Treatment of senepoxide (0.022 g) with $HClO_4$ (2 drops) in CH_3OH (10 mL) for 3 h according to Polonsky[36] afforded *dl*-seneol **19** whose NMR spectrum and behavior on TLC exactly matched those of the natural product.

Acknowledgment. We wish to thank the National Institutes of Health, the American Cancer Society, and Eli Lilly and Co. for generous financial support.

References and Notes

(1) Part 6 in the series "Shikimate-Derived Metabolites". For part 5 see N. Ikota and B. Ganem, "Total Synthesis of Nor-Chorismic Acid", *J. Chem. Soc., Chem. Commun.* in press.
(2) Fellow of the Alfred P. Sloan Foundation, 1978–1980.
(3) E. Boyland and A. A. Levi, *Biochem. J.*, **29**, 2679 (1935).
(4) E. Boyland, *Biochem. Soc. Symp.*, **3**, 40 (1950).
(5) For a recent review see B. Witkop, *Intra-Sci. Chem. Rep.*, **8**, 75 (1974).
(6) For leading references, see (a) World Symposium on Model Studies in Chemical Carcinogenesis, Baltimore, Md., Nov 1972, Marcel Dekker, New York, N.Y., and (b) D. M. Jerina and J. W. Daly, *Science*, **185**, 573 (1974).
(7) E. C. Miller and J. A. Miller in "Molecular Biology of Cancer", H. Busch, Ed., Academic Press, New York, N.Y., 1974, pp 377–402.
(8) E. Vogel and H. Günther, *Angew. Chem., Int. Ed. Engl.*, **6**, 385 (1967).
(9) D. M. Jerina, H. Yagi, and J. W. Daly, *Heterocycles*, **1**, 267 (1973).
(10) (a) J. D. Bu'Lock and C. Leigh, *J. Chem. Soc., Chem. Commun.*, 628 (1975); (b) D. Scholz and U. Schmidt, *Chem. Ber.*, **107**, 2295 (1974).
(11) B. Ganem and G. W. Holbert, *Bioorg. Chem.*, **6**, 393 (1977).
(12) G. W. Holbert, L. B. Weiss, and B. Ganem, *Tetrahedron Lett.*, 4435 (1976).
(13) H. Plieninger and G. Ege, *Chem. Ber.*, **94**, 2095 (1961).
(14) For a preliminary account of this work, see G. W. Holbert and B. Ganem, *J. Am. Chem. Soc.*, **100**, 352 (1978).
(15) M. E. Kuehne and B. F. Lambert, "Organic Syntheses", Collect, Vol. V, Wiley, New York, N.Y., 1973, p 400.
(16) W. E. Barnett and L. L. Needham, *J. Org. Chem.*, **40**, 2843 (1975).
(17) W. E. Barnett and W. H. Sohn, *J. Chem. Soc., Chem. Commun.*, 472 (1972).
(18) H. Plieninger and G. Ege, *Chem. Ber.*, **94**, 2088 (1961).
(19) N. Ikota and B. Ganem, *J. Am. Chem. Soc.*, **100**, 351 (1978).
(20) E. J. Corey and R. Noyori, *Tetrahedron Lett.*, 311 (1970).
(21) Y. Kishi, M. Aratani, H. Tanino, T. Fukuyama, T. Goto, S. Inone, S. Sugiura, and H. Kakoi, *J. Chem. Soc., Chem. Commun.*, 64 (1972).
(22) K. B. Sharpless and R. C. Michaelson, *J. Am. Chem. Soc.*, **95**, 6136 (1973).
(23) W. D. Emmons and A. F. Ferris, *J. Am. Chem. Soc.*, **75**, 4623 (1953).
(24) G. W. Holbert and B. Ganem, *J. Chem. Soc., Chem. Commun.*, 248 (1978).
(25) D. Swern, "Organic Peroxides", Vol. 2, Wiley-Interscience, New York, N.Y., 1971, p 403.
(26) S. A. Cerefice and E. K. Fields, *J. Org. Chem.*, **41**, 355 (1976).
(27) H. B. Henbest and R. A. L. Wilson, *J. Chem. Soc.*, 1958 (1957).
(28) P. Chamberlain, M. L. Roberts, and G. H. Whitham, *J. Chem. Soc. B*, 1374 (1970).
(29) B. A. Chiasson and G. A. Berchtold, *J. Am. Chem. Soc.*, **96**, 2898 (1974).
(30) S. G. Davies and G. H. Whitham, *J. Chem. Soc., Perkin Trans. 1*, 572 (1977).
(31) G. B. Payne, *Tetrahedron*, **18**, 763 (1962).
(32) J. Rebek, Jr., S. F. Wolf, and A. B. Mossman, *J. Chem. Soc., Chem. Commun.*, 711 (1974).
(33) J. Rebek, S. Wolf, and A. Mossman, *J. Org. Chem.*, **43**, 180 (1978).
(34) DBN has recently been reported to undergo nucleophilic additions: D. C. Palmer and M. J. Strauss, *Tetrahedron Lett.*, 1431 (1976)
(35) S. M. Kupchan, R. J. Hemingway, and R. M. Smith, *J. Org. Chem.*, **34**, 3898 (1969).
(36) R. Hollands, D. Becher, A. Gaudemer, and J. Polonsky, *Tetrahedron*, **24**, 1633 (1968).
(37) J. Singh, K. L. Dhar, and C. K. Atal, *Tetrahedron*, **26**, 4403 (1970).
(38) G. O. Schenck and D. E. Dunlap, *Angew. Chem.*, **68**, 248 (1956).
(39) For related examples see (a) G. O. Pierson and O. A. Runquist, *J. Org. Chem.*, **34**, 3654 (1969); (b) J. E. Baldwin and O. W. Lever, *J. Chem. Soc., Chem. Commun.*, 344 (1973).
(40) G. H. Posner and D. Z. Rogers, *J. Am. Chem. Soc.*, **99**, 8214 (1977).
(41) D. L. Whalen, J. A. Montemarano, D. R. Thakkar, H. Yagi, and D. M. Jerina, *J. Am. Chem. Soc.*, **99**, 5523 (1977).

Structure and Absolute Configuration of Thienamycin

Georg Albers-Schönberg,* Byron H. Arison, Otto D. Hensens, Jordan Hirshfield, Karst Hoogsteen,* Edward A. Kaczka, Robert E. Rhodes, Jean S. Kahan, Frederick M. Kahan, Ronald W. Ratcliffe,* Edward Walton, Linda J. Ruswinkle, Robert B. Morin, and Burton G. Christensen

Contribution from the Departments of Biophysics, Exploratory Microbiology, and Synthetic Chemical Research, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065. *Received Octover 3, 1977*

Abstract: The structure and stereochemistry of the potent new β-lactam antibiotic thienamycin were determined as shown in formula **22.**

Thienamycin (**1**) was discovered in fermentation broths of the soil microorganism *Streptomyces cattleya*.[1] It is a novel β-lactam antibiotic[2] of exceptional antibacterial potency and spectrum including activity against *Pseudomonas* and β-lactamase producing species.[3] Since its first publication in the patent literature[4] several closely related structures have been recognized.[5-7] Recently, the first total synthesis of the antibiotic has been reported.[8] In the first part of this paper we describe the chemical and spectroscopic observations which first led to the elucidation of the new structure. In the second part we discuss the relative and absolute stereochemistry at the three chiral centers of thienamycin.

Structure of Theinamycin

Thienamycin is a zwitterionic compound with an acidic dissociation constant of ca. 3.1. Broad infrared absorption at ca. 1580 cm^{-1} is characteristic of a carboxylate anion and a sharper band at 1765 cm^{-1} is reminiscent of the β-lactam carbonyl absorption of cephalosporins and cephamycins.[9,10] The characteristic ultraviolet absorption maximum at 296–297 nm (ε 7900) in the pH range from 4 to 8 shifts to 309 nm at pH 2 and can be abolished together with biological activity by treating the antibiotic with hydroxylamine at neutral pH. ^1H and ^{13}C NMR signals of thienamycin are listed in Table I. The elemental composition $C_{11}H_{16}N_2O_4S$, mol wt 272, was deduced from field-desorption mass spectra of the antibiotic (MH^+ 273) and from high-resolution mass spectra of the derivatives **2** and **3** (Scheme I). These derivatives were first prepared when only small amounts of partially purified antibiotic were available. For each of them several spectra were averaged to obtain the most accurate *m/e* values. The resulting data sets ruled out alternative compositions which have similar fractional masses. Subsequently, the assignments were confirmed by measurements on and derivatization of the purified antibiotic. Molecular weight determination by ultracentrifugation, sulfur analysis by energy dispersive X-ray fluorescence,

0002-7863/78/1500-6491$01.00/0

Table I. ^1H and ^{13}C NMR Data for Thienamycin

assignment	^1H NMR[a]	^{13}C NMR[b]
CH_3-9	1.27, 3 H, d, J = 6.8 Hz	21.0 (q)
CH_2-4,11,12	3.15, 6 H, m	29.4 (t), 39.6 (t), 40.1 (t)
CH-5	4.20, 1 H, m	53.3 (d)
CH-6	3.39, 1 H, dd, J = 2.9	65.8 or 65.9 (d)
CH-8	4.20, 1 H, m	65.9 or 65.8 (d)
C-2,3		132.8 (s), 137.6 (s)
C-7		166.1 (s)
C-10		180.3 (s)

[a] ^1H NMR spectra were recorded at 100 MHz in D_2O; chemical shifts are given in parts per million downfield from internal DSS. [b] ^{13}C NMR spectra were recorded at 20 MHz in buffered (pD 6.8) D_2O solution at 10 °C; chemical shifts are given relative to internal dioxane at 67.4 ppm.

and a combustion analysis[1,4] substantiated the relationship between the derivatives and the natural product.

Acetylation of thienamycin with acetic anhydride in dimethylformamide or methanol at 0 °C, followed by esterification with diazomethane, gave the crystalline *N*-acetylmethyl ester **2** and the 1,7-seco-*N*,*N*-diacetyldimethyl ester **3.** Table II lists the spectroscopic data for both compounds. Both derivatives show the characteristic ultraviolet absorption maximum of thienamycin near 300 nm which in the case of **2** can be extinguished by treatment in methanolic solution with dilute acid or hydroxylamine. The infrared band at 1765 cm^{-1} in the spectrum of thienamycin, tentatively attributed to a β-lactam carbonyl group, appears at 1779 cm^{-1} ($CHCl_3$, Nujol) in the spectrum of the derivative **2.** Similar frequencies have been observed for the β-lactam carbonyl absorption of cephalosporin esters.[10,11] The derivative **3,** which contains the additional elements of methyl acetate, shows no carbonyl adsorption at frequencies higher than the saturated ester absorption at 1735 cm^{-1} which in turn is absent from the spectrum of **2.** The three most important mass spectral fragmentation processes of **2** and **3** are illustrated in Schemes II and III and Table III and are discussed in the following together with the relevant NMR data of Tables I and II.

The spectrum of **2** is dominated by losses of a $C_4H_5O_2$ moiety (**f** − H; e.g., **a, e, i**); those of **3** by losses of $C_5H_9O_3$ (**f** + OCH_3) resulting in fragments (e.g., **n, q, t**) which compared to those of **2** contain the additional elements of ketene in a reagent derived acetyl group (deuterium shifts, Table III). The further loss of ketene from this group leads to fragments which, judging from the overall pattern, are identical with **a, e,** and **i** (only **o** ≡ **a** is shown in Scheme III). These relations have been interpreted in terms of a very reactive β-lactam in the natural product which only partly survives the derivatization conditions and partly undergoes acetolysis to a mixed anhydride which in turn intramolecularly transfers the acetyl group to the ring nitrogen. Esterification with diazomethane then gives **3.** With pure thienamycin the acetolysis reaction can be carried out quantitatively in glacial acetic acid. This explains an increase in the yield of **3** at the expense of **2** with increasing reaction time or temperature during the acetylation step. The mass spectral fragmentation of **2** is analogous to those of penicillins[12] and cephalosporins,[13] which also lead to protonated iminium ions. The interpretation is furthermore consistent with the above-discussed infrared data. The immediate and important consequence of this interpretation is the requirement for a C_2H_5O substituent at C6 instead of the acylamine group of all previously known β-lactam antibiotics.

Scheme I

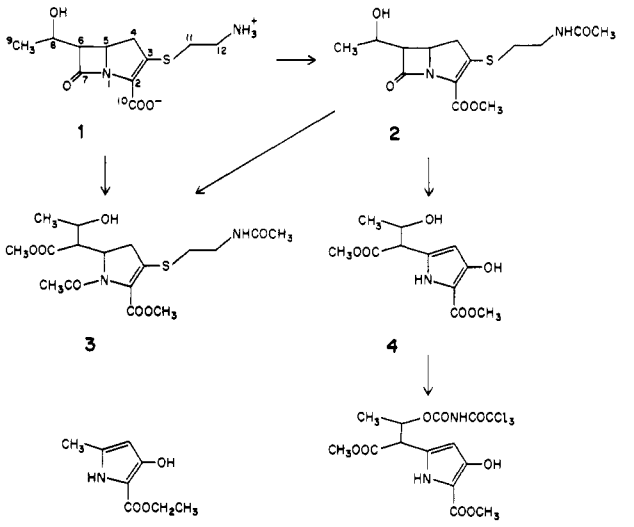
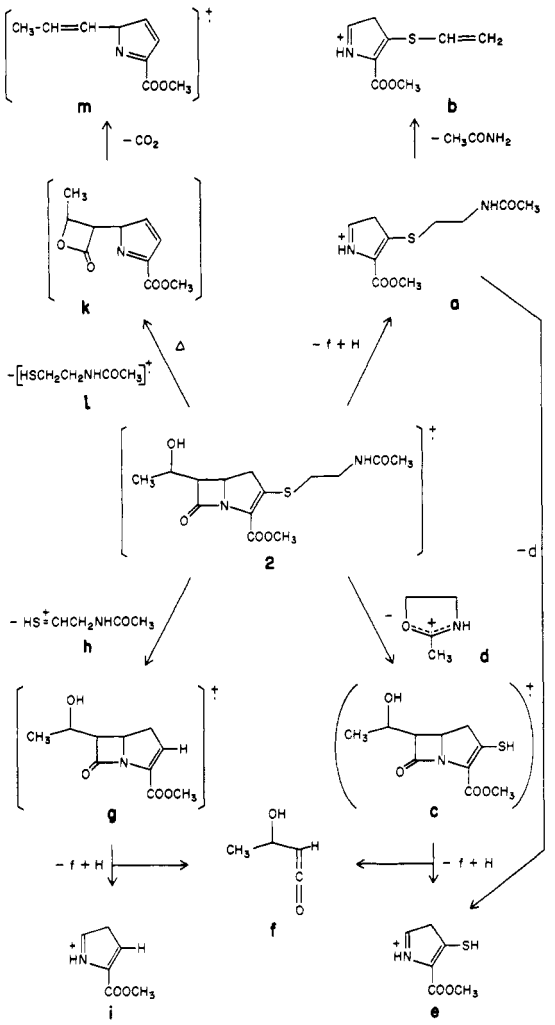


Table II. UV, IR, and ^1H NMR Spectral Data for Derivatives **2, 3, 4,** and **5**[a]

UV: nm (ε) IR: cm^{-1} (state)	H-9	H-8	H-6	H-5	H-4	H-11	H-12	CH_3CO	OCH_3	exchangeable
2, $C_{14}H_{20}N_2O_5S$	1.12, d J = 6.5	3.95, m	3.32 overlapping with H_2O	4.12, dt J = 3, 9	3.18-3.30, m overlapping with H-12	~2.90, m	3.18-3.30, m overlapping with H-4	1.81, s	3.70, s	5.10, d J = 5 8.22, t J = 5
UV: 315 (10 955) IR: 1779, 1692, 1631, 1548 (Nujol)										(OH) (NHCO)
3, $C_{17}H_{26}N_2O_7S$ UV: 305 IR: 1735, 1670, 1630 ($CHCl_3$)	1.18, d J = 6.5	4.07, m D_2O: dq J = 10, 6.5	2.24, dd J = 3.5, 10	5.00, m	2.54-2.78 m (4 H)		3.16, m D_2O: t J = 7	1.66, s 1.90, s	3.34, s 3.57, s	~5.28 m (2 H)
	1.20, d J = 6.5	4.0, m	2.29, dd J = 3.5, 10	5.13, m	2.8—3.3 m (4 H)		~3.50, m	2.02, s 2.08, s	3.53, s 3.88, s	~5.9 m (2 H)
4, $C_{11}H_{15}NO_6$ UV: 270 (16 800) IR: 3425, 2930, 1721, 1664 (Nujol)	1.20, d J = 6.5	4.38, m	3.60, d J = 4		5.75, d J = 2.5 D_2O; s				3.72, s 3.85, s	2.80, d J = 3 (C8-OH)
5	1.34, d J = 6.5	5.65, dq J = 4, 6.5	3.82, d J = 4		5.75, d J = 2.5				3.70, s 3.90, s	

[a] ^1H NMR spectra were recorded at 100 MHz in $CDCl_3$ (**3,** lower row; **4, 5**) and $CDCl_3$-C_6D_6 1:1 (**3,** upper row); the spectrum of **2** at 220 MHz in Me_2SO-d_6. Chemical shifts are given in parts per million relative to Me_4Si as internal standard; coupling constants in hertz. Protons are numbered according to the carbon atoms to which they are attached.

Scheme II



The new lactam substituent is supported and its nature further defined by the ^1H NMR data of Tables I and II and by a spin-decoupling experiment. Double irradiation at the center of the δ 4.20 methine multiplet (area 2 H) of **1** causes the collapse of both the methyl doublet at δ 1.27 and the methine doublet of doublets at δ 3.39 to singlets, thus establishing a $CH_3CH(X)CHCH(Y)$ sequence of hydrogen bearing carbon atoms (X and Y are functional groups of comparable deshielding strength). The assignment of the sequence to the β-lactam and its substituent is supported by the low-field position of the CH-6 signal at δ 3.39 and 3.32 in the spectra of **1** and **2**, respectively, but at δ 2.29 in the spectrum of **3** ($CDCl_3$), after opening of the lactam. The hydroxyethyl structure of the lactam substituent is the only one of five possible combinations of the available atoms which is compatible with the NMR data. Direct evidence for the C8-hydroxy group can, however, be obtained from mass and NMR spectra of **2** and **3**.

The most abundant fragment in the mass spectra of **2** and **3** is **d**, C_4H_8NO, which includes a derivative acetyl group (deuterium shift). The fragmentation leading to **b** indicates a secondary amide (a primary amine in thienamycin) which is confirmed by infrared absorption of **2** at 1631 cm^{-1} (amide I band) and 1548 cm^{-1} (amide II band). The ^{13}C NMR data of Table I then require a $-CH_2CH_2NHCOCH_3$ partial structure for the derivative since the only alternative, $-CH(CH_3)NHCOCH_3$, is preempted by the β-lactam substituent. The assignment is corroborated by the triplet nature of the amidic NH signal at δ 8.22 ($-CH_2NHCOCH_3$) in the NMR spectrum of **2**, and, after equilibration with D_2O, of the methylene signal at δ 3.16 ($-CH_2CH_2NDCOCH_3$) in the

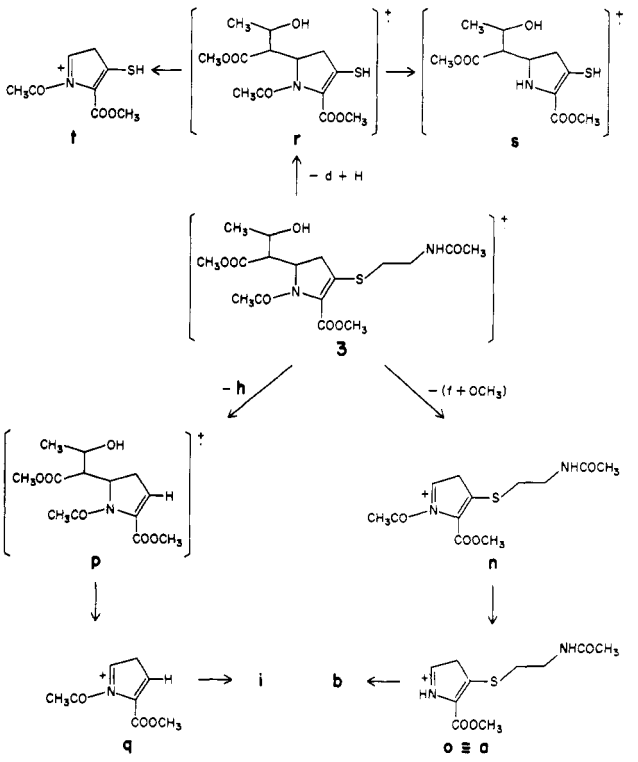
Table III. High-Resolution Mass Spectra and ^2H-Shift Data for **2** and **3**

	composition	calcd mass	found mass	d_3-acetyl analogue
2	$C_{14}H_{20}N_2O_5S$	328.1093	328.1081	331
a	$C_{10}H_{15}N_2O_3S$	243.0803	243.0794	246
b	$C_8H_9NO_2S$	183.0354	183.0352	183
c	$C_{10}H_{13}NO_4S$	243.0565	low abundance uncertain	243
d	C_4H_8NO	86.0606	86.0593 (90%)	89
e	$C_6H_8NO_2S$	158.0276	158.0272	158
f	$C_4H_6O_2$	86.0368	86.0369 (10%)	86
g	$C_{10}H_{13}NO_4$	211.0845	211.0846	211
h	C_4H_8NOS	118.0327	118.0336	121
i	$C_6H_8NO_2$	126.0555	126.0556	126
k	$C_{10}H_{11}NO_4$	209.0688	209.0689	209
l	C_4H_9NOS	119.0405	119.0419	122
m	$C_9H_{11}NO_2$	165.0790	165.0788	165
3	$C_{17}H_{26}N_2O_7S$	402.1461	402.1465	408
n	$C_{12}H_{17}N_2O_4S$	285.0909	285.0927	291
o	$C_{10}H_{15}N_2O_3S$	243.0803	243.0806	247
p	$C_{13}H_{19}NO_6$	285.1212	285.1206	288
q	$C_8H_{10}NO_3$	168.0661	168.0660	171
r	$C_{13}H_{19}NO_6S$	317.0933	317.0919	320
s	$C_{11}H_{17}NO_5S$	275.0827	275.0825	276
t	$C_8H_{10}NO_3S$	200.0381	200.0371	203

spectrum of **3**. The same results have been obtained in equivalent spin-decoupling experiments. Corollary mass spectral fragments of **d** are, for instance, **c** or **e** and **r**.

The fragments **g**, **q**, **p**, and **h**, the latter again including the derivative acetyl group, show that the above-discussed C_4H_8NO moiety is attached to the sulfur atom: $-SCH_2CH_2NHCOCH_3$. This is in good agreement with the ^{13}C NMR data of Table I which suggest two methylene groups bound to heteroatoms (39.6 and 40.1 ppm). The mass spectral fragmentation can be compared with the loss of thioformal-

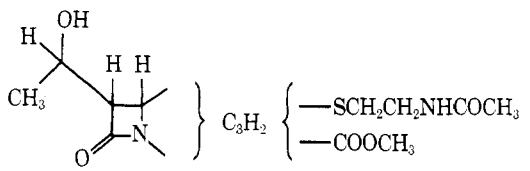
Scheme III



dehyde from thioanisole.[14] Confirmation of these interpretations is provided by amino acid analysis of thienamycin following performic acid oxidation which shows a product which has the chromatographic retention properties of taurine.

β-Lactam compounds have a tendency to undergo thermal degradation reactions during vaporization in the mass spectrometer. This has been observed for derivatives of the cephamycins[10] and is also true for thienamycin derivatives. Two thermal reactions of **2** can be observed in its mass spectra. A lactam–lactone rearrangement leads to the expulsion of *N*-acetylcysteamine and is followed by the electron impact induced loss of carbon dioxide. As a consequence, abundances of the ions **k**, **l**, and **m** (Scheme II) increase with time, particularly if the sample is vaporized at a slow rate. Alternatively, a retro-2,2-cycloaddition reaction of the β-lactam can occur and result in a large increase in abundance at *m/e* 242, $C_{10}H_{14}N_2O_3S$, at the expense of the electron impact induced protonated fragment **a** at *m/e* 243. The same reactions are probably responsible for the decomposition melting point of **2** above 150 °C. Other thienamycin derivatives, as, e.g., the *N*-benzyloxycarbonyl benzyl ester **7** (see below), melt sharply at lower temperature, vaporize at lower temperature, and show little evidence of thermal reactions during mass spectrometric analysis.

The derivative **2** can now be written as the partial structure **2a.** The undefined middle portion comprises the third meth-



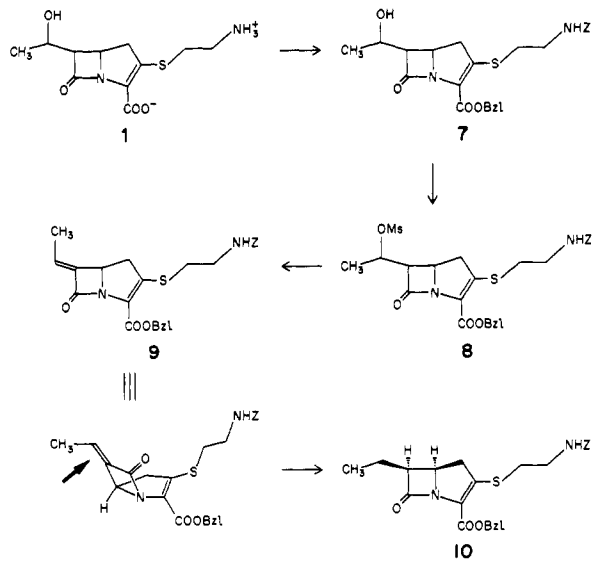
2a

ylene group (^{13}C NMR signals at 29.4 ppm, Table I) and the two carbons of a tetrasubstituted double bond (132.8 and 137.6 ppm); it must provide sites for the attachment of the acetylcysteamine and carboxylic ester groups and allow for the ultraviolet absorption of thienamycin and its derivatives.

Methanolysis of **2** in the presence of air gave *N,N*-diacetylcysteamine disulfide (M^+ 236.0648, calcd 236.0653) and the derivative **4** (Scheme I and Table II). The aliphatic portion of **4** is mass spectrometrically defined by consecutive losses of acetaldehyde (McLafferty rearrangement) leading to an enolized ester ion (*m/e* 213.0633, calcd 213.0637) which in turn loses methanol (*m/e* 181); the aromatic portion shows an equally characteristic loss of methanol from the enolized β-keto ester (*m/e* 149). ^1H NMR data for **4** and for the derivative **5**, which was generated in situ by reaction of the secondary hydroxy group with CCl_3CONCO, are given in Table II. The validity of the assignment of the characteristic aromatic doublet at δ 5.75 is confirmed by the ^1H NMR spectrum of 2-carboethoxy-3-hydroxy-5-methylpyrrole (**6**) (H-4: δ 5.57, 1 H, d, J = 2.8 Hz, s after equilibration with D_2O), which was prepared by reported procedures[15] and in all respects closely resembles **4** (UV, MS, $FeCl_3$). The mechanism of the oxidative methanolysis of **2** has not been investigated in detail.[16]

The analogy between **6** and **4** unambiguously positions the carboxyl group of thienamycin and leaves **1** as the only structure which interprets all data. However, additional spectroscopic evidence is available. The ^1H NMR spectrum of **2** shows the H-5 signal as a doublet of triplets at δ 4.12, thus extending the sequence of hydrogen-bearing carbon atoms from the methyl group to a methylene group beyond C-5. Finally, the ultraviolet absorption maximum of thienamycin at 296 nm is well supported by absorption at the same wavelength which has been reported for a synthetic isocephalosporin.[17] Other examples of this chromophore have since been observed.[18-20]

Scheme IV



Stereochemistry of Thienamycin

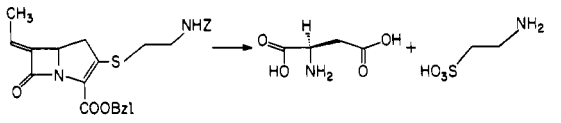
Two independent approaches have been taken to determine the absolute configuration at the three chiral centers of thienamycin. One was aimed at a single-crystal X-ray analysis and, in the event that the absolute configuration could not be obtained in this way, determination of the chirality at C8 by an alternative method. The second approach relied entirely on chemical transformations and spectroscopy. The results of the two approaches confirm each other. We first report on the chemical and spectroscopic observations and conclude our account of this structure determination with the X-ray analysis of *N*-acetylthienamycin methyl ester.

^1H NMR spectra of thienamycin and of its *N*-acetyl methyl ester **2** show small vicinal coupling constants, $J_{5,6} \leq 3$ Hz, for the two β-lactam hydrogens. The epithienamycins A and B,[5] which are stereoisomers of *N*-acetylthienamycin, show $J_{5,6}$ = 5 Hz. Past experience with penicillins and cephalosporins, including 6-hydroxyethylpenam[21] and isocephems,[17] shows the cis relation of the β-lactam hydrogens to be always associated with the larger coupling. On this basis the trans β-lactam configuration was assigned to thienamycin. The correctness of the assignment was demonstrated by X-ray crystallography (see below) and by converting thienamycin to a compound with assured cis configuration of the β-lactam hydrogens.

Successive N-acylation and esterification of thienamycin gave the *N*-benzyloxycarbonyl benzyl ester **7.** This was converted (Scheme IV) to the mesylate **8** which in methanol at room temperature in the presence of sodium bicarbonate underwent elimination to the enelactam **9.** Catalytic reduction of the new double bond in **9** was expected to occur mainly from the less hindered exo face[22] and, indeed, hydrogenation of the enelactam afforded a single 6-ethyl derivative **10.** The large $J_{5,6}$ = 5.7 Hz coupling constant in the NMR spectrum of **10** is consistent with cis stereochemistry. It then follows that **7** ($J_{5,6}$ = 2.5 Hz) and thienamycin are trans substituted. Thus, the above-stated rule, that the cis relation of the β-lactam hydrogens in a bicylcic β-lactam is associated with a larger coupling constant than the trans relation, includes the novel thienamycin ring system.

The absolute configuration of C-5 was determined as shown in Scheme V. A sample of enelactam **9** was treated successively with ozone in methylene chloride at −78 °C, hydrogen peroxide in glacial acetic acid at 25 °C, and 6 N hydrochloric acid at 100 °C. The residue from the hydrolysis was shown by NMR, TLC, and amino acid analysis to be a mixture of aspartic acid (**11,** 69% yield) and taurine (**12,** 82% yield). The

Scheme V



9 11 12

optical rotation of the crude product mixture and CD measurements on the copper complex[23] of the purified product revealed that the aspartic acid had the *R* configuration. Since the aspartic acid is derived from carbon atoms 3, 4, 5, and 6 and the bridgehead nitrogen atom, the isolation of the *R* enantiomer from the degradation sequence demonstrates that the absolute configuration of C-5 in thienamycin is *R*. From the relative C-5, C-6 stereochemistry it follows that C-6 has the *S* configuration.

The absolute configuration at C-8 was determined by applying Mosher's[24] NMR configuration-correlation method for diastereomeric α-methoxy-α-trifluoromethylphenylacetyl (MTPA) esters and by using Horeau's[25] method of partial kinetic resolution of racemic α-phenylbutyric acid anhydride in the formation of diastereomeric esters. Acylation of *N*-benzyloxycarbonylthienamycin benzyl ester **7** with (*R*)-(+)- and (*S*)-(−)-α-methoxy-α-trifluoromethylphenylacetyl chlorides **13** gave the diastereomeric esters **14** and **15** (Scheme VI). Mosher's model predicts that the diastereomer **14** will show the H-9 methyl resonance upfield and the H-5, H-6, and CF_3 resonances downfield relative to the same resonances in diastereomer **15** if the side chain has the *R* configuration. If the configuration were *S*, the prediction would be reversed. The data in Table IV reveal that the hydroxyethyl center in thienamycin has the *R* configuration.

Support for applying Mosher's method in this complex case was provided by similarily derivatizing the penam **16.** The side chain of this compound has been shown[21] to have the *R* configuration by X-ray analysis. The relative chemical shifts for diastereomers **17** and **18** (see Table IV) conform with prediction and demonstrate that the correlation model is valid in a closely related case.

Applying Horeau's method of kinetic resolution of racemic α-phenylbutyric anhydride **19,** which has been used in several

Scheme VI

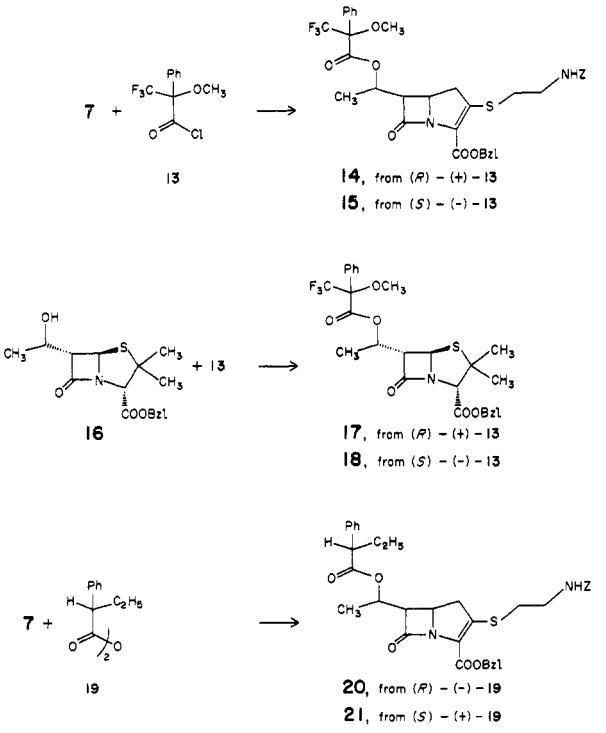


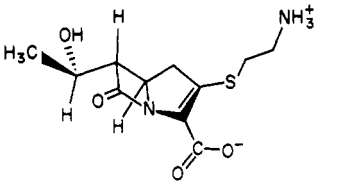
Table IV. NMR Chemical Shifts for MTPA and α-Phenylbutyryl Esters[a]

derivative	$CHCH_3$	H-5	H-6	CF_3
14	1.39	49.0		7.35
15	1.50	3.97		7.30
17	1.38	5.20	3.47	7.30
18	1.47	5.13	3.38	6.96
20	1.36		3.04	
21	1.27		3.20	

[a] Spectra were determined in $CDCl_3$ solution. Proton chemical shifts are reported in parts per million downfield from Me_4Si; fluorine shifts are in parts per million downfield from external TFA. The H-6 resonance in derivatives **14** and **15** is part of a complex, unresolved multiplet.

complex cases,[27-29] the *N*-benzyloxycarbonyl benzyl ester **7** was reacted with excess anhydride in cold pyridine. The diastereomeric esters **20** and **21** were obtained as virtually the only products besides traces of starting material and, after hydrolysis of the recovered excess anhydride, dextrorotatory α-phenylbutyric acid in 18% optical yield. According to Horeau's empirical correlation the configuration at C-8 of **7,** and consequently thienamycin, must be *R*. A NMR configuration correlation model for α-phenylbutyrates analogous to that for MTPA esters has been established using various methylcarbinols of known configuration.[30] This model predicts that the derivative **20** derived from (*R*)-(−)-α-phenylbutyric acid anhydride will show the downfield signal for the C-9 methyl group and upfield signal for the C-6 hydrogen compared to the signals of the same groups in the alternative diastereomer **21** if C-8 has the *R* configuration. The data in Table IV again indicate the *R* configuration at C-8.[31,32]

In summary, thienamycin has the 5*R*,6*S*,8*R* stereochemistry as depicted in structure **22.**



22

X-ray Analysis

Because of the instability of thienamycin, it has not been possible so far to grow crystals of the antibiotic itself or one of its salts. However, during the course of the above-described analyses single crystals in the form of thin needles were obtained by crystallization of the *N*-acetylmethyl ester **2** from acetonitrile–benzene–hexane. The crystals were orthorhombic with $a = 4.726$ (2), $b = 13.881$ (9), and $c = 24.592$ (12) Å. Systematic absences indicated the space group $P2_12_12_1$ with four molecules in the unit cell as estimated by the calculated density. A needle approximately 0.2 cm long and 0.02 cm in diameter was used for data collection. Intensities were measured by θ–2θ scan with a Syntex $P2_1$ automated diffractometer with graphite monochromatized Cu Kα radiation. Data were collected to a 2θ value of 115° with variable scan width and a scan rate of 1°/min. Deterioration of the intensities was not observed during the course of data collection. After Lorentz and polarization correction 722 reflections (20% of the Cu Kα Ewald sphere) with $I > 3\sigma(I)$ were used in the subsequent calculations.

The structure was solved by direct methods[33] and refined[34] by block-diagonal least-squares methods. Hydrogen atoms were located by difference synthesis. Final least-squares re-

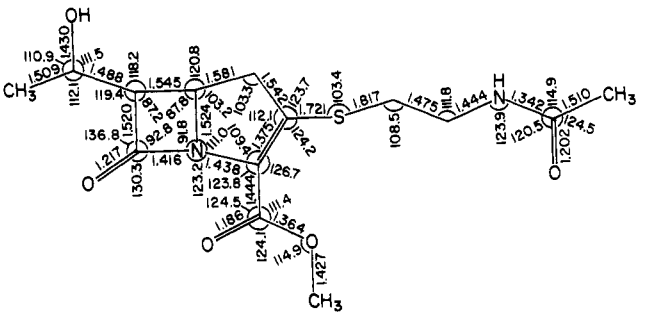


Figure 1. Bond angles and bond distances in *N*-acetylthienamycin methyl ester **2**.

finement with anisotropic temperature factors for the nonhydrogens atom and isotropic temperature factors for the hydrogen atoms lowered the *R* value of 0.067. No attempt was made to determine the absolute configuration with anomalous dispersion. Bond lengths and bond angles for all nonhydrogen atoms are shown in Figure 1. The estimated standard deviations are 0.03 Å and 1°, respectively. Figure 2 presents a stereoscopic ORTEP[35] view of one molecule.

The bicyclic system of the underivatized, zwitterionic antibiotic is likely to have somewhat different dimensions, but the stereochemistry of the lactam nitrogen will not be very different from that in the derivative. The sum of the three nitrogen bond angles (Σ_N) and the distance (D) of the nitrogen from the plane of the attached three carbon atoms, which have been listed for a number of β-lactam compounds[36] and calculated from available X-ray data for a cephamycin derivative,[37] are compared in Table V. The thienamycin derivative with $\Sigma_N = 326°$ and $D = 0.49$ Å is the most folded fused β-lactam ring system for which X-ray data have been reported. In spite of this geometry and of the high chemical reactivity of the thienamycin β-lactam, the β-lactam infrared band for **2** and **7** in Nujol or chloroform solution appears at the relatively low frequency of 1779 cm^{-1}. For comparison, 6β-acylaminopenem esters[18] in methylene chloride absorb near 1800 cm^{-1} and even the more planar and less reactive cephalosporin esters in solution absorb at slightly higher frequency.[11] The shift can probably be attributed to the replacement of the ring heteroatom by a methylene group.[38]

In summary, thienamycin has the structure and complete stereochemistry as shown in formula **22**. The absolute configuration about the bridgehead carbon and nitrogen atoms is the same as in other naturally occurring fused β-lactam compounds (penicillin, cephalosporin, cephamycin, clavulanic acid[42]). Sterically, the hydroxyethyl side chain appears to be related to the methoxy group of the cephamycins.[43] The discovery of thienamycin is not only important because of its high potency and wide spectrum as an antibiotic; it also represents, together with clavulanic acid, the first biogenetically novel,[44] fused β-lactam antibiotic since the discovery of penicillin. The biosynthesis of the thienamycin ring system and its possible biogenetic relationship to clavulanic acid will be discussed in a forthcoming paper.

Table V. Σ_N and D Values[a] Determined from X-ray Crystallographic Data

	Σ_N, deg	D, Å
N-acetylthienamycin methyl ester (**2**)	325.9	0.49
penicillin V[35]	337	0.40
ampicillin[35]	339	0.38
6-APA[35]	343	0.32
cephaloridine[35]	350.7	0.24
7-phenylacetamido-7-methoxy-3-methyl-3-cephem *tert*-butyl ester[36]	356.7	0.15

[a] See text.

Experimental Section

Infrared spectra were recorded on either a Perkin-Elmer 137, 267, or 421 spectrometer, and ultraviolet spectra on a Perkin-Elmer 202 or Cary 15 instrument. ^1H NMR spectra were recorded on Varian T-60, HA-100, and SC-300 instruments with Me_4Si as internal standard (δ 0 ppm), and ^{13}C NMR spectra on a Varian FT-20 with dioxane as internal standard (δ 67.4 ppm). Low-resolution mass spectra were obtained on a LKB-9000 and high-resolution spectra on a MS-902 spectrometer.

***N*-Acetylthienamycin Methyl Ester (2) and 1,7-Seco-*N,N*-diacetylthienamycin Dimethyl Ester (3).** Ca. 30% pure[45] antibiotic (11.5 mg), showing a single ultraviolet absorption maximum at 296 nm, was suspended and stirred at room temperature in dry dimethylformamide (0.5 mL) and freshly distilled acetic anhydride (1 mL). After 1 h, solvent and excess reagent were removed by repeated extraction wtih 50-mL portions of hexane and, finally, a small amount of ether and hexane. The semisolid residue was dissolved in ice-cold methanol, briefly treated with freshly prepared ethereal diazomethane solution, and evaporated to dryness under reduced pressure at room temperature. The residue was partly soluble in methylene chloride. The insoluble material appeared to be polymeric. The soluble material, by TLC analysis on silica gel GF(254) plates (Analtech) in chloroform–methanol (9:1), showed fluorescence quenching products, coinciding with most of the iodine straining material, at R_f 0.65, 0.54, and 0.41. Preparative chromatography on thoroughly prewashed (methylene chloride–methanol, 4:1) silica gel HF(254) plates (Analtech) gave 1,7-seco-*N,N,O*-triacetylthienamycin dimethyl ester[46] (1.5 mg) as an oil (R_f 0.65; M^+ 444.1549, $C_{19}H_{28}N_2O_8S$, calcd 444.1567), 1,7-seco-*N,N*-diacetylthienamycin dimethyl ester (**3**, 2.5 mg, R_f 0.54), and *N*-acetylthienamycin methyl ester (**2**, 1.3 mg, R_f 0.41). **3** was obtained in spectral purity by repeated chromatography

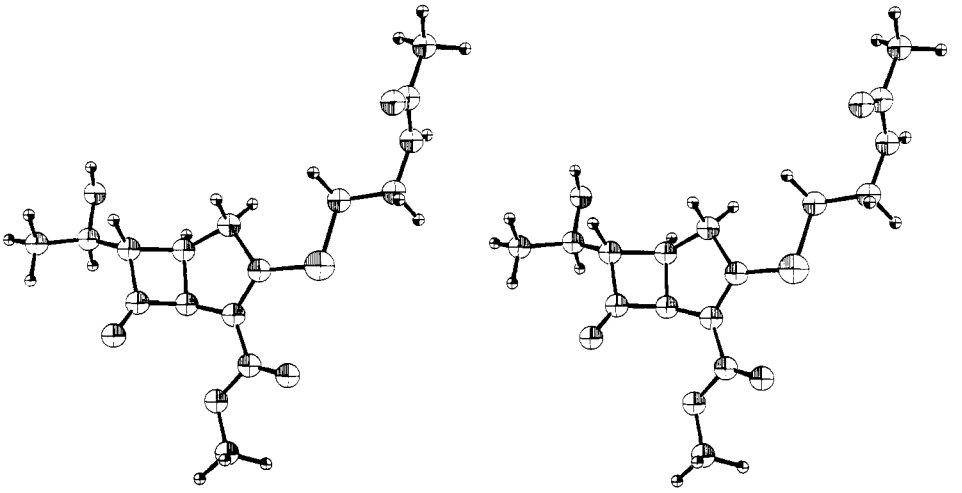


Figure 2. ORTEP view of *N*-acetylthienamycin methyl ester **2**.

on plates in methylene chloride–2-propanol solvents. **2** was crystallized by trituration of the residue of an evaporated chloroform–methanol solution with acetone.

Seven lyophilized fractions of the antibiotic from a final Biogel P-2 chromatogram in 2,6-lutidine acetate buffer (0.1 M, pH 7) containing most of the bioactivity and totaling 73.3 mg of E% 215 at 296 nm, were each stirred for 15–20 min at 0 °C in dimethylformamide–acetic anhydride (2:1, 3 mL) under a cover of hexane. After removal of solvent and reagent as described above, the residues were dissolved in ice-cold methanol, combined, and briefly treated with ethereal diazomethane solution (74.8% yield by UV). The solution was evaporated to dryness and the methylene chloride soluble portion chromatographed in chloroform–methanol (9:1) on four prewashed SiO_2 HF(254) plates (20 × 20 × 0.025 cm). The zones corresponding to **2** and **3** were eluted with the same solvent. The crude **3** was rechromatographed on two plates yielding spectroscopically pure 1,7-seco-*N,N*-diacetylthienamycin dimethyl ester (6.5 mg). The crude **2** (20.6 mg), after two crystallizations of an almost completely evaporated methanol solution from acetone, gave pure *N*-acetylthienamycin methyl ester (12.3 mg, 18.9% yield): UV (MeOH) 315 nm (max, ϵ 10 955), 247 nm (min, ϵ 1334); mp 161–181 °C dec.

Pure **2** (10 mg) was dissolved by warming in dry acetonitrile (0.5 mL) and the solution immediately diluted first with benzene (2.5 mL) and subsequently hexane (10 mL). After several days, thin but relatively sturdy and well-formed needles had grown which proved suitable for single-crystal X-ray analysis.

Methanolysis of *N*-Acetylthienamycin Methyl Ester 2. Mother liquors of **2** in methanol–acetone contained a UV fluroescence quenching material of $R_f \sim 0.6$ on silica gel GF(254) plates in chloroform–methanol (9:1). The combined mother liquors of several derivatization experiments yielded a total of 7.7 mg of this material which after two further chromatograms on silica gel HF(254) plates gave spectroscopically pure **4** (1.8 mg): M^+ 257; UV (MeOH) 270 nm ($\epsilon \sim 17\,000$); $FeCl_3$ green-blue.

Solutions of pure **2** of 1 mg/mL in methanol were kept for 14 h at room temperature without loss of optical density at 315 nm. In 0.02 M methanolic H_3PO_4 solution under nitrogen the 315-nm absorption rapidly diminished without appearance of a new absorption. Subsequent admission of air to the solution caused the appearance of a sizable maximum near 270 nm. In 0.02 M methanolic H_3PO_4 solution under air the 315-nm absorption of **2** disappeared while simultaneously a new maximum at 269–270 nm appeared reaching $\epsilon \sim 16\,500$ (based on mol wt 257) after standing overnight.

Pure **2** (3.4 mg) was left for several days in a concentrated methanol–acetone (5:95) solution in an attempt to achieve slow crystallization. No crystallization occurred. The sample was found completely degraded to **4** and a product devoid of UV-fluorescence quenching properties but staining with iodine ($R_f \sim 0.55$): mass spectrum *m/e* 86.0601, 118.0340 (117–119), 236.0648 (M^+), identical by TLC and MS with $(CH_3CONHCH_2CH_2S)_2$.

2-Carboethoxy-3-hydroxy-5-methylpyrrole (6). Glycine ethyl ester (2.07 g, 0.02 mol), ethyl acetoacetate (2.60 g, 0.02 mol), and a catalytic amount of glycine ethyl ester hydrochloride were refluxed in benzene (100 mL) with azeotrope distillation of the liberated water. After 2 h the solvent was evaporated under reduced pressure and the residue chromatographed on silica gel in hexane–ether (4:1) to give pure Schiff base (M^+ 215). The solidified Schiff base (243 mg, 1.09 mmol) was added to a suspension of freshly prepared sodium ethoxide (10.9 mmol) in ether and stirred for 24 h. The precipitate was filtered, washed with ether, and treated with dilute acetic acid, forming two layers. A small amount of insoluble material was filtered off, and the organic layer was taken up in ether, separated, washed with water and saturated sodium chloride solution, dried over anhydrous potassium carbonate, and evaporated. A portion of the residue was purified from minor contaminants by TLC on silica gel in hexane–ether (4:1): UV (CH_3OH) 270 nm (ϵ 23 150); $FeCl_3$ green-blue, on silica gel plate gray; M^+ 169; ^1H NMR (100 MHz, $CDCl_3$) δ 1.35 (t, 3, J = 7 Hz, OCH_2CH_3), 2.23 (s, 3, $=CCH_3$), 4.31 (q, 2, J = 7 Hz, OCH_2CH_3), 5.58 (d, 1, J = 2.5 Hz, =CH, s after exchange with D_2O), ~7.8 (exchangeable).

***N*-Benzyloxycarbonylthienamycin Benzyl Ester (7).** A solution of benzyl chloroformate (1.02 g, 6 mmol) in dioxane (10 mL) was added over 10 min to an ice-cold solution of thienamycin (1.10 g, 4.05 mmol) and sodium bicarbonate (3.40 g, 40.5 mmol) in water (100 mL) and dioxane (90 mL). After having been stirred for an additional 10 min in the cold, the mixture was acidified to pH 7 with cold 1 M sulfuric acid and extracted with cold ether (4 × 100 mL). The aqueous portion was layered with ice-cold ethyl acetate (100 mL) and vigorously stirred while acidifying to pH 3 with cold 1 M sulfuric acid. The layers were separated and the aqueous phase was extracted with more ethyl acetate (2 × 20 mL). The combined ethyl acetate solution was washed with cold brine and extracted throughly with ice-cold 0.05 N lithium hydroxide solution (80 mL). The aqueous phase was concentrated under vacuum to remove organic solvents and lyophilized to provide crude *N*-benzyloxycarbonylthienamycin lithium salt (1.21 g) as a yellow solid.

The salt was suspended in hexamethylphosphoramide (14 mL) and treated with benzyl bromide (0.88 mL, 7.4 mmol). The mixture was stirred at room temperature for 2.5 h, diluted with ethyl acetate, washed thoroughly with water, 5% sodium bicarbonate solution, and brine, dried ($MgSO_4$), and evaporated under vacuum. The residue was triturated with hexane to remove excess benzyl bromide, dissolved in warm ethyl acetate (20 mL), diluted with ether (20 mL), and left in a refrigerator for several days. The off-white, fibrous needles (0.62 g) of **7** were collected, washed with ether, and dried under vacuum: mp 119–121 °C; IR ($CHCl_3$) 3460, 1779, 1722, 1713, 1510, 1333, 1136 cm^{-1}; UV (MeOH) 317 nm (ϵ 11 600); ^1H NMR (300 MHz, CD_3OD spiked $CDCl_3$) δ 1.28 (d, 3, J = 6.5 Hz, $CHCH_3$), 2.95 (m, 2, SCH_2), 3.08 (dd, 1, J = 9, 18 Hz, H-4a), 3.15 (dd, 1, J = 2.5, 7 Hz, H-6), 3.35 (dd, 1, J = 9, 18 Hz, H-4b), 3.37 (m, 2, NCH_2), 4.13 (dq, 1, J = 7, 6.5 Hz, $CHCH_3$), 4.19 (dt, 1, J = 2.5, 9 Hz, H-5), 5.08 (s, 2, NCO_2CH_2Ph), 5.23 and 5.31 (AB q, 2, J = 12.5 Hz, CO_2CH_2Ph), 5.80 (m, 1, NH), 7.34 (m, 10, phenyl); mass spectrum *m/e* 496 (M^+), 478, 452, 410, 361, 318, 91.

***N*-Benzyloxycarbonyl-*O*-methanesulfonylthienamycin Benzyl Ester (8).** Methanesulfonyl chloride (17.2 mg, 0.15 mmol) in methylene chloride (0.39 mL) was added dropwise over 5 min to a solution of **7** (50 mg, 0.1 mmol) and triethylamine (0.028 mL, 0.2 mmol) in ice-cold methylene chloride (3 mL). The solution was stirred for an additional 90 min in the cold, then diluted with methylene chloride, washed with H_2O, 0.1 M pH 3 buffer, and 5% sodium bicarbonate solution, dried ($MgSO_4$), and evaporated under vacuum. The residual oil was lyophilized from benzene to afford mesylate **8** (53 mg) as a pale yellow, amorphous powder: IR (CH_2Cl_2) 3445, 1782, 1722, 1512, 1334, 1179, 1137 cm^{-1}; UV (dioxane) 317 nm (ϵ 11 100); ^1H NMR (100 MHz, $CDCl_3$) δ 1.56 (d, 3, J = 6.5 Hz, $CHCH_3$), 2.8–3.5 (m, 7, CH_2, SCH_2, NCH_2, H-6), 3.00 (s, 3, OSO_2CH_3), 4.28 (dt, 1, J = 2.5, 9 Hz, H-5), 5.08 (q, 1, J = 6.5, $CHCH_3$), 5.09 (s, 2, NCO_2CH_2Ph), 5.26 (AB q, J = 14 Hz, CO_2CH_2Ph), 7.32 (m, 10, phenyl); mass spectrum *m/e* 574 (M^+), 478, 410, 259, 91.

***N*-Benzyloxycarbonyl-6,8-anhydrothienamycin Benzyl Ester (9).** A mixture of mesylate **8** (44 mg, 0.077 mmol), sodium bicarbonate (12.9 mg, 0.154 mmol), and methanol (2.5 mL) was stirred at room temperature for 100 min, then diluted with methylene chloride and washed thoroughly with water. The methylene chloride solution was dried ($MgSO_4$) and evaporated under vacuum. The residual oil (33 mg) was twice chromatographed on silica gel GF plates, using 3:1 ethyl acetate–chloroform as developing solvent, to afford enelactam **9** (16 mg) as a pale yellow oil: IR ($CHCl_3$) 3480, 1779, 1715, 1508, 1330, 1271, 1178, 1121 cm^{-1}; UV (MeOH) 306 nm; ^1H NMR (100 MHz, $CDCl_3$) δ 1.80 (d, 3, J = 7 Hz, $CHCH_3$), 2.6–3.7 (m, 6, CH_2, SCH_2, NCH_2), 4.68 (dt, 1, J = 2, 9 Hz, H-5), 5.07 (s, 2, NCO_2CH_2Ph), 5.28 (AB q, 2, J = 12 Hz, CO_2CH_2Ph), 6.36 (dq, 1, J = 2, 7 Hz, $CHCH_3$), 7.30 (m, 10, phenyl); mass spectrum *m/e* 478 (M^+), 410, 343, 269, 210.

Hydrogenation of Enelactam 9. A mixture of enelactam **9** (15 mg), platinum oxide (10 mg), and ethyl acetate (1.5 mL) was hydrogenated at 40 psi for 2 h. Additional catalyst (5 mg) was added and the hydrogenation was continued for an additional 2 h. The mixture was filtered through a pad of 1:1 silica gel G–magnesium sulfate (1 g of each) using ethyl acetate as eluent. Evaporation of the solvent under vacuum left an oil (10 mg) which was purified by chromatography on a silica gel GF plate using 3:1 ethyl acetate–chloroform as developing solvent. The major UV-visible band at R_F 0.67 provided the ethyl derivative **10** (4 mg) as an oil: IR ($CHCl_3$) 3470, 1776, 1715, 1505, 1325, 1276, 1230, 1125 cm^{-1}; UV (MeOH) 316 nm; ^1H NMR (100 MHz, $CDCl_3$) δ 1.01 (t, 3, J = 7.5 Hz, CH_2CH_3), 1.77 (m, 2, CH_2CH_3), 2.8–3.6 (m, 7, CH_2, SCH_2, NCH_2, H-6), 4.19 (dt, 1, J = 5.7, 9.5 Hz, H-5), 5.08 (s, 2, NCO_2CH_2Ph), 5.27 (AB q, 2, J = 13 Hz, CO_2CH_2Ph), 7.32 (m, 10, phenyl); mass spectrum *m/e* 480 (M^+), 410, 91.

Oxidative Degradation of Enelactam 9. A solution of enelactam **9**

(12 mg, 0.025 mmol) in methylene chloride (2 mL) was stirred under an atmosphere of ozone for 1 h at −78 °C. Excess ozone and solvent were removed in a stream of nitrogen while warming to room temperature. The residue in acetic acid (2 mL) was treated with 30% hydrogen peroxide solution (0.3 mL) and kept at room temperature for 2 h. The solution was evaporated under vacuum to a residue which was taken up in 6 N hydrochloric acid (3 mL) and heated at 100 °C for 2 h. After cooling, the reaction mixture was evaporated under vacuum and stripped with water. The residual solid was dissolved in water (1.00 mL).

TLC analysis of the aqueous solution on silica gel using 2:2:1 chloroform–methanol–acetic acid as developing solvent showed two ninhydrin-positive zones of identical mobility with authentic samples of aspartic acid (R_f 0.33) and taurine (R_f 0.62). Amino acid analysis of an aliquot of the aqueous solution gave values of 0.0172 mmol/mL of aspartic acid and 0.0214 mmol/mL of taurine. An aliquot (0.88 mL) of the aqueous solution was evaporated to dryness and the residue was dissolved in 6 N hydrochloric acid. The optical rotation of this solution was $[\alpha]_D$ −17.6° (*c* 0.1 based on amino acid analysis, lit. value for (*R*)-Asp −23°).

The rotation solution was concentrated under vacuum and the residue lyophilized from water to give a solid (6.6 mg). This material was purified by column chromatography on silica gel (4 g). Elution with 2:2:1 chloroform–methanol–ammonium hydroxide provided taurine (2.2 mg) and aspartic acid (2.2 mg, ^{1}H NMR in D_2O identical with that of an authentic sample). The aspartic acid sample was dissolved in water (1.00 mL). Amino acid analysis indicated an aspartic concentration of 0.009 22 mmol/mL. An aliquot (0.605 mL) of the solution was treated with copper(II) chloride monohydrate (0.86 mg) and diluted to 10 mL with 0.1 M pH 8.4 phosphate buffer (final concentration 0.28 mM in $(Asp)_2Cu$). This solution showed molar ellipticities of $[\theta]^{221}$ −1150 and $[\theta]^{260}$ +1650 deg cm^2/dmol. Standard 0.5 mM solutions of (*R*)- and (*S*)-$(Asp)_2Cu$ gave values of −1600 and +2000 and +1800 and −1920, respectively.

Preparation of α-Methoxy-α-trifluoromethylphenylacetyl (MTPA) Esters 14 and 15. (*S*)-(+)-α-Methoxy-α-trifluoromethylphenylacetyl chloride (MTPA-Cl, 0.026 mL, 0.14 mmol) was added to a solution of alcohol **7** (50 mg, 0.03 mmol) in anhydrous pyridine (0.3 mL) and carbon tetrachloride (0.3 mL). The reaction mixture was then shaken and left to stand at room temperature for 2 h. The mixture was diluted with methylene chloride, washed with water, 1 N hydrochloric acid, 1 M dipotassium hydrogen phosphate solution, and brine, dried ($MgSO_4$), and concentrated under vacuum. Chromatography of the residual oil (62 mg) on a silica gel GF plate using 2:1 chloroform–ethyl acetate as solvent provided MTPA ester **14** (38 mg) as a clear oil: IR (CH_2Cl_2) 3450, 1778, 1745, 1718, 1506, 1328, 1134, 1016 cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.39 (d, 3, *J* = 6.5 Hz, $CHCH_3$), 2.7–3.5 (m, 7, CH_2, SCH_2, NCH_2, H-6), 3.49 (q, 3, *J* = 1 Hz, OCH_3), 4.09 (dt, 1, *J* = 3, 9 Hz, H-5), 5.07 (s, 2, NCO_2CH_2Ph), 5.25 (AB q, 2, *J* = 12 Hz, CO_2CH_2Ph), 5.47 (p, 1, *J* = 6.5 Hz, $CHCH_3$), 7.34 (m, 15, phenyl); mass spectrum *m/e* 712 (M^+), 478, 410, 259, 189, 91.

Treatment of alcohol **7** with (*R*)-(−)-MTPA-Cl as described above provided MTPA ester **15** (31 mg) as a clear oil: IR (CH_2Cl_2) 3425, 1783, 1745, 1721, 1715, 1511, 1330, 1282, 1136, 1020 cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.50 (d, 3, *J* = 6.5 Hz, $CHCH_3$), 2.65–3.55 (m, 7, CH_2, SCH_2, NCH_2, H-6), 3.49 (q, 3, *J* = 1 Hz, OCH_3), 3.97 (dt, 1, *J* = 3, 9.5 Hz, H-5), 5.08 (s, 2, NCO_2CH_2Ph), 5.26 (AB q, 2, *J* = 14 Hz, CO_2CH_2Ph), 5.45 (m, 1, $CHCH_3$), 7.33 (m, 15, phenyl); mass spectrum *m/e* 712 (M^+), 478, 410, 259, 189, 91.

Preparation of MTPA Esters 17 and 18. (*S*)-(+)-MTPA-Cl (0.019 mL, 0.10 mmol) was added to a solution of penam alcohol **16** (24 mg, 0.072 mmol) in anhydrous pyridine (0.22 mL) and carbon tetrachloride (0.22 mL). The mixture was swirled briefly and then kept at room temperature for 2 h. The mixture was diluted with methylene chloride and washed with water, 1 N hydrochloric acid, 1 M dipotassium hydrogen phosphate solution, and brine. The organic phase was dried ($MgSO_4$) and evaporated under vacuum to an oil (37 mL) which was purified by chromatography on a silica gel GF plate using 3:1 benzene–ethyl acetate as developing solvent. MTPA ester **17** (30 mg) was obtained as a clear oil: IR (CCl_4) 1783, 1751, 1171, 1015, 908 cm^{-1}; ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.33 (s, 3, 2α-CH_3), 1.38 (d, 3, *J* = 6.5 Hz, $CHCH_3$), 1.57 (s, 3, 2β-CH_3), 3.47 (dd, 1, *J* = 2, 6.5 Hz, H-6), 3.48 (q, 3, *J* = 1 Hz, OCH_3), 4.47 (s, 1, H-3), 5.15 (s, 2, CO_2CH_2Ph), 5.20 (d, 1, *J* = 2 Hz, H-5), 5.53 (p, 1, *J* = 6.5 Hz, $CHCH_3$), 7.36 (m, 10, phenyl); mass spectrum *m/e* 551 (M^+), 318, 250, 189, 91.

Treatment of penam alcohol **16** with (*R*)-(−)-MTPA-Cl as described above gave MTPA ester **18** (28 mg) as a clear oil: IR (CCl_4) 1783, 1750, 1171, 1016, 908 cm^{-1}; ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.33 (s, 3, 2α-CH_3), 1.47 (d, 3, *J* = 6.5 Hz, $CHCH_3$), 1.53 (s, 3, 2β-CH_3), 3.38 (dd, 1, *J* = 2, 6.5 Hz, H-6), 3.48 (q, 3 *J* = 1 Hz, OCH_3), 4.43 (s, 1, H-3), 5.13 (d, 1, *J* = 2 Hz, H-5), 5.15 (s, 2, CO_2CH_2Ph), 5.53 (p, 1, *J* = 6.5 Hz, $CHCH_3$), 7.36 (m, 10, phenyl); mass spectrum *m/e* 551 (M^+), 318, 250, 189, 91.

Application of Horeau's Method to *N*-Benzyloxycarbonylthienamycin Benzyl Ester (7). The general procedure of Herz and Kagan[27] was slightly modified. A solution of racemic α-phenylbutyric acid anhydride **19** (120 mg, 0.39 mmol) and **7** (19.5 mg, 0.039 mmol) in anhydrous pyridine (1, mL) was allowed to stand at room temperature for 3 h. The product was precipitated with petroleum ether and further washed with the same solvent. The washings were combined and concentrated, water (1 mL) was added to the pyridine solution, and the mixture was allowed to stand for 4 h to destroy excess anhydride. The mixture was taken up in ethyl acetate and washed with water, thrice with sodium bicarbonate solution, and again with water. The combined washings were extracted twice with chloroform, acidified with dilute hydrochloric acid to pH 2, and extracted thrice with chloroform. The chloroform extract was dried (Na_2CO_3), the solvent removed, and the residual acid weighed to constant weight after drying in vacuo. This afforded α-phenylbutyric acid (66 mg), α_D +0.035 ± 0.002° (1.8 mL of benzene, 10-cm tube), $[\alpha]_D$ +0.95°, which corresponds to an optical yield of 18%.

The petroleum ether insoluble fraction was shown by TLC to be essentially pure apart from traces of starting material and pyridine. Preparative TLC gave the mixture of diastereomeric esters **20** and **21** as an oil: mass spectrum *m/e* 642 (M^+); ^{1}H NMR (see text).

Esterification of 7 with (*S*)-(+)-α-Phenylbutyric Acid Anhydride. α-Phenylbutyric acid was resolved and converted to the anhydride as described in ref 25. Esterification of *N*-benzyloxycarbonylthienamycin benzyl ester (**7**) by the above procedure gave the (*S*)-(+)-α-phenylbutyric ester **21** in good yield as an oil: ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3, *J* = 7.5 Hz, CH_2CH_3), 1.28 (d, 3, *J* = 6.5 Hz, $CHCH_3$), 3.21 (dd, 1, *J* = 3 and 8 Hz, H-6), 3.99 (dt, 1, *J* = 3, 9.5 Hz, H-5), 5.10 (m, 1, $CHCH_3$); mass spectrum *m/e* 642 (M^+).

Supplementary Material Available: Tables of crystallographic data (5 pages). Ordering information is given on any current masthead page.

References and Notes

(1) J. S. Kahan, F. M. Kahan, R. Goegelman, S. A. Currie, M. Jackson, E. O. Stapley, T. W. Miller, A. K. Miller, D. Hendlin, S. Mochales, S. Hernandez, and H. B. Woodruff, Abstracts XVI, Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Ill., 1976, No. 277. (A detailed report on the isolation and physical and microbiological characterization of the antibiotic is in preparation.)
(2) G. Albers-Schonberg, B. H. Arison, E. Kaczka, F. M. Kahan, J. S. Kahan, B. Lago, W. M. Maiese, R. E. Rhodes, and J. L. Smith, ref 1, No. 229.
(3) H. Kropp, J. S. Kahan, F. M. Kahan, J. Sundelof, G. Darland, and J. Birnbaum, ref 1, No. 228.
(4) Merck & Co., Inc., U.S. Patent 3 950 357 (April 12, 1976).
(5) E. O. Stapley, P. Cassidy, S. A. Currie, D. Daoust, R. Goegelman, S. Hernandez, M. Jackson, J. M. Mata, A. K. Miller, R. L. Monaghan, J. B. Tunac, S. B. Zimmerman, and D. Hendlin, Abstracts XVII, Interscience Conference on Antimicrobial Agents and Chemotherapy, New York, N.Y., 1977, No. 80. P. J. Cassidy, E. O. Stapley, R. Goegelman, T. W. Miller, B. H. Arison, G. Albers-Schonberg, S. B. Zimerman, and J. Birnbaum, *ibid.*, No. 81.
(6) A. G. Brown, D. F. Corbett, A. J. Eglington, and T. T. Howarth, *J. Chem. Soc., Chem. Commun.*, 523 (1977).
(7) K. Meada, S. Takahashi, M. Sezaki, K. Iinuma, H. Naganowa, S. Kondo, M. Ohno, and H. Umezawa, *J. Antibiot.*, **30**, 770 (1977).
(8) D. B. R. Johnston, S. M. Schmitt, F. A. Bouffard, and B. G. Christensen, *J. Am. Chem. Soc.*, **100**, 313 (1978).
(9) E. P. Abraham and G. G. F. Newton, *Biochem. J.*, **79**, 377 (1961).
(10) G. Albers-Schonberg, B. H. Arison, and J. L. Smith, *Tetrahedron Lett.*, 2911 (1972); structure determination of cephamycin C, unpublished results.
(11) R. B. Morin, B. G. Jackson, R. A. Mueller, E. A. Laragrino, W. B. Scanlon, and S. L. Andrews, *J. Am. Chem. Soc.*, **91**, 1401 (1969).
(12) W. Richter and K. Biemann, *Monatsh. Chem.*, **95**, 766 (1964).
(13) W. Richter and K. Biemann, *Monatsh. Chem.*, **96**, 484 (1965).
(14) *m/e* 78, C_6H_6: E. Stenhagen, S. Abrahamsson, and F. W. McLafferty, "Registry of Mass Spectral Data," Vol. 1, Wiley-Interscience, New York, N.Y., 1974, p 199, No. AA-517-2.
(15) A. Treibs and A. Ohorodnik, *Justus Liebigs Ann. Chem.*, **611**, 139, 149 (1958).
(16) The ultraviolet spectrum of **4** does not appear in methanolyses under exclusion of oxygen; also, nonoxygenated pyrrole, dihydropyrrole, and 3-methoxypyrrole analogues have been observed in methanolyses of the

N-benzyloxycarbonyl benzyl ester **7**.
(17) D. M. Brunwin and G. Lowe, *J. Chem. Soc., Perkin Trans. 1,* 1321 (1973).
(18) R. B. Woodward, "Recent Advances in the Chemistry of β-Lactam Antibiotics", *Chem. Soc., Spec. Publ.,* **No. 28,** 167–180 (1977).
(19) D. B. Bryan, R. F. Hall, K. G. Holden, W. F. Huffman, and J. G. Gleason, *J. Am. Chem. Soc.,* **99,** 2353 (1977).
(20) T. W. Doyle, J. L. Douglas, B. Beleau, J. Mennier, and B. Luh, *Can. J. Chem.,* **55,** 2873 (1977).
(21) F. DiNinno, T. R. Beattie, and B. G. Christensen, *J. Org. Chem.,* **42,** 1960 (1977).
(22) Related reductions in penicillin derivatives give predominantly cis-substituted products: J. C. Sheehan and Y. S. Lo, *J. Org. Chem.,* **38,** 3227 (1973); F. DiNinno, unpublished results.
(23) K. M. Wellman, S. Bogdansky, W. Mungall, T. G. Mecca, and C. R. Hare, *Tetrahedron Lett.,* 3607 (1967).
(24) J. A. Dale and H. S. Mosher, *J. Am. Chem. Soc.,* **95,** 512 (1973); G. R. Sullivan, J. A. Dale, and H. S. Mosher, *J. Org. Chem.,* **38,** 2143 (1973).
(25) R. Weidman and A. Horeau, *Bull. Soc. Chim. Fr.,* 117 (1967), and references cited therein.
(26) According to Mosher's nomenclature,[24] **14** and **15** were prepared from (*R*)-(+)- and (*S*)-(−)-α-methoxy-α-trifluoromethylphenylacetic acids (MTPA), respectively, which correspond to the (*S*)-(+) and (*R*)-(−) acid chlorides.
(27) W. Herz and H. B. Kagan, *J. Org. Chem.,* **31,** 681 (1966); **32,** 216 (1967). (Hydroxylated sesquiterpene lactones).
(28) H. Kakisawa, T. Kosima, M. Yanai, and K. Nakarishi, *Tetrahedron,* **21,** 3091 (1965). (Grayanatoxins).
(29) J. B. McAlpine and N. V. Riggs, *Aust. J. Chem.,* **28,** 211 (1975). (Leucodrin and derivatives).
(30) O. D. Hensens, manuscript in preparation.
(31) It should be noted that in this model the chemical shift differences are opposite to those in Mosher's MTPA ester model, but the same as in the case of mandelate and *o*-methylmandelate derivatives.[24]
(32) Trans coplanar elimination of methanesulfonic acid from **8** having side chain *R* configuration affords enelactam **9** having the *E* configuration.
(33) G. Germain, P. Main, and H. M. Woolfson, *Acta. Crystallogr., Sect. A,* **27,** 368 (1971).
(34) Computer programs used for these calculations were the X-ray 1972 system, Technical Report TR-192, Computer Science Center, University of Maryland, College Park, Md.
(35) C. K. Johnson, ORTEP, Oak Ridge National Laboratory, Report ORNL-3794.
(36) R. M. Sweet in "Cephalosporins and Penicillins", E. H. Flynn, Ed., Academic Press, New York, N.Y., 1972, p. 297.
(37) X-ray data from H. E. Applegate, J. E. Dolfini, M. S. Puar, W. A. Slusarchyk, and B. Toeplitz, *J. Org. Chem.,* **39,** 2794 (1974).
(38) Nonfused 4-thioazetidin-2-one intermediates in the synthesis of 6β-acylaminopenem esters, e.g., 4β-acetylthio-3β-phenoxyacetylaminoazetidin-2-one or *tert*-butyl 2-(4β-acetylthio-2-oxo-3β-phenoxyacetylamino-1-azetidinyl)-2-hydroxyacetate in CH_2Cl_2, show the β-lactam band at 1782 cm^{-1} [39] while dethiopenicillin G methyl ester in solution absorbs at about 1736 cm^{-1}.[40] Frequency differences of about 20 cm^{-1} have also been observed in the spectra of carba- and oxacephalosporin esters.[41]
(39) I. Ernest, J. Gosteli, and R. B. Woodward, U. S. Patent 4,070,477 (1978), Ciba-Geigy.
(40) E. Kaczka and K. Folkers in "The Chemistry of Penicillin", H. T. Clarke, J. R. Johnson, and R. Robinson, Ed., Princeton University Press, Princeton, N.J., 1949, p 249; H. W. Thompson, R. R. Brattani, H. M. Randall, and R. S. Rasmussen, *ibid.,* p 409.
(41) R. A. Firestone, J. L. Fahey, N. S. Maciejewicz, and B. G. Christensen, *J. Med. Chem.,* **20,** 551 (1977).
(42) T. T. Howarth and A. G. Brown, *J. Chem. Soc., Chem. Commun.,* 266 (1976).
(43) R. W. Ratcliffe and B. G. Christensen, *Tetrahedron Lett.,* 2907 (1972).
(44) G. Albers-Schonberg, W. Maiese, R. S. Dewey, B. Lago, E. Kaczka, I. D. Ihnan, and J. L. Smith, International Union of Pure and Applied Chemistry Conference on Mass Spectrometry of Natural Products, Weizmann Institute, Israel, 1977 (manuscript in preparation).
(45) The purity was estimated on the basis of in vitro bioactivity against *Staph. aureus* in comparison to the potency of subsequently obtained pure antibiotic.
(46) This derivative was subsequently almost completely avoided. Its mass spectra below *m/e* 402 are largely identical with those of **3**.

Communications to the Editor

An ab Initio Molecular Orbital Calculation of the Structure of Silabenzene

Sir:

Silabenzene has proven to be an elusive molecule[1-3] despite the relative stability of similar heteroatomic analogues of benzene such as pyridine, phosphabenzene, arsabenzene, and stibabenzene.[4] Attempts have been made to generate a substituted silabenzene with the following rearrangement of a singlet carbene:[3]

(1)

X = C, Si

Although this reaction occurs readily for carbon,[5] the silicon analogue does not appear to yield a silabenzene. In this communication we wish to report an ab initio investigation of the structure of silabenzene.[6]

The calculations were performed with the GAUSSIAN 70[7] and the FORCE[8] programs using the standard STO-3G basis set[9] on the IBM 360/91 computer at Princeton. The force method[10] was used to optimize fully all geometrical parameters in the structures reported. Because of difficulties associated with choosing a suitable, nonredundant internal coordinate system for cyclic molecules, the optimization was based on cartesian energy derivatives. An initial force constant matrix was obtained by transforming a simple, empirical valence force field using redundant internal coordinates to the cartesian coordinates system. (The accuracy of the initial force constant matrix affects only the rate of convergence, but not the final outcome of the geometry optimization.) The force method, especially when combined with an empirically estimated force constant matrix, can be an order of magnitude more efficient than optimization methods based only on the energy. In addition, magnitudes of the residual forces at the optimized geometry provide an important check on the quality of the optimization (convergence level of $\sim 5 \times 10^{-3}$ mdyn for the root-mean-square energy gradient in cartesian coordinates). The results are summarized in Table I. To serve as a reference point, the structures for silaethylene[11a-c] and the associated carbenes[11d] were also optimized fully with the STO-3G basis.[12]

Inspection of the geometrical parameters for singlet silabenzene reveals that only small changes in the bond angles (relative to benzene) are necessary to accommodate the larger silicon. The C–C bond lengths are essentially the same as in benzene,[13] and the Si–C bond length lies between the values for a single bond[14] and a double bond,[12] displaying almost the same relative shortening found in benzene. Thus, the calculated structures would suggest that silabenzene possesses some degree of aromaticity. In the triplet state, these bond lengths increase somewhat; but, unlike silaethylene,[11b] where the triplet undergoes a large conformational change, triplet silabenzene remains planar.

Further support for the aromatic character can be found in the molecular orbital (MO) energies and MO coefficients. The usual benzenoid pattern of orbital energies (−0.461, 2 × (−0.283), 2 × (0.271), 0.507 au calcd for C_6H_6 at STO-3G) is not strongly perturbed (−0.416, −0.278, −0.200, 0.256, 0.284, and 0.494 au), although the energy of the highest occupied MO is above that of benzene, suggesting less resonance

0002-7863/78/1500-6499$01.00/0