 4.63; N, 8.04; S, 9.19. Found: C, 55.02; H, 4.52; N, 7.96; S, 9.21.

The methyl ester was prepared in a similar manner to that of methyl ester of phenoxymethyl penicillin sulfoxide. The product was recrystallized from CH_2Cl_2–Et_2O: mp 109–110°; ir 1780 (S), 1750, and 1695 cm^{-1}; nmr δ 1.95 (s, 3), 3.85 (s, 3), 4.58 (S, 2), 4.83 (br s, 1), 5.31 (d, 1, J = 4 Hz), 5.76 (q, 1, J = 4 and 9 Hz), 5.98 (br s, 1), and 7.2 (m, 6).

Anal. Calcd for $C_{17}H_{18}N_2O_5S$: C, 56.34; H, 5.00; N, 7.73; S, 8.85. Found: C, 56.13; H, 5.12; N, 7.60; S, 8.68.

Preparation of 3-Methyl-7-(2-phenoxyacetamido)-3-cephem (17). Phenoxymethyl penicillin sulfoxide acid (10 g) was heated in tetrachloroethane (100 ml) at reflux temperature for 5 min. The solvent was removed *in vacuo*. The product was partitioned into acidic and neutral materials. The neutral material was recrystallized from $CHCl_3$–petroleum ether, yielding 800 mg of the decarboxylated cephalosporin derivative **17**: mp 173.5–174.5°; $[\alpha]D$ −35.3°; uv λ_{max} 256 mμ (ϵ 9150); ir 1770, 1692, and 1658 (sh) cm^{-1}; nmr δ 1.78 (s, 3), AB pattern, 3.45, 2.98 (m, 2, J = 18 Hz), 4.52 (s, 2), 4.97 (d, 1, J = 4.5 Hz), 5.77 (d, 1, J = 4.5 and 9.5 Hz), 6.48 (s, 1), and 7.2 (m, 6).

Anal. Calcd for $C_{15}H_{16}N_2O_3S$: C, 59.19, H, 5.29; N, 9.20; S, 10.53. Found: C, 59.43; H, 5.44; N, 9.20; S, 10.70.

The acidic material was converted to methyl ester and chromatographed. Phenoxyacetic acid methyl ester, compounds **6** and **1**,

were isolated. The yield of **6** from the starting sulfoxide was approximately 2%.

Compound **17** could also be obtained by heating phenoxymethyl penicillin sulfoxide in a variety of solvents (or reagents) including xylene Ac_2O, and pyridine.

Preparation of Phenoxymethyl Penicillin Sulfoxide Benzhydryl Ester. The acid (15 g) was suspended in 200 ml of ethyl acetate; excess diphenyldiazomethane in ether was added with stirring. After 20 min a clear red solution resulted. After standing overnight at room temperature, the solution was evaporated *in vacuo* to a red gum. This crystallized from Me_2CO–petroleum ether to give 17.4 g, mp 155–156°. Some preparations of this material melted at 93–94°; $[\alpha]D$ +191°.

Anal. Calcd for $C_{29}H_{28}N_2O_6S$: C, 65.39; H, 5.30; N, 5.26. Found: C, 65.61; H, 5.29; N, 4.92.

Preparation of Δ^3-Cephem Benzhydryl Ester (19b). The sulfoxide benzhydryl ester (5 g) and 140 mg of dry *p*-toluenesulfonic acid were suspended in 100 ml of xylene. The solution was refluxed for 30 min, then evaporated and worked up to give 4.7 g of crude product. Chromatography over a silicic acid column with cyclohexane methyl isobutyl ketone (4:1) as eluting solvent yielded 422 mg of product (recrystallized from methanol): mp 156–157°; $[\alpha]D$ +30.3°.

Anal. Calcd for $C_{29}H_{28}N_2O_5S$: C, 67.88; H, 5.09; N, 5.44; S, 6.23. Found: C, 67.63; H, 5.20; N, 5.26; S, 6.30.

Treatment of the benzhydryl ester with Ac_2O under similar conditions used for the methyl ester gave an inseparable mixture of the benzhydryl esters corresponding to **4** and **5**. Catalytic hydrogenolysis of the mixture failed.

Preparation of the Acid 19c by Hydrogenolysis of 19b. The above ester (100 mg) was dissolved in dioxane (6 ml) to which a trace of dry HCl had been added. This solution was added to a suspension of 200 mg of prereduced 10% Pd–C in dioxane. The reduction proceeded at room temperature. The product, after filtration and evaporation, was separated into neutral and acidic fractions. The neutral product, 40 mg, was identical with the starting ester. The acidic material, 16 mg, was the Δ^3-cephem acid, mp 185–187°, pK_a = 5.7 in 66% DMF. The structure was confirmed by nmr signals at δ 2.20 (s, 3), AB pattern 3.27, 3.54 (m, 2, J = 18 Hz), 4.60 (s, 2), 5.07 (d, 1, J = 5 Hz), 5.86 (q, 1, J = 5 and 10 Hz), 6.47 (m, 2, NH and COOH), and 7.1 (M, 5).

Anal. Calcd for $C_{16}H_{16}N_2O_5S$: C, 55.17; H, 4.63; N, 8.04. Found: C, 55.32; H, 4.93; N, 7.63.

Preparation of Methyl 7-Phenoxyacetamidocephalosporanate (7). Phenoxyacetyl chloride (17.0 g) was added to a cooled solution of 27.3 g of 7-ACA and 20.0 g of $NaHCO_3$ in 200 ml of H_2O and 100 ml of Me_2CO. After 2 hr the solution was concentrated, layered with ethyl acetate, and the pH lowered to 2.0 with stirring. The ethyl acetate solution was washed with H_2O, and the product was titrated from this solution into H_2O by addition of dilute KOH. The salt, after evaporation, was recrystallized from MeOH, 24.49 g, $[\alpha]D$ +106°.

Anal. Calcd for $C_{18}H_{17}N_2O_4SK$: C, 48.63; H, 3.8; N, 6.30;5 S, 7.21. Found: C, 48.79; H, 3.91; N, 6.27; S, 7.04.

The methyl ester was prepared by adding excess CH_2N_2 in ether to an ethyl acetate solution of the acid, obtained by titrating the above salt with dilute HCl in a mixture of H_2O–ethyl acetate. The product was recrystallized from MeOH, mp 149–150°, $[\alpha]D$ +53°; ir 1792, 1750, and 1692 cm^{-1}; nmr δ 2.05 (s, 3), AB pattern 3.37, 3.53 (m, 2, J = 18 Hz), 3.83 (s, 3), 4.52 (s, 2), AB pattern, 4.87, 5.07 (m, 2, J = 14 Hz), 5.04 (d, 1, J = 4.5 Hz), 5.92 (*q*, 1, J = 4.5 and 9 Hz), and 7.1 (m, 6 H).

Anal. Calcd for $C_{19}H_{20}N_2O_7S$: C, 54.27; H, 4.79; N, 6.66. Found: C, 54.56; H, 4.91; N, 6.48.

Isomerization of the Double Bond in Methyl 7-Phenoxyacetamidocephalosporanate (7). **7** (3 g) was allowed to stand in a pyridine–H_2O (1:1) solution for 18 hr at room temperature. After removal of solvent the residue was separated into neutral (2.29 g) and acidic (0.48 g) fractions. The latter constituted the Δ^2 acid **21b** which melted at 181.5–182.5° after several crystallizations from ethyl acetate–H_2O.

Anal. Calcd for $C_{18}H_{18}N_2O_7S$: C, 53.19; H, 4.46; N, 6.89; S, 7.89. Found: C, 53.12; H, 4.64; N, 6.68; S, 7.57.

The neutral material was a 70:30 mixture of Δ^2 and Δ^3 esters by nmr analysis. These could be separated on a silica column using 10% ethyl acetate in benzene as eluent. The Δ^2-methyl ester (**22**) crystallized from CH_2Cl_2–petroleum ether, mp 137.5–138.5°.

Anal. Calcd for $C_{19}H_{20}N_2O_7S$: C, 54.27; H, 4.79; N, 6.66; S, 7.63. Found: C, 54.12; H, 5.01; N, 6.39; S, 7.51.

Catalytic Reduction of Methyl 7-Phenoxyacetamidocephalosporanate (7). The methyl ester of 7-phenoxyacetamidocephalosporanic acid (549 mg) was reduced with 2.0 g of 10% Pd–C at 1200 psi of hydrogen in dioxane solution (45 ml). The product, after filtration and evaporation, was chromatographed on 10 g of silica gel G (E. Merck) using 5% ethyl acetate in $CHCl_3$ as elution solvent. Fractions 5–6, 97 mg, were a mixture of desired product (**6**) and starting material. Rechromatography using 2% ether in $CHCl_3$ as elution solvent gave, after recrystallization, 11 mg of product identical in every way (ir, uv, melting point, nmr, X-ray) with 6 prepared by the sulfoxide rearrangement.

Fractions 7–9, 275 mg, of the original chromatogram contained pure starting ester. Later fractions 11–14 yielded 50 mg of a derivative of the starting ester in which the double bond has been reduced. Crystallization from $CHCl_3$–petroleum ether gave material melting at 153–153.5°, $[\alpha]D$ +129°; ir 1776, 1740, and 1690 cm^{-1}; nmr δ 2.03 (s, 3), 2.8 (m, 3), 3.85 (s, 3), 4.37 (d, 2, J = 7 Hz), 4.60 (s, 2), 4.71 (br s, 1), 5.25 (d, 1, J = 4.5 Hz), 5.70 (q, 1, J = 4.5 and 9 Hz), and 7.2 (m, 6 H).

Anal. Calcd for $C_{19}H_{22}N_2O_7S$: C, 54.01; H, 5.24; N, 6.63; S, 7.59. Found: C, 53.72; H, 5.43; N, 6.34; S, 7.66.

The potassium salt (5 g) in aqueous solution was reduced with hydrogen under high pressure over Pt at 60°. The crude product (2.45 g), isolated as the acid, was chromatographed on 150 g of silica using Et_2O–AcOH–H_2O (20:2:1) as eluent, collecting 20-ml fractions. Fractions 80–98 provided 450 mg of the dihydro acid, mp 227–229°; a sample of this was converted to the above ester for further characterization. Earlier fractions contained the acid **19c**.

Reduction of 250 mg of the Δ^2 isomer of methyl phenoxyacetamidocephalosporanate with hydrogen over a Pt catalyst at 60° under low pressure conditions in dioxane–H_2O gave, after chromatography, 22 mg of the same dihydro ester. The isolation of the Δ^3 isomer in addition to starting material restricts the interpretations that can be made regarding this experiment.

Biological Activities of Acetoxy Derivatives and Compounds 4, 5, and 6. Equal samples of **4**, **5**, and **6** were incubated at pH 7 in phosphate buffer for 20 hr at 37° with shaking. At the end of the incubation, samples were plated *vs.* various microorganisms; they were also examined by paper electrophoresis using bioautography for detection. Solutions from **4** and **5** showed activity against *S. aureus* and *B. subtilis* but not against Gram-negative organisms. The zone size produced by **5** was approximately one-half that produced by **4**. On paper electrophoresis, acid antibiotics were seen which had the same mobility as phenoxymethyl penicillin. The zone size produced by **4** on electrophoresis was comparable to that produced by phenoxymethyl penicillin methyl ester treated in an analogous manner. The antibacterial activity produced from both **4** and phenoxymethyl penicillin ester was destroyed by penicillinase. Under these conditions **6** was inactive. Incubation of **6** at high levels in pH 9 phosphate buffer, however, did give penicillinase-insensitive antibacterial activity against *S. aureus* and *B. subtilis*.

In a separate experiment, hydrolysis of **5** under conditions employed earlier with **6** gave a mixture of acids, the major component of which was **20b**. A neutral material recovered in 30% yield was compound **6**.

Acknowledgment. We are grateful to the members of the Molecular Structure Research group for physical chemical measurements and for microanalyses. We also wish to acknowledge the many helpful discussions with our colleagues and consultants and, in particular, Dr. C. C. Price for his strong encouragement in the initial phases of this work.