Anal. Calcd for $C_{19}H_{19}N_3O_5S \cdot 0.5C_6H_6$: C, 60.00; H, 5.03; N, 9.54; S, 7.28. Found: C, 60.22; H, 4.92; N, 9.33; S, 7.20.

B. From the Aldehyde 6.—A solution of 0.51 g (1.0 mmol) of **6**, 0.13 g (1.0 mmol) of sodium thiophenoxide,[14] and 1.0 ml of *N,N*-dimethylformamide was stirred for 40 min. Water (25 ml) was added and the resulting mixture was extracted with 3 × 10 ml of methylene chloride. The methylene chloride solution was washed with 2 × 7 ml of water and dried. The solvent was evaporated and residual oil was chromatographed on a silicic acid (100 mesh) column with ethyl acetate. The effluent was separated into a homogeneous fraction which crystallized from a mixture of benzene and petroleum ether after a seed of the phenylthioazetidinone **9** was added, 87.6 mg (19.9%), tlc R_f was identical with that of **9** in ethyl acetate.

C. From the *trans*-Phenylsulfonylazetidinone 10.—A stirred solution of 0.284 g (0.656 mmol) of *trans*-phenylsulfonylazetidinone **10** (see below for preparation), 0.067 ml (0.072 g, 0.66 mmol) of benzenethiol, 20 ml of ethanol, and 10 ml of water was prepared under nitrogen. The pH was held at 9.0 (1 *N* sodium hydroxide) for 40 min. Water (65 ml) was added and the mixture was extracted with 3 × 40 ml of methylene chloride. The organic solution was washed with 2 × 25 ml of water, dried, and evaporated under reduced pressure. The residue (85.3 mg) contained two components according to thin layer chromatography (benzene–ethyl acetate, 1:1). Only one of these was obtained pure by chromatography on a 2-mm thick silica gel coated plate (Brinkmann Instruments) (same solvent as above). Crystallization from benzene gave 8.9 mg, mp 76.0–78.5°; tlc R_f was the same as that of the phenylthioazetidinone **9**.

(14) Prepared from benzenethiol and sodium methoxide in anhydrous methanol. Addition of ether caused separation of the product.

***trans*-3-[2-Methyl-2-(*o*-nitrophenoxy)propionamido]-4-phenylsulfonyl-2-azetidinone (10).**—A solution of 0.88 g (5.6 mmol) of potassium permanganate in 8.4 ml of water was added dropwise over a 10-min period to a solution of 1.23 g (2.8 mmol) of phenylthioazetidinone **9** in 20 ml of 80% acetic acid. The resultant mixture was stirred for 45 min, and 30% hydrogen peroxide was added until all color was discharged. Water (100 ml) was added and the mixture was extracted with three portions (80, 40, 40 ml) of methylene chloride. The organic solution was washed with 2 × 20 ml of water and dried. The methylene chloride was removed and the residual oil was slowly crystallized from benzene. This was recrystallized from benzene–ethanol (4:1) to yield the pure trans isomer, **10**: 0.33 g; mp 158.0–159.0°; $[\alpha]^{25}D$ −32.5° (*c* 1, $CHCl_3$); tlc R_f 0.59 (benzene–ethyl acetate, 1:1); ir (CH_2Cl_2) 3395 (NH), 1815 (β-lactam), 1695 (amide I), 1525 (amide II), 1150 and 1335 (nitro), and 1300 cm^{-1} (sulfone); nmr (acetone-d_6) δ 6.9–8.7 (m, 11, aromatic and amide), 5.0–5.4 (dd overlapped by d, 2, J_1 of dd = 2 Hz, H-5 and H-6), 1.52, 1.49 (singlets, 6, methyl).

Anal. Calcd for $C_{19}H_{19}N_3O_7S$: C, 52.65; H, 4.42; N, 9.70; S, 7.37. Found: C, 52.41; H, 4.47; N, 9.56; S, 7.43.

Registry No.—**1**, 10514-63-3; **2**, 37696-07-4; **5**, 37696-08-5; **6b**, 37696-09-6; **6** 2,4-DNPH, 37696-10-9; **7**, 37818-75-0; **8**, 37696-11-0; **9**, 37755-01-4; **10**, 37755-02-5; **12**, 37696-12-1.

Synthesis of 6-Methylthiopenicillins and 7-Heteroatom-Substituted Cephalosporins

WILLIAM A. SLUSARCHYK,* HAROLD E. APPLEGATE, PHILLIP FUNKE, WILLIAM KOSTER, MOHINDAR S. PUAR, MARIAN YOUNG, AND JOSEPH E. DOLFINI

The Squibb Institute for Medical Research, Princeton, New Jersey 08540

Received September 29, 1972

A number of 6α-methylthiopenicillins and 7α-methylthiocephalosporins have been prepared from intermediates obtained by methylthiolation of Schiff bases of 6-aminopenicillanic acid esters and 7-aminocephalosporanic acid esters. Fluorination with perchloryl fluoride gave a 7α-fluorocephalosporin Schiff base that could be solvolyzed to 7α-methoxy- and 7α-methylthiocephalosporin intermediates. The same 7α-methoxycephalosporin Schiff base intermediate could be obtained by mercuric acetate catalyzed methanolysis of the corresponding 7α-methylthio Schiff base. Reaction of a 7α-methylthiocephalosporin with mercuric acetate in methanol gave a mixture of 7α- and 7β-methoxycephalosporins from which pure 7β-methoxy epimer could be isolated. The same reaction with methanol replaced by dimethoxyethane or acetic acid yielded a 7α-acetoxycephalosporin. Nuclear Overhauser studies performed on the 7-substituted cephalosporins led to assignments of configuration at C-7, which were supported by single-crystal X-ray analysis of 7α-methylthio-7-phenylacetamidodeacetoxycephalosporanic acid *tert*-butyl ester.

Previous investigations have shown that neither the introduction of a 7α-methyl group into a cephalosporin nor of a 6α-methyl group into a penicillin results in improved antimicrobial activity.[1,2] Similar results were found when the 7(6)-α-methyl substituents were replaced by α-acetyl groups.[3] As part of the biological study of cephalosporins and penicillins possessing substituents at the C-7(6) position, it seemed reasonable for us to examine the effects of electron-withdrawing substituents other than acetyl. Heteroatom substituents were an obvious choice; thus, we report now our synthesis of 7-acetoxy-, 7-methoxy-, and 7-methylthiocephalosporins and 6-methylthiopenicillins.[4] Key compounds and the general synthetic schemes are outlined below (1).

The methylthio group was introduced stereospecifically into the 7 position of the Δ^3-cephem nucleus by two routes. Using a one-step method, the anion of the benzaldehyde Schiff base (I) of 7-aminodeacetoxycephalosporanic acid *tert*-butyl ester, prepared by using 1 equiv of KO-*t*-Bu in dimethoxyethane at −20°, was methylthiolated with methyl methanethiolsulfonate ($CH_3SSO_2CH_3$)[5] or methylsulfenyl chloride (CH_3SCl)[5] to give the crystalline 7α-methylthio Schiff base II in 40% yield. In an alternative procedure, the anion of the Schiff base I was fluorinated, using perchloryl fluoride, to give the 7α-fluoro Schiff base III, which could be solvolyzed with methanethiol under acidic conditions to the 7α-methylthio Schiff base II. Schiff bases ob-

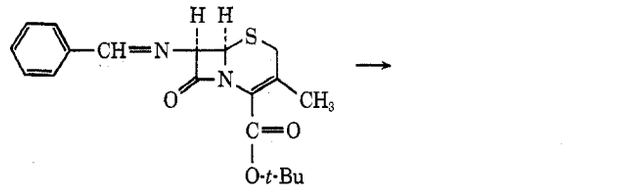
(1) E. H. W. Böhme, H. E. Applegate, B. Toeplitz, J. E. Dolfini, and J. Z. Gougoutas, *J. Amer. Chem. Soc.*, **93**, 4324 (1971).

(2) R. A. Firestone, N. Schelechow, D. B. R. Johnston, and B. G. Christensen, *Tetrahedron Lett.*, 375 (1972).

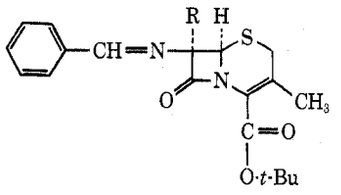
(3) E. H. W. Bohme, H. E. Applegate, J. B. Ewing, P. T. Funke, M. S. Puar, and J. E. Dolfini, *J. Org. Chem.*, **38**, 230 (1973).

(4) During these studies, there appeared reports of 7-methoxycephalosporins obtained from fermentations[4a,b] and by an elegant synthesis[4c] of these and related analogs: (a) R. Nagarajan, L. D. Boeck, M. Gorman, R. L. Hamill, C. E. Higgens, M. M. Hoehn, W. M. Stark, and J. G. Whitney, *J. Amer. Chem. Soc.*, **93**, 2308 (1971); (b) S. Karady, S. H. Pines, L. M. Weinstock, F. E. Roberts, G. S. Brenner, A. M. Hoinowski, T. Y. Cheng, and M. Sletzinger, *ibid.*, **94**, 1410 (1972); (c) L. D. Cama, W. J. Leanza, T. R. Beattie, and B. G. Christensen, *ibid.*, **94**, 1408 (1972).

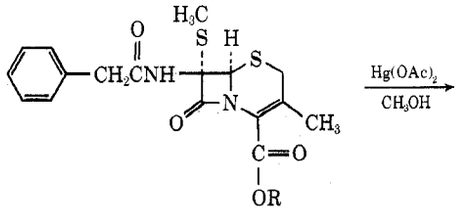
(5) I. B. Douglass, *J. Org. Chem.*, **24**, 2004 (1959).



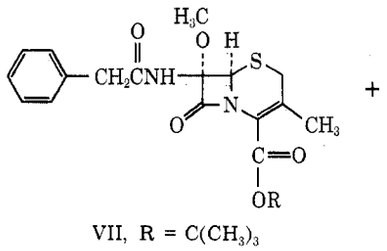
I



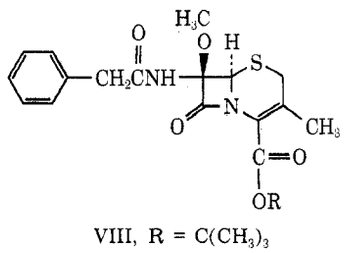
II, R = SCH_3
III, R = F
IV, R = OCH_3



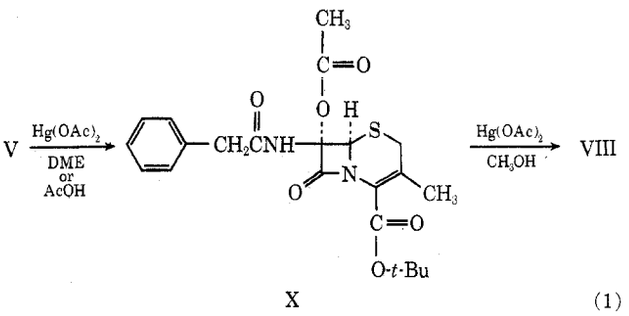
V, R = $C(CH_3)_3$
VI, R = H



VII, R = $C(CH_3)_3$



VIII, R = $C(CH_3)_3$
IX, R = H



X (1)

tained by either method were found to be identical by pmr, ir, and tlc comparisons. Direct acylation of the Schiff base II, using phenylacetyl chloride and water in dichloromethane, provided the amide V; subsequent removal of the *tert*-butyl protecting group with trifluoroacetic acid afforded 7α-methylthio-7-phenylacetamidodeacetoxycephalosporanic acid (VI).

Evidence that the methylthio group was introduced from the less hindered side of the Schiff base I, the α side, and that the methylthio group is as shown in II was obtained by single-crystal X-ray analysis of the 7-methylthio-7-phenylacetamido-Δ^3-cephem V. The X-ray determination indicated that the methylthio group is cis to the C-6 proton in the Δ^3-cephem V and, since the conversion of the methylthio Schiff base II to the Δ^3-cephem V involves no reaction at the 7 position, the methylthio group must also be cis to the C-6 proton in the Schiff base II.

In addition to the X-ray analysis, nuclear Overhauser effect (NOE) studies[6] were performed on the Δ^3-cephem V. Double irradiation of the methylthio protons showed a 5% NOE for the C-6 proton, whereas double irradiation of the amide NH gave a 0% NOE for the C-6 proton. These findings were consistent with those of the X-ray determination; nuclear Overhauser effect studies were used to assign structures to other new C-7 substituted cephalosporins (Table II).

In one approach to 7α-methoxy-7-phenylacetamidodeacetoxycephalosporanic acid, the 7α-methylthio cephem V was solvolyzed with methanol in the presence of 1 equiv of mercuric acetate, yielding essentially a quantitative conversion to a mixture of 7-methoxy epimers, VII and VIII. On the basis of nuclear Overhauser effect studies, the epimer obtained in yields of 10–30% was assigned structure VII, having the 7α-methoxy substituent, whereas the epimer obtained in 70–90% yield was assigned structure VIII, possessing the 7β-methoxy configuration. The 7β-methoxy epimer VIII could be isolated readily in yields of 50% or more by fractional crystallization. The 7α-methoxy epimer VII, however, was not obtained as a single component, but as a mixture consisting of 7α-methoxy epimer (75%) and 7β-methoxy epimer (25%), after partial removal of 7β-methoxy epimer, either by repeated preparative tlc on silica gel or by crystallization. When silver tetrafluoroborate was substituted for mercuric acetate, only the 7β-methoxy epimer VIII was isolated (44% yield). Treatment of the 7β-methoxy epimer VIII with trifluoroacetic acid gave the Δ^3-cephem acid IX, in 90% acid yield, which could be isolated from acetone–hexane as a crystalline product containing one acetone per equivalent of acid.

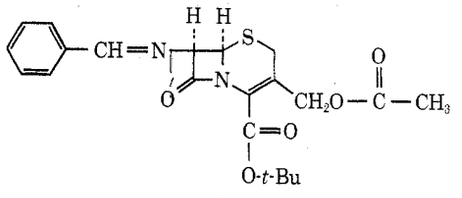
In a second approach to the 7α-methoxy-7-phenylacetamidodeacetoxycephalosporanic acid, the 7α-fluoro Schiff base III was solvolyzed with methanol to give the Schiff base IV, which was assigned the 7α-methoxy configuration by analogy with the methanethiol solvolysis of the fluoro Schiff base III, which yielded the 7α-methylthio Schiff base II. The 7α-methoxy Schiff base IV was obtained alternatively in 74% yield from the 7α-methylthio Schiff base II by methanolysis in the presence of mercuric acetate.

As mentioned previously, methanolysis of the 7α-methylthiocephem V in the presence of mercuric acetate leads to a mixture of 7-methoxy epimers VII and VIII. However, treatment of the Δ^3-cephem V with 1 equiv of mercuric acetate not in the presence of methanol, but in the presence of dimethoxyethane or acetic acid, for 20 min at room temperature, afforded a quantitative conversion to an acetoxy compound that was assigned structure X. Although the oily acetoxy com-

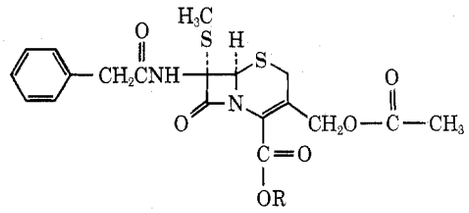
(6) R. A. Bell and J. K. Saunders, *Chem. Rev.*, **71**, 617 (1971).

pound could not be crystallized, its spectral properties (pmr and ir) were consistent with structure X, and the results of nuclear Overhauser effect studies were in agreement with the 7α-acetoxy configuration. Attempted removal of the *tert*-butyl protecting group from the acetoxy compound, using trifluoroacetic acid at 0°, gave a complex mixture of acidic components that could not be readily separated. Treatment of the acetoxy compound with methanol and 1 equiv of mercuric acetate at room temperature, however, gave the 7β-methoxy cephem VIII in quantitative yield. Spectral (pmr and ir) and mixture melting point comparisons of this product with the 7β-methoxycephem VIII, obtained by mercuric acetate methanolysis of 7α-methylthio cephem V, revealed no differences between the samples.

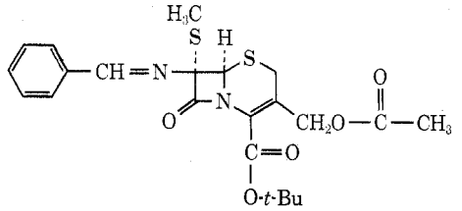
The 7α-methylthiocephalosporanic acid XIV was prepared by procedures similar to those described for the synthesis of the 7α-methylthiodeacetoxycephalosporanic acid VI. Treatment of the Schiff base XI with potassium *tert*-butoxide and methyl methanethiolsulfonate gave the expected methylthio Schiff base XII, whose configuration at C-7 is assigned, as shown, by analogy with the direction of methylthiolation of the deacetoxycephalosporin Schiff base I. Direct acylation of XII, using phenylacetyl chloride–water (10% excess) in dichloromethane and subsequent removal of the *tert*-butyl protecting group with trifluoroacetic acid gave, finally, the 7α-methylthiocephalosporanic acid (XIV).



XI

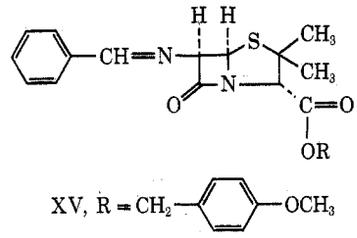


XIII, R = $C(CH_3)_3$
XIV, R = H

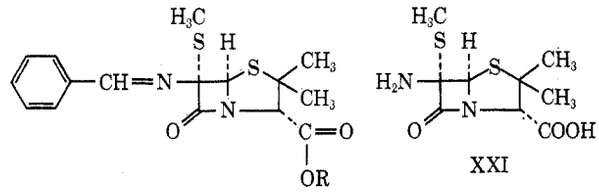


XII

In an initial approach to the synthesis of 6-methylthiopenicillins, the Schiff base XV of 6-aminopenicillanic acid *p*-methoxybenzyl ester was methylthiolated with potassium *tert*-butoxide and methyl methanethiolsulfonate to give, in 95% yield, the 6α-methylthio Schiff base XVIII, whose configuration at C-6 is assigned by analogy with alkylation and acylation reactions of penicillin Schiff bases that have been shown to occur

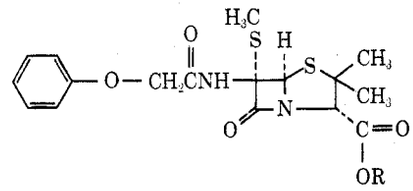


XV, R = CH_2–C_6H_4–OCH_3
XVI, R = $Si(CH_3)_3$
XVII, R = H

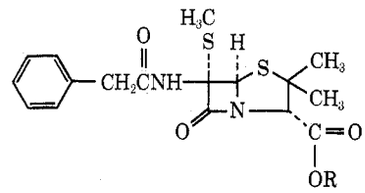


XXI

XVIII, R = CH_2–C_6H_4–OCH_3
XIX, R = $Si(CH_3)_3$
XX, R = H



XXII, R = CH_2–C_6H_4–OCH_3
XXIII, R = H
XXIV, R = $Si(CH_3)_3$



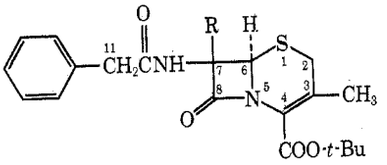
XXV, R = CH_2–C_6H_4–OCH_3
XXVI, R = H
XXVII, R = $Si(CH_3)_3$

from the α side. Direct acylation of XVIII with phenoxyacetyl chloride or phenylacetyl chloride gave the amides XXII and XXV, respectively, which could not be readily hydrogenolyzed to the corresponding free acids.

The 6α-methylthiopenicillanic acids XXIII and XXVI were prepared by an alternative approach, utilizing the trimethylsilyl group as a protecting agent. Treatment of the Schiff base XVII of 6-aminopenicillanic acid, sequentially, with equimolar amounts of potassium *tert*-butoxide, trimethylsilyl chloride, potassium *tert*-butoxide, and methyl methanethiolsulfonate gave 6α-methylthio-6-aminopenicillanic acid (XXI) in 23% yield, along with the 6α-methylthio Schiff base XX in 19% yield. Acylation of the *N*,*O*-bistrimethylsilyl derivative of XXI with phenoxyacetyl chloride or phenylacetyl chloride gave after hydrolysis and acidification the corresponding 6α-methylthiopenicillanic acids XXIII and XXVI in yields of 38 and 78%, re-

TABLE I

NMR DATA ($CDCl_3$, τ) FOR CEPHALOSPORIN *tert*-BUTYL ESTERS[a]



Compd	R Group	H-6	R-7	H-2[b]	H-11	NH	C-3 Methyl
V	SCH_3	5.08	7.75	6.82, 6.72	6.34	3.64	7.87
VII	OCH_3	4.86	6.58	6.86, 6.68	6.37	~2.77[c]	7.92
VIII	OCH_3	4.98	6.55	6.90, 6.72	6.32	3.40	7.89
X	$OCOCH_3$	4.90	7.93	7.04, 6.92	6.34	~2.9[c]	7.88

[a] Measurements were made using a Varian XL-100 nmr spectrometer; tetramethylsilane was used as an internal standard. [b] AB quartet (J = 18.0 Hz). [c] NH signal is under phenyl resonance.

spectively. Although the acids XXIII and XXVI and the Schiff base acid XX were amorphous and, therefore, difficult to analyze, their spectral properties (pmr and ir) and the mass spectra of their trimethylsilyl esters were consistent with the assigned structures.

Assignment of Configurations.—Single-crystal X-ray analysis of needles of 7-methylthio-7-phenylacetamidodeacetoxycephalosporanic acid *tert*-butyl ester that had been recrystallized from methanol indicated that they were of the monoclinic space group $P2_1$, having unit cell parameters a = 10.18 Å, b = 5.84 Å, c = 18.54 Å, β = 95°27′, two molecules per unit cell. A Patterson map was used to locate the two sulfur atoms, and Fourier maps were used to locate other atoms. An R factor of 0.20 for the 1600 nonzero reflections was obtained without any refinement, thereby establishing the absolute configuration of the molecule that verified the suspected cis relationship for the methylthio group at C-7 and the proton at C-6.

In addition to the X-ray study of the 7-methylthiocephem V, nuclear magnetic resonance measurements were used to determine the stereochemical relationships at C-6 and C-7 in the Δ^3-cephems synthesized. Pmr assignments for the Δ^3-cephems V, VII, VIII and X are shown in Table I; corresponding nuclear Overhauser effect data are given in Table II. Nuclear Overhauser effects for the C-6 proton in the methylthiocephem V after double irradiation of the methylthio protons and the amide protons have already been discussed. The assignment of the cis relationship to the 7-acetoxy and the C-6 proton, as shown in structure X, is based on findings similar to those found for the methylthiocephem V. Double irradiation of the acetoxy protons gave a 13% NOE for the C-6 proton, which is consistent with the acetoxy group being cis to the C-6 proton; yet, in the case of a group such as acetoxy, this finding does not exclude entirely the possibility of a trans acetoxy C-6 proton relationship. Double irradiation of the amide proton, on the other hand, showed a 0% NOE for the C-6 proton, which is consistent only with the amido group being trans to the C-6 proton and the acetoxy group, therefore, being cis.

TABLE II

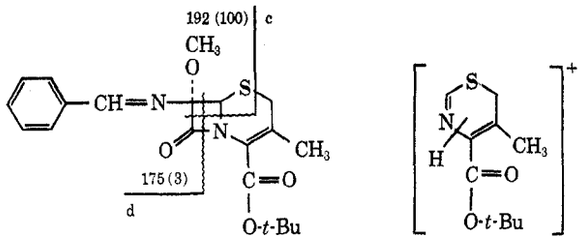
NUCLEAR OVERHAUSER EFFECT DATA[a]

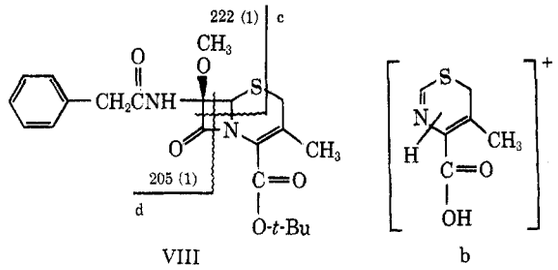
Compd	R Group	Group irradiated	% Enhancement of C-6 proton	Stereochemical assignment of C-6 H and C-7 R
V	SCH_3	SCH_3 at 7.75	5	α cis
V	SCH_3	NH at 3.64	0	β trans
VII[b]	OCH_3	OCH_3 at 6.58	14	α cis
VII[b]	OCH_3	NH at 2.77	1	β trans
VIII	OCH_3	OCH_3 at 6.55	5	β trans
VIII	OCH_3	NH at 3.40	10	α cis
X	$OCOCH_3$	$OCOCH_3$ at 7.93	13	α cis
X	$OCOCH_3$	NH at 2.90	0	β trans

[a] The nuclear Overhauser effect measurements were carried out on deoxygenated, sealed $CDCl_3$ solutions containing tetramethylsilane as an internal standard. The nmr spectrometer (Varian XL-100-15) was internally locked to ^2H of the solvent. The C-6 protons of the cephalosporins were integrated at least ten times with maximum irradiation of the C-7 substituent protons or the amido protons. The irradiating frequency was moved 30 Hz off resonance and the procedure was repeated.[6] [b] This data was obtained on a mixture (60:40) of VII and VIII.

The stereochemical assignments of the 7-methoxy epimers VII and VIII were made by comparison of the respective NOEs obtained for the C-6 protons after saturation of the methoxy proton and amide proton resonances. Two samples of 7-methoxy epimers were examined. One contained the more adundant epimer, the crystalline one, and the other contained both the noncrystalline and crystalline epimers (noncrystalline: crystalline 60:40). As shown in Table II, the crystalline epimer gave 5 and 10% NOEs for the methoxy–C-6 proton and amide proton–C-6 proton interactions, respectively, whereas the noncrystalline epimer gave 14 and 1% NOEs for the same interactions, respectively. A 10% NOE for the amide proton–C-6 proton interaction in the crystalline epimer strongly suggests that the methoxy group is trans to the C-6 proton in this epimer. A 0% NOE should be expected for the amide proton–C-6 proton interaction in the epimer where the methoxy group is cis to the C-6 proton; however, errors of ±3% in the determination of NOEs are quoted in the literature, and, in the measurement of NOEs for the noncrystalline epimer using the 60:40 mixture, the error might be even higher because of difficulties inherent in the integration. The order of the NOEs is consistent, however, with assignment of the trans methoxy structure VIII for the crystalline epimer and the cis methoxy structure VII for the noncrystalline epimer.

The mass spectral fragmentation patterns of the 7-heteroatom-substituted Schiff bases were found to differ considerably from those of the corresponding 7-substituted 7-phenylacetamido compounds, as illustrated by differences in intensities for molecular ions and for frag-

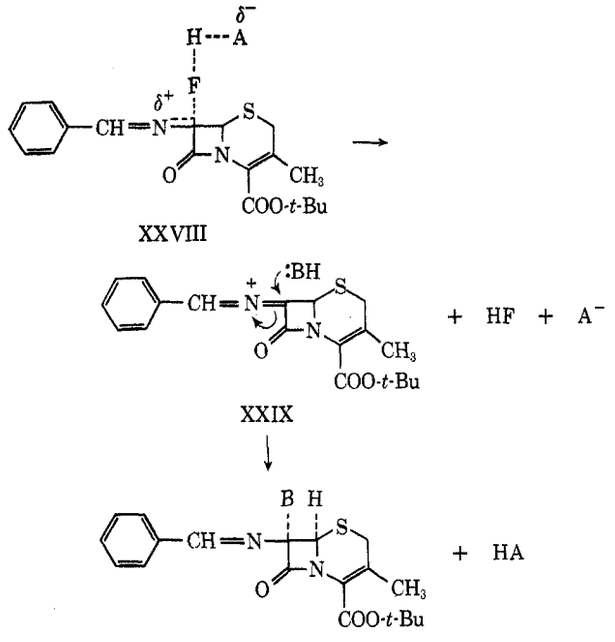




ments a, b, and c derived from the 7-methoxy *tert*-butyl esters IV and VIII. Similar differences were found for the 7-fluoro- and 7-methylthiocephems and the 6-methylthiopenicillins.

The mechanism for the solvolysis of the fluoro Schiff base III appears to proceed by acid catalysis, since reaction occurred only under acidic conditions. No reaction occurred at room temperature when methanol and triethylamine in deuteriochloroform, methanethiol in dimethoxyethane, or water in acetone-d_6 were used, whereas reaction with each of these mixtures did occur after the addition of trifluoroacetic acid. Methanolysis of III proceeded readily in a chloroform–methanol mixture (4:1); however, it is believed that the small amount of acid present in commercially available chloroform was sufficient to catalyze the reaction.

Acid catalysis of the solvolysis reaction should be expected, since incipient fluoride ions are known to form strong hydrogen bonds, and since acid catalysis has been demonstrated for many fluorides, including benzyl fluoride and methyl fluoroacetate.[7] A possible mechanism for the solvolysis is one in which an acid (HA) hydrogen bonds with III to give an intermediate XXVIII; the developing charge at C-7 could be stabilized by the imine nitrogen, leading to the planar intermediate XXIX and, finally, attack by solvent (BH) (methanethiol or methanol) would then be expected to occur from the less hindered exo face (α side). Attack from the exo face was established in the case of solvolysis of the fluoro compound III with methanethiol, which gave material that was identical with 7α-methylthiocephem II, that had been prepared by methylthiolation of the anion of I with methyl methanethiolsulfonate or methylsulfenyl chloride. Presumably, solvolysis of the fluoro compound III with methanol proceeds in an analogous manner to give the 7-methoxy Schiff base IV whose methoxy group is considered to be α. It is possible that mercuric acetate–methanol solvolysis of the methylthio Schiff base II proceeds in a similar fashion, since only one 7-methoxy product is formed, the presumed 7α-methoxy epimer.



The mechanism of the mercuric acetate solvolysis of the 7α-methylthio-7-phenylacetamido-Δ^3-cephem V remains obscure. It has been shown that (1) treatment of 7α-methylthiocephem V with mercuric acetate in methanol at room temperature for 30 min or at reflux for 10 min yields a mixture of 7α-methoxy and 7β-methoxy epimers (ratio 1:4); (2) treatment of 7α-methylthiocephem V with mercuric acetate in dimethoxyethane at room temperature for 15 min yields, exclusively, 7α-acetoxycephem X; (3) treatment of 7α-acetoxycephem X with mercuric acetate in methanol for 30 min gives, exclusively, 7β-methoxy VIII, whereas treatment of X with methanol or methanol–acetic acid for several hours at room temperature results in no reaction; (4) treatment of 7α-methylthiocephem V with mercuric acetate in methanol for 15 min at 0°, followed by immediate work-up, leads, as shown by pmr examination, to the presence of a large amount of 7α-acetoxycephem X and lesser amounts of the methoxy epimers VII and VIII; (5) treatment of the 7β-methoxycephem VIII with mercuric acetate and methanol yields no 7α-methoxy cephem VII.

These data suggest that more than one mechanism is in operation in the mercuric acetate methanolysis of 7α-methylthiocephem V to the mixture of 7-methoxy epimers VII and VIII. Formation of a major portion of the 7β-methoxycephem VIII probably proceeds through the intermediate, 7α-acetoxycephem X; by what mechanism the 7α-methoxycephem VII is formed remains unclear.

The substituted penicillins and cephalosporins described here were tested for antimicrobial activity *in vitro*. The 6α-methylthio penicillins XXIII and XXVI and the 7α-methylthiocephalosporins VI and XIV had activities considerably less than those found for corresponding compounds that are unsubstituted at C-6 or C-7. The 7β-methoxycephalsoprin IX, at a concen-

(7) M. Stacey, J. C. Tatlow, and A. G. Sharpe, *Advan. Fluorine Chem.*, 3, 63 (1963).

tration of 100 μg/ml, showed no activity against any gram-positive and gram-negative microorganism tested.

Experimental Section

The pmr spectra were determined on Varian nuclear magnetic resonance spectrometers (Models T-60 and XL-100-15) using tetramethylsilane as an internal standard, and chemical shifts are reported on the τ scale. Infrared spectra were recorded on Perkin-Elmer spectrometers (Models 257 and 621), and the mass spectra were obtained on an AEI-MS-902 mass spectrometer. Melting points are uncorrected.

Methylsulfenyl Chloride and Methyl Methanethiolsulfonate.—Methyl methanethiosulfonate was prepared by a modification of the procedure of Douglas.[5] Liquid chlorine (50.3 g, 0.71 mol) at Dry Ice–acetone temperature was allowed to warm carefully and distil into dimethyl sulfide (61 g, 0.647 mol), and was then stirred at −20° and protected from moisture to give methylsulfenyl chloride sufficiently pure for methylthiolations or for conversion to methyl methanesthiolsulfonate. Water (23.3 ml, 1.29 mol) was slowly added dropwise to the methylsulfenyl chloride; the temperature was maintained at −20° for 30 min and then allowed to rise to room temperature. The mixture was stirred overnight, during which time HCl was evolved and the color turned from orange to yellow. Distillation of the mixture gave an initial fraction of water–dimethyl sulfide and, finally, one of methyl methanethiolsulfonate (32.4 g), bp 96–97° (4.5 mm). Ir and pmr spectra were consistent with the desired product.

7α-Methylthio-7-benzaliminodeacetoxycephalosporanic Acid *tert*-Butyl Ester (II) from Methylthiolation of Schiff Base I. Method A. Methyl Methanethiolsulfonate Procedure.—To a stirred solution of Schiff base I (13.5 g, 0.377 mmol) in 200 ml of dimethoxyethane at −20° under N_2 was added potassium *tert*-butoxide (4.22 g, 0.377 mmol). The deep-red solution was stirred for 1.5 min and methyl methanethiolsulfonate (4.75 g, 0.377 mmol) was added. As soon as the color of the solution had turned from deep red to yellow, the reaction mixture was poured into pH 6.5 buffer (300 ml). The mixture was extracted with $CHCl_3$, and the $CHCl_3$ extract was washed with saturated NaCl solution, dried (Na_2SO_4), and evaporated to a residue. Recrystallization of the residue from acetone–hexane gave 5.38 g (35% yield) of 7α-methylthio Schiff base II: ir ($CHCl_3$) 1764 (β-lactam C=O), 1715 (conjugated ester C=O), 1628 (C=N), and 1130 cm^{-1} (SCH_3); pmr ($DCCl_3$) τ 8.45 (9 H, s, *tert*-butyl), 7.93 (3 H, s, C=CCH_3), 7.70 (C-6), 2.0–2.8 (5 H, m, aromatics), 1.91 (1 H, s, CH=N); mass spectrum molecular ion at 404.1206 (calcd for $C_{20}H_{24}N_2O_3S_2$: 404.1226). An analytical sample that was recrystallized from CH_2Cl_2–petroleum ether (bp 30–60°) had mp 165°. *Anal.* Calcd for $C_{20}H_{24}N_2O_3S_2$: C, 59.38; H, 5.98; N, 6.93; S, 15.85. Found: C, 59.48; H, 6.08; N, 6.90; S, 15.69.

Method B. Methylsulfenyl Chloride Procedure.—The procedure in part A was followed, with methylsulfenyl chloride used in place of methyl methanethiolsulfonate. From 20.3 g (56.5 mmol) of Schiff base I, 6.33 g (56.5 mmol) of potassium *tert*-butoxide, 4.6 g (56.5 mmol) of methylsulfenyl chloride, and 250 ml of dimethoxyethane was obtained 7.70 g (34% yield) of crystalline Schiff base. The 7α-methylthio Schiff bases prepared by methods A and B were found to be identical by ir, pmr, melting point, and mixture melting point comparisons.

7-Benzalimino-7-fluorodeacetoxycephalosporanic Acid *tert*-Butyl Ester (III).—To a stirred solution of the Schiff base I (218 mg, 0.611 mmol) in anhydrous dimethoxyethane (40 ml) at −50° under nitrogen was added sublimed potassium *tert*-butoxide (67 mg, 0.611 mmol), yielding a dark red mixture. Perchloryl fluoride, diluted in a stream of nitrogen, was passed slowly through the solution until the red color was discharged. Nitrogen was bubbled through the solution as it was warmed to room temperature. The mixture was cooled to −20°, diluted with an equal volume of $CHCl_3$, and passed rapidly through silica gel. Removal of solvent under reduced pressure gave the ester III as a yellow oil (259 mg): ir (neat) 1780 (β-lactam C=O), 1720 (*tert*-butyl ester C=O), 1640 cm^{-1} (C=N); pmr ($DCCl_3$) τ 8.35 (9 H, *tert*-butyl CH_3), 7.87 (3 H, s, CH_3), 6.68 (2 H, m, C-2), 4.80 (1 H, s, J_{H-F} = 8.0 Hz, C-6), 2.03–2.63 (5 H, m, aromatic); M^+, *m/e* 376.1209 ($C_{19}H_{21}N_2O_3SF$, 346.1255). A diagnostic peak at *m/e* 180.0261 (C_9H_7NSF, 180.0283) is consistent with PhCH=NCF=CHS^+.

7-Benzalimino-7-methylthiodeacetoxycephalosporanic Acid *tert*-Butyl Ester (II) from Solvolysis of the Fluoro Schiff Base III.—To a solution of the fluoro Schiff base III (110 mg, 0.293 mmol) in anhydrous dimethoxyethane at 0° was added a solution of methanethiol (0.2 g) in dry dimethoxyethane (5 ml) and trifluoroacetic acid (0.023 ml, 0.30 mmol). The mixture was stirred at 0° for 1 hr and allowed to warm to room temperature for another hour. After being purged with nitrogen, the solution was diluted with benzene and washed with saturated aqueous sodium bicarbonate and water. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to a yellow oil (101 mg), which was purified by silica gel tlc with $CHCl_3$:hexane (3:1). The resulting solid (46 mg) had spectral (pmr and ir) and R_f values identical with those of a sample of II obtained by methylthiolation of I.

7-Benzalimino-7-methoxydeacetoxycephalosporanic Acid *tert*-Butyl Ester (IV). Procedure A.—A mixture of mercuric acetate (431 mg, 1.35 mmol) and the methylthio Schiff base II (500 mg, 1.24 mmol) in anhydrous methanol (15 ml) was stirred at room temperature for 1 hr. Dilution with anhydrous ether (75 ml) and filtration through Celite removed insoluble material. After solvent had been stripped under reduced pressure, the residue was taken up in ether and washed with 5% bicarbonate solution and water. The organic layer was treated with Norit, and the volume of solvent was reduced, yielding IV as colorless crystals, 305 mg, mp 141–142°. A second crop of IV was obtained from the filtrate, 520 mg, mp 137.5–139° (total yield 74%). Recrystallization from methanol yielded analytically pure material: mp 142–143°; ir ($CHCl_3$) 1770 (β-lactam C=O), 1715 (*tert*-butyl ester C=O), 1635 cm^{-1} (C=N); pmr ($DCCl_3$) τ 8.47 (9 H, s, *tert*-butyl C=O), 7.78, (3 H, s, CH_3), 6.98 (1 H, d, J_{gem} = 17 Hz, C-2), 6.55 (1 H, d, J_{gem} = 17 Hz, C-2), 6.39 (3 H, s, OCH_3), 4.92 (1 H, s, C-6), 1.97–2.75 (5 H, complex m, aromatic), 1.30 (1 H, s, azomethine CH). *Anal.* Calcd for $C_{20}H_{24}N_2O_4S$: C, 61.84; H, 6.23; N, 7.21. Found: C, 61.60; H, 6.18; N, 7.24.

Procedure B.—A solution of the fluoro Schiff base III (297 mg) in chloroform (6 ml) and anhydrous methanol (2 ml) was stirred at room temperatue for 10 min. Solvent was removed *in vacuo*, and residual methanol was azeotropically distilled with benzene under reduced pressure. The pmr and ir spectra of the yellow oily residue were identical with those obtained for material prepared by procedure A. The mass spectrum exhibited a parent peak at *m/e* 388 ($C_{20}H_{24}N_2O_4S$, 388).

7α-Methylthio-7-phenylacetamidodeacetoxycephalosporanic Acid *tert*-Butyl Ester (V).—To a stirred solution of 7α-methylthio Schiff base II (2.54 g, 6.28 mmol) in 30 ml of CH_2Cl_2 at room temperature under N_2 was added phenylacetyl chloride (0.84 ml, 6.28 mmol) and water (0.15 ml, 8.34 mmol). The mixture was stirred for 18 hr, diluted with CH_2Cl_2, and poured into water. The pH was adjusted to 7.5, and the CH_2Cl_2 layer was washed successively with water, dilute aqueous $NaHSO_3$, and water. The CH_2Cl_2 solution was dried (Na_2SO_4) and evaporated *in vacuo* to a residue that crystallized from Et_2O–CH_2Cl_2 to give 1.18 g (43% yield) of V: ir ($CHCl_3$) 1775 (β-lactam C=O), 1712 (conjugated C=O), 1675 (amide C=O), 1480 ("amide II" band), and 1130 cm^{-1} (SCH_3); pmr ($DCCl_3$) τ 8.50 (9 H, s, *tert*-butyl), 7.92 (3 H, s, C=CCH_3), 7.75 (3 H, s, $-SCH_3$), 6.82 (2 H, broad singlet, C-2), 6.36 (2 H, broad singlet, $ARCH_2C$=O), 5.09 (1 H, s, NH). An analytical sample that was recrystallized from Et_2O–$CHCl_3$ had mp 174–175°. *Anal.* Calcd for $C_{21}H_{26}N_2O_4S_2$: C, 58.04; H, 6.03; N, 6.45; S, 14.76. Found: C, 58.03; H, 5.86; N, 6.41; S, 14.56.

7α-Methylthio-7-phenylacetamidodeacetoxycephalosporanic Acid (VI).—To 7α-methylthio amide V (652 mg, 1.5 mmol) cooled in an ice–water bath was added 2 ml of trifluoroacetic acid. The stoppered mixture was removed from the cooling bath and left at room temperature for 20 min. The mixture was evaporated *in vacuo* to a residue that was taken up in $CHCl_3$–H_2O. The pH was adjusted to 7.5 with 0.5 *N* NaOH, and the $CHCl_3$ was removed. The aqueous portion was layered with $CHCl_3$ and adjusted to pH 2.0 with 1 *N* HCl. Sodium chloride was added, and the mixture was extracted repeatedly with $CHCl_3$. Evaporation of the dried (Na_2SO_4) $CHCl_3$ extract gave 459 mg of acid VI (81% yield) as a colorless residue: ir ($CHCl_3$) 3500–3100 and 2700–2400 (acid OH), 1774 (β-lactam C=O), 1715 (conjugated C=O), and 1680 cm^{-1} (amide C=O); pmr ($DCCl_3$) τ 7.77 (6 H, s, $-SCH_3$ and C=CCH_3), 6.77 (2 H, s, C-2), 6.32 (2 H, s, $ArCH_2C$=O), 5.07 (1, H, s, C-6), 3.07 (1 H, s, NH), 2.68 (5, H, s, aromatic), 1.04 (1 H, s, COOH). Recrystallization from acetone–hexane gave an analytical sample, mp 105–116°

dec, containing 1 equiv of acetone, which was verified by its pmr spectrum ($DCCl_3$): τ, 7.83 [6 H, s, $(CH_3)_2C{=}O$ and $C{=}CCH_3$], 7.7 (6 H, s, SCH_3 and $C{=}CCH_3$). *Anal.* Calcd for $C_{17}H_{18}N_2O_4S_2 \cdot C_3H_6O$: C, 55.02; H, 5.54; N, 6.42; S, 14.69. Found: C, 54.66; H, 5.73; N, 6.44; S, 14.51.

7α-Methoxy-7-phenylacetamidodeacetoxycephalosporanic Acid *tert*-Butyl Ester (VII) and 7β-Methoxy-7-phenylacetamidodeacetoxycephalosporanic Acid *tert*-Butyl Ester (VIII).—To a suspension of methylthioamide V (652 mg, 1.5 mmol) in 5 ml of refluxing CH_3OH under N_2 was added mercuric acetate (478 mg, 1.5 mmol). The mixture was stirred under reflux for 10 min, cooled to room temperature, and evaporated *in vacuo* to a residue. The residue was taken up in benzene–water, and the benzene layer was washed three times with water, dried (Na_2SO_4), and evaporated to a residue. The residue was subjected to slow fractional crystallization from small amounts of CH_3OH, which yielded 420 mg of pale-yellow crystalline β-methoxy epimer (VIII), a residue from crystal washings, and 130 mg of mother liquor whose pmr spectrum indicated a 60:40 mixture of α-methoxy and β-methoxy epimers, respectively. Slow crystallization of this mixture of epimers gave additional crystalline β-methoxy epimer and 76 mg of mother liquor whose pmr spectrum indicated a 70:30 mixture of α-methoxy and β-methoxy epimers.

The crystalline β-methoxy epimer VIII, on recrystallization from CH_3OH, had mp 175–176°; ir spectrum ($CHCl_3$) 1770 (β-lactam C=O), 1710 (conjugated C=O), 1690 (amide C=O), 1158, 1134, 1106, and 1086 cm^{-1} (COC and CSC); pmr ($DCCl_3$, 60 MHz) τ 8.50 (9 H, s, *tert*-butyl), 7.90 (3 H, s, $C{=}CCH_3$), 6.55 (3 H, s, OCH_3), 6.75, 6.95 (2 H, q, J = 17 Hz, C-2), 6.33 (3 H, s, $-CH_2C{=}O$), 4.98 (1 H, s, C-6), 3.32 (1 H, b, NH), 2.67 (5, H, s, aromatics); mass spectrum molecular ion at m/e 418.1584 (calcd, for $C_{21}H_{26}N_2O_5S$, 418.1560).

The mother liquor containing 70% of α-methoxy epimer showed ir ($CHCl_3$) 1770 (β-lactam C=O), 1710 (conjugated C=O), 1690 (amide C=O), and 1155, 1138, 1100, and 1090 cm^{-1}; pmr ($DCCl_3$) τ 8.50 (9, H, s, *tert*-butyl), 7.93 (3 H, s, $C{=}CCH_3$), 6.68 and 6.88 (2 H, q, J = 17 Hz, C-2), 6.58 (3 H, s, $-OCH_3$), 6.37 (2 H, s, $-CH_2C{=}O$), 4.83 (1 H, s, C-6), 2.67 (5 H, s, aromatics).

7β-Methoxy-7-phenylacetamidodeacetoxycephalosporanic Acid (IX).—To the 7β-methoxy *tert*-butyl ester VIII (145 mg, 0.346 mmol) cooled in an ice bath was added 2 ml of trifluoroacetic acid. The stoppered mixture was stirred for several seconds, removed from the bath, and left at room temperature for 20 min. Excess trifluoroacetic acid was removed *in vacuo*, and the residue was taken up in $CHCl_3$–H_2O and adjusted to pH 7.5 with 0.5 *N* NaOH. The $CHCl_3$ layer was removed, discarded, and replaced with fresh $CHCl_3$. The pH was adjusted to 2.0 with 1 *N* HCl and, after the addition of NaCl, the mixture was extracted repeatedly with $CHCl_3$. The $CHCl_3$ extract was dried (Na_2SO_4) and evaporated to give the acid IX (113 mg, 90% yield) as a colorless residue: ir ($CHCl_3$) 1774 (β-lactam C=O), 1710 (sh) (conjugated C=O), and 1690 cm^{-1} (amide C=O), in addition to absorptions at 3500–3100 and 2700–2400 cm^{-1} (NH and acidic OH); pmr ($DCCl_3$) τ 7.80 (3, H, s, $C{=}CCH_3$), 6.87 (2 H, s, C-2), 6.55 (3 H, s, OCH_3), 6.30 (2 H, s, $-CH_2C{=}O$), 4.95 (1 H, s, C-6), 2.67 (5 H, s, aromatics), 0.02 (1 H, s, COOH). Recrystallization of the residue from acetone–hexane gave colorless crystals, mp 92–94°, containing one acetone of crystallization, as evidenced by its pmr spectrum and elemental analysis. *Anal.* Calcd for $C_{17}H_{18}N_2O_5S \cdot C_3H_6O$: C, 57.13; H, 5.75; N, 6.66; S, 7.63. Found: C, 57.25; H, 5.55; N, 6.44; S, 7.62.

7α-Acetoxy-7-phenylacetamidodeacetoxycephalosporanic Acid *tert*-Butyl Ester (X).—To a suspension of the 7α-methylthio ester V (651 mg, 1.5 mmol) in 5 ml of dimethoxyethane was added mercuric acetate (478.5 mg, 1.5 mmol). The mixture was stirred under nitrogen for 20 min at room temperature. The precipitate was filtered and washed with dimethoxyethane, yielding 413 mg of pale-yellow powder. The filtrate was evaporated to a residue, which was taken up in benzene–water. The benzene layer was washed with water, dried (Na_2SO_4), and evaporated to give 620 mg (93% yield) of 7α-acetoxy-*tert*-butyl ester X, as an almost colorless oil: ir ($CHCl_3$) 1785 (β-lactam C=O), 1750 (sh) (ester C=O), 1720–1685 (broad band, conjugated C=O and amide C=O), and 1480 cm^{-1} (amide II band); pmr ($DCCl_3$) τ 8.48 (9 H, s, *tert*-butyl), 7.90 [3 H, s, $-OC(=O)CH_3$], 7.87 (3, H, s, $C{=}CCH_3$), 6.98 (2 H, s, C-2), 6.32 (2 H, s, $-CH_2C{=}O$), 4.87 (1 H, s, C-6), 2.85 (1 H, s, NH), 2.67 (5 H, s, aromatics); mass spectrum, no molecular ion, but m/e 344 (M − CH_3COOH).

Stereospecific Conversion of 7α-Acetoxy Ester X to 7β-Methoxy Ester VIII.—To a solution of 7α-acetoxy ester X (81 mg, 0.182 mmol) in 2 ml of anhydrous methanol was added mercuric acetate (58 mg, 0.182 mmol). The mixture was stirred under nitrogen for 30 min at room temperature. The methanol was removed under reduced pressure, and the residue was taken up in benzene–water. The benzene layer was washed with water, dried (Na_2SO_4), and evaporated, leaving 75 mg of 7β-methoxy ester VIII as a colorless residue having pmr and ir spectra like those of pure 7β-methoxy ester VIII. Recrystallization from Et_2O–CH_2Cl_2 gave colorless crystals, mp 175–176°.

7α-Methylthio-7-benzaliminocephalosporanic Acid *tert*-Butyl Ester (XII).—To a stirred solution of Schiff base XI (2.43 g, 5.84 mmol) in 50 ml of dry dimethoxyethane at −5° under N_2 was added potassium *tert*-butoxide (655 mg, 5.84 mmol). The solution was stirred for 1 min, and methyl methanethiolsulfonate (7.37 g, 5.84 mmol) was added. After being stirred for 5 min, the solution was poured into pH 6.5 buffer. The mixture was extracted with $CHCl_3$, and the $CHCl_3$ was washed with saturated NaCl solution, dried (Na_2SO_4), and evaporated to a residue. Chromatography of the residue on a column of silica gel (200 g) using hexane–$CHCl_3$ (60:40) and subsequent purification by silica gel tlc in the system 1,2-dichloroethane–benzene–acetone (75:25:1) afforded 570 mg of XII (21% yield) as a crystalline product: ir ($CHCl_3$) 1770 (β-lactam C=O), 1720 (ester C=O), 1625 (C=N), and 1125 cm^{-1} (SCH_3); pmr ($DCCl_3$) τ 8.43 (9 H, s, *tert*-butyl), 7.93 (3 H, s, *O*-acetyl), 7.72 (3 H, s, SCH_3), 6.47, 6.67 (2 H, AB quartet, J = 18 Hz, C-2), 4.98, 5.15 (2 H, AB quartet, J = 13 Hz, CCH_2O), 4.93 (1 H, s, C-6), 2.66–2.92 (5 H, m, aromatics), and 1.14 (1 H, s, CH=N). An analytical sample that was recrystallized from hexane–acetone had mp 124–125°. *Anal.* Calcd for $C_{22}H_{26}N_2O_5S_2$: C, 57.12; H, 5.67; N, 6.06; S, 13.86. Found: C, 56.91; H, 5.50; N, 6.31; S, 13.70.

7α-Methylthio-7-phenylacetamidocephalosporanic Acid *tert*-Butyl Ester (XIII).—To a stirred solution of Schiff base XII (262 mg, 0.566 mmol) in 10 ml of CH_2Cl_2 at room temperature under N_2 was added phenylacetyl chloride (94 mg, 0.566 mmol) followed by water (11.5 mg, 0.624 mmol). The mixture was stirred for 18 hr, diluted with CH_2Cl_2, and poured into water. The CH_2Cl_2 layer was washed with water, dried (Na_2SO_4), and evaporated *in vacuo* to a residue that was fractionated by silica gel tlc in the system $CHCl_3$–hexane (9:1), yielding 184 mg of XIII (66% yield) as a residue: pmr ($DCCl_3$) τ 7.92 (3 H, s, *O*-acetyl), 7.77 (3 H, s, SCH_3), 6.60 (2 H, broad singlet, C-2), 6.34 (2 H, s, $ArCH_2$), and 5.07 (1 H, s, C-6).

7α-Methylthio-7-phenylacetamidocephalosporanic Acid (XIV).—To 7α-methylthioamide XIII (184 mg, 0.374 mmol) cooled in an ice–water bath was added 2 ml of trifluoroacetic acid. The stoppered mixture was removed from the bath and stirred at room temperature for 15 min. The mixture was evaporated *in vacuo* to a residue that was taken up in EtOAc–H_2O. The pH was adjusted to 7.5 with 0.5 *N* NaOH, and the EtOAc was removed. Sodium chloride was added, and the mixture was extracted repeatedly with EtOAc. Evaporation of the dried (Na_2SO_4) EtOAc extract gave a residue that was taken up in $CHCl_3$–MeOH. Evaporation of the solvent gave 101 mg (62% yield) of crystalline XIV: ir (KBr) 1775 (β-lactam C=O), 1730 (ester C=O), 1700 (acid C=O), and 1640 cm^{-1} (amide C=O); pmr ($DCCl_3$–D_3COD) τ 7.92 (3 H, s, *O*-acetyl), 7.73 (3 H, s, SCH_3), 6.60 (2 H, broad singlet, C-2), 6.37 (2 H, s, $ArCH_2$), 5.08 (1 H, s, C-6), 5.00 (2 H, AB quartet, J = 14 Hz, $-CH_2OAc$), and 2.67 (5 H, s, aromatics). Recrystallization from acetone–hexane gave an analytical sample, mp 156–157°. *Anal.* Calcd for $C_{19}H_{20}N_2O_6S_2$: C, 52.28; H, 4.62; N, 6.42. Found: C, 52.50; H, 4.83; N, 6.43.

6α-Methylthio-6-benzaliminopenicillanic Acid *p*-Methoxybenzyl Ester (XVIII).—To a stirred solution of Schiff base XV (1.04 g, 2.43 mmol) in dimethoxyethane (150 ml) at −10° was added potassium *tert*-butoxide (272 mg, 2.43 mmol). The orange solution was stirred for 2 min, and methyl methanethiolsulfonate (306 mg, 2.43 mmol) was added. After stirring for 1 hr at −10°, the mixture was poured into pH 6.6 buffer (300 ml) and extracted with ethyl acetate. Evaporation of the dried ($MgSO_4$) extract gave 1.10 g of XVII (95% yield) as a yellow oil: ir ($CHCl_3$) 1765 (β-lactam C=O), 1740 (ester C=O), and 1610 cm^{-1} C=N); pmr ($DCCl_3$) τ 8.67 (3 H, s, $-CH_3$), 8.57 (3 H, s, $-CH_3$), 7.83 (3 H, s, $-SCH_3$), 6.37 (3 H, s, $-OCH_3$), 5.57 (1 H, s, C-3), 4.93 (2 H, s, $-OCH_2$), 4.43 (1 H, s, C-5), 2.93 (9 H, m, aromatic), and 1.33 (1 H, s, CH=N); mass spectrum molecular ion at m/e 470, base peak at m/e 121.

6α-Methylthio-6-phenoxyacetamidopenicillanic Acid *p*-Methoxybenzyl Ester (XXII).—To a solution of methylthio Schiff base XVII (104 mg, 2.45 mmol) in 4 ml of dimethoxyethane was added phenoxyacetyl chloride (33.5 μl, 2.45 mmol), followed by water (4 μl, 2.45 mmol). The mixture was stirred for 40 min at room temperature and poured into water. Extraction with ethyl acetate gave a yellow oil (61 mg) that was purified by tlc on Quantum PQlF silica gel in the system hexane–ethyl acetate (4:1), to give 32 mg (25% yield of XXII as a colorless oil: ir ($CHCl_3$) 1780 (β-lactam C=O), 1745 (ester C=O), and 1692 cm^{-1} (amide C=O); pmr ($DCCl_3$) τ 8.67 (3, H, s,$-CH_3$), 8.53 (3 H, s, $-CH_3$), 7.73 (3 H, s, $-SCH_3$), 6.20 (3 H, s, $-OCH_3$), 5.63 (1 H, s, C-3), 5.50 (2 H, s, $-CH_2C=O$), 4.90 (2 H, s, $-OCH_2$), 4.45 (1 H, s, C-5), 3.00 (9 H, s, aromatic), and 1.93 (1 H, m, NH).

The amide XXII was also prepared in 20% yield by treating XVIII with equivalent amounts of *p*-toluenesulfonic acid monohydrate, triethylamine, and phenoxyacetyl chloride in EtOAc.

6α-Methylthio-6-phenylacetamidopenicillanic Acid *p*-Methoxybenzyl Ester (XXV).—The 6α-methylthioamide XXV was obtained in 14% yield by the procedure described for the preparation of amide XXII: ir ($CHCl_3$) 1775 (β-lactam C=O), 1740 (ester C=O), and 1680 cm^{-1} (amide C=O); pmr ($DCCl_3$) τ 8.70 (3 H, s, $-SCH_3$), 7.83 (3 H, s, $-SCH_3$), 6.32 (2 H, s, $-CH_2C=O$), 6.18 (3 H, s, $-OCH_3$), 5.63 (1 H, s, C-2), 4.88 (2 H, s, $-OCH_2$), 4.45 (1 H, s, C-5), and 3.30–2.57 (10 H, m, NH and aromatics).

6α-Methylthio-6-benzaliminopenicillanic Acid (XX) and 6α-Methylthio-6-aminopenicillanic Acid (XXI).—To a slurry of 6-benzaliminopenicillanic acid (XVII) (5.11 g, 16.9 mmol) in dry dimethoxyethane (200 ml) at room temperature was added potassium *tert*-butoxide (1.89 g, 16.9 mmol). The mixture turned orange, and complete solution occurred after 3 min. Trimethylsilyl chloride (1.83 g, 16.9 mmol) was added, and the mixture was stirred for 12 min as it cooled to −10°. Potassium *tert*-butoxide (1.89 g, 16.9 mmol) was added, and the solution turned red. After 15 min, methyl methanethiolsulfonate (2.12 g, 16.9 mmol) was added, and stirring was continued for 30 min at −10°. The dimethoxyethane was removed *in vacuo*, and the residue was taken up in pH 7.8 phosphate buffer and EtOAc. The EtOAc layer was discarded, and the aqueous layer was washed repeatedly with EtOAc. The EtOAc washings were discarded, and the aqueous part was layered with EtOAc and adjusted to pH 4.0 with dilute HCl. Extraction with $CHCl_3$ and EtOAc gave a residue, after drying ($MgSO_4$) and concentration. Trituration of the residue with $CHCl_3$ gave 240 mg of amino acid XXI as a solid, and a supernate. Evaporation of the supernate gave 650 mg of Schiff base XX (19% yield) as an oil. Adjustment of the pH 4 aqueous solution to pH 1.9 and extraction with EtOAc gave a further quantity of XXI (800 mg), for a total yield of 23%.

The amorphous amino acid XXI could not be recrystallized: ir (Nujol) 1755 (β-lactam C=O) and 1715 cm^{-1} (acid C=O); mp 172–176° dec; pmr (DMSO-d_6) τ 8.60 (3 H, s, $-CH_3$), 8.53 (3, H, s, $-CH_3$), 7.85 (3 H, s, $-SCH_3$), 5.82 (1 H, s, C-5), and 3.90 (3 H, broad, NH_3^+); mass spectrum, molecular ion *m/e* 262, base peak *m/e* 160. *Anal.* *Calcd* for $C_{19}H_{14}N_2O_3S_2$: C, 41.22; H, 5.38; N, 10.68. Found: C, 41.88; H, 5.78; N, 10.00.

The Schiff base XX had ir ($CHCl_3$) 1760 (β-lactam C=O), 1720 (COOH), and 1622 cm^{-1} (C=N); pmr ($DCCl_3$) τ 8.43 (6 H, s, 2-CH_3), 7.73 (3, H, s, SCH_3), 5.60 (1 H, s, C-3), 4.45 (1 H, s, C-5) 4.60 (5 H, m, aromatics), 1.57 (1 H, broad, COOH); mass spectrum of trimethylsilyl ester, molecular ion at *m/e* 422.

6α-Methylthio-6-phenoxyacetamidopenicillanic Acid (XXIII).—To a stirred suspension of the methylthioamino acid XXI (127 mg, 0.485 mmol) in dimethoxyethane (12 ml) was added *N,O*-bistrimethylsilylacetamide (100 μl, 0.485 mmol). Solution occurred after 15 min of stirring. Triethylamine (68 μl, 0.485 mmol) and phenoxyacetyl chloride (67 μl, 0.485 mmol) were added sequentially, and the mixture was stirred for 1.5 hr at room temperature and concentrated under vacuum to a residue. The residue was taken up in EtOAc–H_2O, and the water layer was discarded. Water was added to the EtOAc layer, and the pH was adjusted to 7.5. The EtOAc layer was discarded, and the aqueous solution was covered with EtOAc and adjusted to pH 3.2 with dilute HCl. The resulting EtOAc extract was dried (Na_2SO_4) and evaporated to a residue. Trituration with hexane–benzene gave 72 mg of amorphous XXIII (38% yield): ir ($CHCl_3$) 1780 (β-lactam C=O), 1730 (COOH), and 1690 cm^{-1} (amide C=O); pmr ($DCCl_3$) τ 8.47 (6 H, s, 2 CH_3), 7.70 (3 H, s, $-SCH_3$), 5.50 (1 H, s, C-3), 5.33 (2 s, OCH_2), 4.35 (1 H, s, C-5), 2.83 (5 H, m, aromatics), and 2.30 (1 H, s, NH); mass spectrum of trimethylsilyl ester, molecular ion at *m/e* 468.

6-Methylthio-6-phenylacetamidopenicillanic Acid (XXVI).—The acid XXVI was obtained in 78% yield by the method described for the preparation of the acid XXIII. The amorphous acid XXVI had ir ($CHCl_3$) 1777 (β-lactam C=O), 1725 (COOH), and 1680 cm^{-1} (amide C=O); pmr ($DCCl_3$) τ 8.57 (6 H, m, 2 CH_3), 7.83 (3 H, s, $-SCH_3$), 6.38 (2 H, s, $-CH_2C=O$), 5.67 (1 H, s, C-3), 4.48 (1 H, s, C-5), 2.67 (5 H, m, aromatics), 2.17 (1 H, m, NH); mass spectrum of trimethylsilyl ester, molecular ion at *m/e* 452.

Registry No.—I, 36954-81-1; II, 37786-92-8; III, 37786-93-9; IV, 37786-94-0; V, 37786-95-1; VI, 37786-96-2; VII, 37786-97-3; VIII, 37786-98-4; IX, 37786-99-5; X, 37787-00-1; XI, 36954-82-2; XII, 37787-02-3; XIII, 37787-03-4; XIV, 37787-04-5; XV, 36954-77-5; XVII, 21019-16-9; XVIII, 37787-07-8; XX, 37787-08-9; XXI, 37787-09-0; XXII, 37787-10-3; XXIII, 37787-11-4; XXV, 37787-12-5; XXVI, 37787-13-6; methylsulfenyl chloride, 5813-48-9; methyl methanethiolsulfonate, 2949-92-0.

Acknowledgments.—We wish to thank Mrs. B. Keeler-Toeplitz and Professor J. Z. Gougoutas, University of Minnesota, for the X-ray crystallographic determination, and we wish to thank Professors J. Z. Gougoutas and J. Strominger, Harvard University, for very helpful discussions during this investigation.