Structural Characterization of an Anhydropenicillin and Its Stereochemical Relationship to Penicillins

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Abstract: The molecular structure of phenoxymethylanhydropenicillin has been determined by single-crystal X-ray diffraction techniques. The molecule crystallizes with an ethanol molecule of crystallization in the orthorhombic space group $P2_12_12_1$. The latter parameters are $a = 8.4565$ (5), $b = 6.9243$ (4), and $c = 32.5349$ (15) Å. The experimental density of 1.33 g cm^{-3} agrees well with the value of 1.32 g cm^{-3} calculated for four $C_{16}H_{15}N_2O_4S \cdot C_2H_5OH$ species per unit cell. Refinement by full-matrix least squares based on 1532 independent diffractometry-collected data with $I > 2\sigma(I)$ resulted at convergence in final discrepancy indices of $R_1 = 5.4\%$ and $R_2 = 6.4\%$. The structure is analogous to that of the active penicillins with respect to the resulting stereochemistry caused by the fusion of the four-membered β-lactam ring to the five-membered ring; *viz.*, a thiazolidinone ring for the anhydropenicillins *vs.* a thiazolidine ring for the penicillins. As in the penicillins, the large pyramidal character of the nitrogen atom in the β-lactam ring of phenoxymethylanhydropenicillin (as evidenced by the nitrogen atom being perpendicularly displaced by 0.41 Å out of the plane of its three bonded constituents) is likewise assumed to inhibit considerably any delocalization of its lone pair of electrons. Hence, the chemical stability of the anhydropenicillins to hydrolysis of the β-lactam ring (in contrast to that of the penicillins) cannot apparently be simply ascribed (as previously suggested) to extensive delocalization in the ground electronic state of the electron pair on the nitrogen atom into the adjacent α,β-unsaturated system. This conclusion is in accord with the observed N–C and C–O bond lengths of 1.415 (7) and 1.183 (7) Å, respectively, in the β-lactam ring and the N–C bond length of 1.431 (7) Å in the thiazolidinone ring. The ethanol molecule of crystallization is involved in hydrogen bonding with the carbonyl oxygen atom of the peptide linkage in the side chain.

The relationship between the structure and activity of antibiotics and other compounds of biological interest has generated a great deal of interest and research over the years. One of the most studied of these relationships has been that of the β-lactam antibiotics; *viz.*, the penicillins and cephalosporins (Figures 1a,b).[2-8] It has been shown that these antibiotics act to inhibit the synthesis of the bacterial cell wall. More specifically, they reduce the amount of terminal cross-linking by the peptidoglycan strands, which causes the cell to lose structural strength and ultimately to rupture.[6-8] Tipper and Strominger[6] suggested that the conformation of the penicillins is similar to D-Ala-D-Ala, which is the substrate upon which the enzyme peptidoglycan transpeptidase acts in the cross-linking process. The enzyme therefore mistakes the penicillin or cephalosporin molecule for its normal substrate and binds with the antibiotic, thus preventing the cross-linking process.

Further studies by Lee[9] have shown that the conformation of penicillin is most similar to that of D-Ala-D-Ala when the dihedral angle around the peptide bond in D-Ala-D-Ala is distorted by approximately 45°. This deformed conformation of the dipeptide portion has been postulated to be the transition state during cleavage or formation of a peptide bond,[10] and the peptidoglycan transpeptidase is presumed to bond preferentially with such a distorted D-Ala-D-Ala geometry which closely resembles that of the penicillins. Kinetic considerations indicate, however, that (since the penicillins do not have to expend energy in order to distort to this favorable conformation) the activation energy for the reaction of the enzyme with a penicillin is lower than with the normal D-Ala-D-Ala substrate, and, hence, the rate of reaction with a penicillin is correspondingly higher. Thus, the penicillins are able, at concentration levels acceptable to the organism, to compete effectively with the substrate for the transpeptidase enzyme.

The relationship between the structure of these molecules and their chemical reactivity has also been extensively studied.[2-4,11,12] It has been shown that both the biological activity and the rate of hydrolysis can be correlated with the β-lactam carbonyl stretching frequency and the extent of nonplanarity of the β-lactam nitrogen.[2,13] The nonplanarity of the β-lactam nitrogen has been attributed to the steric strain which results from fusion of its four-membered ring to the five-membered thiazolidine ring.

Anhydropenicillins (Figure 1c), first synthesized by Wolfe and coworkers,[14,15] were presumed[2] to have a molecular conformation similar to that of the penicillins with a nonplanar β-lactam atom. Furthermore, since the carbonyl stretching frequencies for anhydropenicillins[15] are considerably greater than those found for the normal penicillins,[16] a labile system would be ex-

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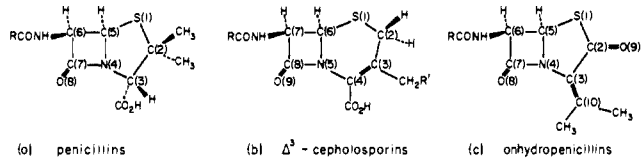

(a) penicillins (b) Δ^3-cepholosporins (c) onhydropenicillins

Figure 1. Formulas of the molecular nuclei of the biologically active pencillins and Δ^3-cephalosporins, and the biologically inactive anhydropenicillins.

pected for an anhydropenicillin.[17-20] Thus, it is surprising to learn that this system is remarkably stable in that it can be recovered intact either from a reflux in various solvents (*viz.*, toluene, lower alcohols, aqueous dioxane, or xylene) or from a melt.[15]

The loss of biological activity by the anhydropenicillins is readily rationalized by the loss of the carboxylate group. The peptidoglycan transpeptidase enzyme, with its very specific steric requirements for its substrate, presumably no longer mistakes this molecule for D-Ala-D-Ala and hence does not combine with it. A rationale for the loss of chemical activity is not, however, immediately obvious. Wolfe, *et al.*,[15] suggested that the less basic character of the nitrogen atom of an anhydropenicillin and the high infrared stretching frequency of the β-lactam carbonyl can be rationalized on the basis that the electron pair on the nitrogen atom is delocalized in the ground state (as well as in the excited state) into the adjacent α,β-unsaturated side chain.

The primary goals of our X-ray investigation of the solid-state structure of phenoxymethylanhydropenicillin, a representative member of this family of compounds, were to establish the absolute configuration of an anhydropenicillin molecule and to provide operational evidence from an analysis of the bond lengths and angles for any extensive delocalization of the electron pair on the β-lactam nitrogen atom (in the ground electronic state). It was further hoped that a comparison of the molecular parameters obtained in this analysis with those of the normal penicillins would give insight into the unexpected stability of anhydropenicillin.

Experimental Section

White needle-like crystals of phenoxymethylanhydropencillin[21] containing ethanol of crystallization were obtained by slow evaporation of a cold ethanolic solution. Most crystals examined under the petrographic microscope were unsuitable for X-ray examination, while others deemed suitable had split spots on the X-ray photographs indicating twinned crystals. A small single crystal of dimensions 0.11 × 0.18 × 0.35 mm (corresponding to the [100], [001], and [010] directions, respectively) was finally found. Preliminary X-ray photographs indicated the orthorhombic crystal system (Laue symmetry D_{2h}-2/*m*2/*m*2/*m*); systematic absences of $\{h00\}$ for $h = 2n + 1$, $\{0k0\}$ for $k = 2n + 1$, and $\{00l\}$ for $l = 2n + 1$ uniquely determined the space group as the acentric $P2_12_12_1$. The crystal was aligned on a Datex-automated General Electric diffractometer equipped with a pulse height analyzer designed to admit 90% of the Ni-filtered Cu Kα radiation.[22] Twenty independent reflections were carefully centered, and the angle settings were refined with ANGSET[23] to give lattice parameters of a = 8.4565 (5), b = 6.9243 (4), and c = 32.5349 (15) Å.[24] The volume of the unit cell is 1905.1 Å^3. The experimental density of 1.33 g cm^{-3} agrees well with a calculated density of 1.32 g cm^{-3} based on four $C_{16}H_{16}O_4N_2S \cdot C_2H_5OH$ species per unit cell.

Data were collected *via* the θ–2θ scan technique with a scan speed of 2°/min for $2\theta < 125.0°$ for two of the four intensity-weighted reciprocal lattice octants related by twofold symmetry. A (stationary crystal)–(stationary counter) background measurement of 12.5 sec was made on each side of the scan. The scan width was 1.2° except for $2\theta < 15°$, where a 1.5° scan range was used. Throughout the data collection four reflections were sampled every 3 hr for the purpose of monitoring crystal decay and alignment. The crystal began to decay slightly (~7%) during the collection of the second asymmetric unit. A correction was applied which assumed that the decay was linear over short periods, and the data were rescaled accordingly.

The criteria used for considering a reflection to be observed was that $I \geq 2\sigma(I)$, $I = S - B(T/t)$, and $\sigma(I) = [S + B(T/t)^2 + 0.002I^2]^{1/2}$ where I is the net intensity, S is the total scan count for time T, and B is the total accumulated background count for time t. The data were reduced to structure factor amplitudes according to the following formulas: $|F_o| = (I/Lp)^{1/2}$ and $\sigma(|F_o|) = \sigma(I)/2|F_o|Lp$, where Lp is the Lorentz polarization correction.

The linear absorption coefficient for $C_{16}H_{16}O_4N_2S \cdot C_2H_5OH$ with Cu Kα radiation (λ = 1.5418 Å) is 17.39 cm^{-1}. Since the calculated transmission factors ranged from 0.70 to 0.84, an absorption correction was applied to the two octants of data, which were then merged into a single asymmetric octant[25,26] of 1532 observed reflections. No corrections for extinction were made in that the $|F_c|$'s for the strong reflections at low sin θ/λ showed no systematic deviations from the $|F_o|$'s.

A three-dimensional Patterson function calculated from the 1000 largest normalized structure factors yielded the location of the sulfur atom.[27,28] Although a Fourier synthesis phased on the sulfur atom coordinates had an unweighted discrepancy index (R_1) of 59%,[29] a number of additional atoms in both the thiazolidinone ring and the phenyl ring were identified. Two successive Fourier syntheses provided the positions for all the nonhydrogen atoms in the molecule, as well as positions corresponding to the oxygen and carbon atoms of a C_2H_5OH molecule of crystallization. At this point in the structural determination, the residuals R_1 and R_2 were, respectively, 49 and 67%. A series of isotropic full-matrix least squares[30,31] reduced these values to 9.9 and 11.1%. A difference Fourier synthesis, calculated from atomic parameters obtained from the output of the last isotropic least-squares cycle, yielded the location of 16 of the 22 hydrogen atoms in the structure. Idealized coordinates calculated with the program MIRAGE[32] were used for all

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(30) ORFLSD, a local modification of the program by W. R. Busing, K. O. Martin, and H. A. Levy, "ORFLS, A Fortran Crystallographic Least-Squares Program," ORNL-TM-305, Oak Ridge National Laboratory, 1962.

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Table I

	x	y	z	$\beta_{11} \times 10^4$	$\beta_{22} \times 10^4$	$\beta_{33} \times 10^4$	$\beta_{12} \times 10^4$	$\beta_{23} \times 10^4$	$\beta_{12} \times 10^4$
	A. Atomic Parameters with Estimated Standard Deviation for Anhydropenicillin–Ethanol[a,b]								
S(1)	0.2224 (2)	0.6462 (2)	0.3744 (0.4)	171 (2)	230 (3)	10 (1)	4 (3)	6 (1)	−5 (1)
C(2)	0.1614 (7)	0.5045 (9)	0.4177 (2)	155 (9)	202 (14)	11 (1)	−5 (11)	−3 (2)	−3 (2)
C(3)	0.0729 (6)	0.6251 (8)	0.4477 (1)	141 (8)	203 (14)	9 (1)	−15 (10)	−2 (2)	0 (2)
N(4)	0.0552 (5)	0.8169 (7)	0.4320 (1)	182 (8)	184 (11)	8 (1)	−1 (9)	−1 (2)	−3 (2)
C(5)	0.1513 (6)	0.8673 (8)	0.3961 (1)	152 (8)	193 (13)	10 (1)	−12 (10)	0 (2)	−4 (2)
C(6)	0.0042 (7)	0.9725 (7)	0.3776 (2)	203 (9)	157 (12)	9 (1)	−19 (10)	−3 (2)	1 (2)
C(7)	−0.0853 (8)	0.8917 (8)	0.4146 (2)	210 (11)	185 (14)	9 (1)	35 (11)	2 (2)	−9 (2)
O(8)	−0.2182 (5)	0.8847 (7)	0.4258 (1)	185 (7)	331 (14)	14 (1)	59 (9)	7 (2)	−4 (2)
O(9)	0.1911 (5)	0.3354 (6)	0.4199 (1)	260 (9)	191 (10)	15 (1)	29 (9)	2 (2)	−1 (2)
C(10)	0.0191 (7)	0.5721 (10)	0.4844 (2)	152 (9)	343 (20)	10 (1)	14 (12)	−2 (2)	8 (3)
C(11)	0.0292 (9)	0.3708 (11)	0.5001 (2)	251 (14)	410 (26)	15 (1)	10 (17)	5 (3)	28 (4)
C(12)	−0.0602 (8)	0.7124 (14)	0.5131 (2)	254 (14)	531 (28)	9 (1)	48 (18)	0 (2)	4 (4)
N(13)	0.0535 (5)	0.9127 (6)	0.3381 (1)	211 (9)	153 (10)	10 (1)	−18 (8)	−5 (2)	1 (2)
C(14)	−0.0909 (7)	1.0419 (8)	0.3091 (2)	152 (9)	181 (14)	10 (1)	−5 (10)	4 (2)	6 (2)
O(15)	−0.0755 (5)	1.2156 (5)	0.3122 (1)	244 (8)	144 (9)	13 (1)	−4 (7)	−5 (2)	1 (2)
C(16)	−0.1517 (7)	0.9610 (8)	0.2686 (2)	191 (10)	178 (14)	10 (1)	−27 (11)	−2 (2)	3 (2)
O(17)	−0.1766 (5)	0.7602 (5)	0.2714 (1)	240 (8)	178 (9)	10 (1)	−13 (7)	−11 (1)	3 (2)
C(18)	−0.2608 (6)	0.6751 (8)	0.2399 (1)	3.9 (1)[c]					
C(19)	−0.3094 (6)	0.4868 (9)	0.2466 (2)	4.6 (1)[c]					
C(20)	−0.3955 (7)	0.3932 (10)	0.2172 (2)	5.3 (1)[c]					
C(21)	−0.4371 (7)	0.4832 (10)	0.1810 (2)	5.4 (1)[c]					
C(22)	−0.3874 (7)	0.6698 (10)	0.1742 (2)	5.1 (1)[c]					
C(23)	−0.2972 (7)	0.7657 (8)	0.2037 (2)	4.6 (1)[c]					
C(24)	−0.3473 (8)	0.3933 (11)	0.4065 (2)	6.3 (2)[c]					
C(25)	−0.3140 (7)	0.5118 (10)	0.3696 (2)	5.5 (1)[c]					
C(26)	−0.1493 (4)	0.5321 (6)	0.3614 (1)	5.2 (1)[c]					
	B. Positional Parameters for the Hydrogen Atoms Obtained from a Fourier Difference Map[d]								
H(5)	0.2610	0.9417	0.4022						
H(6)	0.0352	1.0990	0.3746						
H1(11)	0.0345	0.2976	0.4873						
H2(11)	−0.0125	0.3426	0.5231						
H3(11)	−0.1625	0.3830	0.5377						
H1(12)	−0.0295	0.7172	0.5372						
H2(12)	−0.0296	0.8121	0.5076						
H3(12)	−0.1508	0.6900	0.5061						
H(13)	−0.0749	0.8153	0.3365						
H1(15)	−0.2368	1.0156	0.2594						
H2(15)	−0.0853	0.9929	0.2484						
H(19)	−0.2743	0.4325	0.2747						
H(20)	−0.4875	0.2498	0.2253						
H(21)	−0.4992	0.3833	0.1604						
H(22)	−0.3979	0.7330	0.1437						
H(23)	−0.2532	0.9134	0.1977						
H1(24)	−0.4512	0.4241	0.4244						
H2(24)	−0.3079	0.2931	0.3978						
H3(24)	−0.2794	0.4396	0.4303						
H1(25)	−0.3633	0.6327	0.3736						
H2(25)	−0.3521	0.4640	0.3425						
H(26)	−0.1051	0.4462	0.3449						

[a] In this and subsequent tables the esd's of the last significant figures are given in parentheses. [b] The anisotropic thermal parameters are of the form $\exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl)]$. [c] Isotropic thermal parameters were used in the refinement of the six phenyl carbon atoms and the three nonhydrogen atoms of the ethanol molecule. [d] The Fourier difference map was calculated from the atomic parameters given in part A.

hydrogen atoms in the subsequent refinement. Isotropic temperature coefficients of 3.0 Å^2 were assigned to the hydrogen atoms, and neither their positional nor thermal parameters were allowed to vary during the subsequent refinement. After each cycle new idealized positions for the hydrogen atoms were calculated, based on the shifts of the nonhydrogen atoms.

The absolute configuration of the molecule was determined by correction of the scattering factors of sulfur and oxygen for anomalous dispersion.[33] Discrepancy indices of $R_1 = 7.5\%$ for one configuration *vs.* $R_1 = 7.0\%$ for its mirror image established the enantiomorph at the 0.01 level of significance.[34] The anomalous dispersion corrections were used throughout the final refinement.

Four full-matrix least-squares cycles with anisotropic temperature coefficients utilized for all nonhydrogen atoms except for those atoms comprising the phenyl ring and C_2H_5OH molecule resulted in convergence at $R_1 = 5.4\%$ and $R_2 = 6.4\%$. A semiempirical method was used in the refinement to correct the weighting scheme. In this method the observed values of $||F_o| - |F_c||/\sigma(|F_o|)$ were fitted to a fifth-order polynomial in $|F_o|$. This procedure led to a "goodness-of-fit" of 0.87, which indicates that there is a slight overestimation in the standard deviation of an observation of unit weight.

The atomic parameters from the last least-squares cycle are listed in Table I, along with the positions of all 22 hydrogen atoms found from a difference Fourier map based on the final nonhydrogen atomic coordinates.[35] Interatomic distances and angles with

(33) For Cu Kα radiation the values of the real and imaginary anomalous dispersion corrections to the atomic scattering factors are: $\Delta f' = 0.3$, $\Delta f'' = 0.6$ for S; $\Delta f' = 0.0$, $\Delta f'' = 0.1$ for O ("International Tables for X-Ray Crystallography," Vol III, The Kynoch Press, Birmingham, England, 1962, p 214).

(34) W. C. Hamilton, "Statistics in Physical Science," Ronald Press, New York, N. Y., 1964, p 159.

(35) A listing of the structural factor amplitudes will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036, by referring to code

estimated standard deviations are given in Table II, while Table III contains some least-squares planes of interest.[36,37] A stereoscopic drawing[38] of the molecule is exhibited in Figure 2.

Table II. Interatomic Distances and Bond Angles for Phenoxymethylanhydropenicillin–Ethanol

	A. Interatomic Distances, Å		
S(1)–C(2)	1.792 (6)	N(13)–C(14)	1.338 (6)
S(1)–C(5)	1.790 (6)	C(14)–O(15)	1.214 (6)
C(2)–C(3)	1.488 (7)	C(14)–C(16)	1.523 (7)
C(3)–N(4)	1.431 (7)	C(16)–O(17)	1.409 (6)
N(4)–C(5)	1.466 (6)	O(17)–C(18)	1.380 (6)
N(4)–C(7)	1.415 (7)	C(18)–C(19)	1.384 (8)
C(5)–C(6)	1.562 (7)	C(19)–C(20)	1.366 (7)
C(6)–C(7)	1.527 (8)	C(20)–C(21)	1.379 (8)
C(7)–O(8)	1.183 (7)	C(21)–C(22)	1.377 (9)
C(2)–O(9)	1.199 (6)	C(22)–C(23)	1.395 (8)
C(2)–C(10)	1.328 (7)	C(23)–C(19)	1.369 (6)
C(10)–C(11)	1.487 (9)	C(24)–C(25)	1.483 (8)
C(10)–C(12)	1.507 (10)	C(25)–O(26)	1.424 (6)
C(6)–N(13)	1.435 (6)		
	B. Bond Angles, Degrees		
C(2)–S(1)–C(5)	93.6 (3)	C(3)–C(10)–C(11)	123.3 (6)
S(1)–C(2)–O(9)	121.4 (5)	C(3)–C(10)–C(12)	122.1 (6)
S(1)–C(2)–C(3)	110.7 (4)	C(11)–C(10)–C(12)	114.6 (6)
C(3)–C(2)–O(9)	127.9 (6)	C(6)–N(13)–C(14)	121.2 (4)
C(2)–C(3)–N(4)	109.8 (4)	N(13)–C(14)–O(15)	125.4 (5)
C(2)–C(3)–C(10)	127.4 (6)	N(13)–C(14)–C(16)	116.4 (5)
N(4)–C(3)–C(10)	122.8 (6)	C(16)–C(14)–O(15)	118.1 (5)
C(3)–N(4)–C(5)	116.6 (4)	C(14)–C(16)–O(17)	110.9 (5)
C(3)–N(4)–C(7)	124.8 (5)	C(16)–O(17)–C(18)	116.8 (5)
C(5)–N(4)–C(7)	93.4 (4)	O(17)–C(18)–C(19)	116.0 (5)
S(1)–C(5)–C(6)	121.0 (4)	O(17)–C(18)–C(23)	124.0 (5)
S(1)–C(5)–N(4)	107.3 (4)	C(19)–C(18)–C(23)	120.0 (5)
N(4)–C(5)–C(6)	88.6 (4)	C(18)–C(19)–C(20)	119.6 (5)
C(5)–C(6)–C(7)	85.5 (4)	C(19)–C(20)–C(21)	121.4 (6)
C(5)–C(6)–N(13)	118.7 (4)	C(20)–C(21)–C(22)	118.9 (6)
C(7)–C(6)–N(13)	115.5 (4)	C(21)–C(22)–C(23)	120.2 (6)
C(6)–C(7)–N(4)	91.9 (5)	C(22)–C(23)–C(18)	119.8 (5)
C(6)–C(7)–O(8)	136.9 (6)		
N(4)–C(7)–O(8)	131.2 (5)	C(25)–C(24)–O(26)	113.1 (5)

Results and Discussion

General Description of the Crystal Structure. The most interesting structural parameters in this molecule are those of the β-lactam and thiazolidinone rings, which presumably reflect (at least partially) the dramatic changes in the chemical properties which are due to the anhydropenicillin rearrangement. The β-lactam nitrogen, N(4), is definitely nonplanar with respect to the three atoms connected to it, lying 0.41 Å out of the plane defined by C(3), C(5), and C(7). In normal amides the nitrogen atom is coplanar with its substituents such that the lone electron pair of the nitrogen atom is available for π bonding with the carbonyl group. The net effect is an electron delocalization through the N(4)–C(7)–O(8) system, which results in a slight lengthening of the C(7)–O(8) bond and a corresponding decrease in the N(4)–C(7) bond length. In the anhydropenicillins, however, the nonplanar character of the β-lactam severely restricts the amount of delocalization involving the unshared electron pair of the nitrogen atom. This is reflected in the N(4)–C(7) bond length of 1.415 (7) Å being considerably larger than the average value of 1.32 Å found in normal amides.[39]

In accord with these above-mentioned planarity considerations of the β-lactam nitrogen atom, there is relatively little shortening of the N(4)–C(3) bond of the thiazolidine ring. The observed value of 1.431 (7) Å, when compared to the estimated N–C (trigonal) single-bond length of 1.44 Å,[40] indicates that there must be relatively little delocalization of the unshared electron pair of N(4) with the π system of the isopropylidene group attached to the thiazolidinone ring at

Table III. Equations of Least-Squares Planes and Perpendicular Distances (Å) of Selected Atoms from These Planes[a]

1. Plane containing C(3), C(5), and C(7)
$-0.2727X - 0.6251Y - 0.7314Z + 13.5276 = 0$

C(3)	0	C(6)	0.323
C(5)	0	C(10)	−0.519
C(7)	0	O(8)	0.069
N(4)	−0.415		

2. Plane containing S(1), C(2), and C(5)
$-0.8897X - 0.1507Y - 0.4309Z + 7.5960 = 0$

S(1)	0	N(4)	0.272
C(2)	0	C(6)	1.256
C(5)	0	O(9)	−0.078
C(3)	0.118		

3. Plane containing S(1), C(2), C(3), N(4), and C(5)
$-0.8455X - 0.1942Y - 0.4974Z + 8.5715 = 0$

S(1)	−0.054	C(6)	1.123
C(2)	−0.020	C(7)	1.273
C(3)	−0.036	O(9)	−0.040
N(4)	0.088	C(10)	−0.173
C(5)	−0.086		

4. Plane containing N(4), C(5), C(6), and C(7)
$-0.2151X - 0.8552Y - 0.7150Z + 11.5237 = 0$

N(4)	−0.041	S(1)	1.550
C(5)	0.037	C(3)	0.822
C(6)	−0.035	O(8)	0.150
C(7)	0.039	N(13)	0.030

5. Plane containing N(4), C(6), C(7), and O(8)
$-0.1327X - 0.8605Y - 0.4918Z + 11.8437 = 0$

N(4)	0.002	C(3)	0.873
C(6)	0.002	C(5)	0.168
C(7)	−0.008	N(13)	1.055
O(8)	0.004		

6. Plane containing C(2), C(3), C(10), C(11), C(12), and O(9)
$0.8762X - 0.2181Y - 0.4299Z + 7.7828 = 0$

C(2)	−0.016	O(9)	−0.012
C(3)	0.037	S(1)	−0.077
C(10)	0.002	N(4)	0.099
C(11)	0.012	C(5)	−0.187
C(12)	−0.024		

7. Plane containing C(6), N(13), C(14), O(15), and C(16)
$0.9357X - 0.0592Y - 0.3477Z + 4.6334 = 0$

C(6)	−0.004	C(16)	0.000
N(13)	0.010	C(5)	0.994
C(14)	−0.011	C(7)	−1.098
O(15)	0.006	O(17)	−0.146

8. Plane containing C(18), C(19), C(20), C(21), C(22), and C(23)
$0.8415X - 0.3417Y - 0.4186Z + 6.7111 = 0$

C(18)	−0.009	C(22)	−0.003
C(19)	−0.001	C(23)	0.011
C(20)	0.009	O(17)	−0.040
C(21)	−0.007		

[a] The equation of the plane is expressed in orthogonal ångström coordinates X, Y, Z which are related to the fractional crystallographic coordinates x, y, z by the transformation: $X = ax$, $Y = by$, and $Z = cz$. All atoms were assigned unit weights in the calculation.

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(36) W. R. Busing, K. O. Martin, and H. A. Levy, "ORFFE, a Fortran Crystallographic Function and Error Program," ORNL-TM-306, Oak Ridge National Laboratory, 1964.

(37) D. L. Smith, PLANES, Ph.D. Thesis (Appendix IV), University of Wisconsin, Madison, Wis., 1962.

(38) C. K. Johnson, "ORTEP, A Fortran Thermal-Ellipsoid Plot Program for Crystal Structure Illustration," ORNL-3794, Oak Ridge National Laboratory, 1964.

(39) R. E. Marsh and J. Donohue, *Advan. Protein Chem.*, 22, 235 (1967).

(40) D. R. Lide, Jr., *Tetrahedron*, 17, 125 (1962).

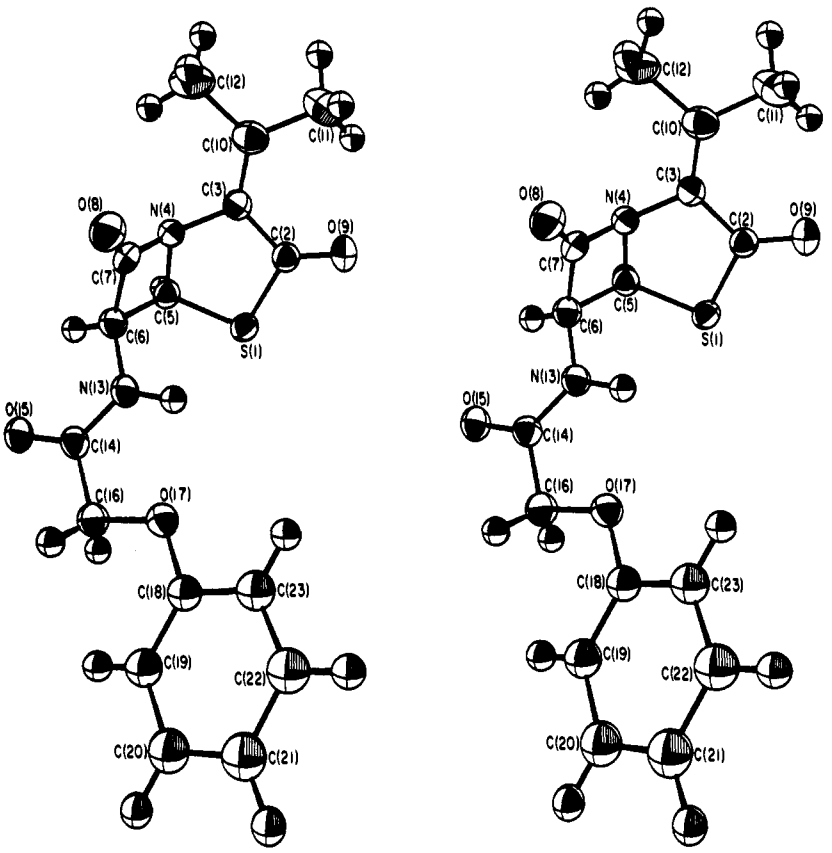


Figure 2. Stereoscopic view of the conformation of phenoxymethylanhydropenicillin. Thermal ellipsoids representing 50% probability are shown.

C(3). The N(4)–C(3) bond distance is significantly longer than the observed N–C (olefin) distances of range 1.36–1.39 Å[2,41,42] found in those compounds where the introgen atom is trigonal and its unshared electron pair is capable of considerable π delocalization with the olefinic bond. The C(2)–C(3) bond length of 1.488 (7) Å is not significantly different from the estimated C (trigonal)–C (trigonal) single-bond length of 1.48 Å,[40] as expected for an α,β-unsaturated thiolactone.

The crystallographic data indicate that the peptide linkage attached to the β-lactam at C(6) exhibits the normal amide type of resonance. The observed N(13)–C(14) bond length of 1.338 (6) Å is considerably shorter than both the estimated N–C (trigonal) single-bond distance of 1.44 Å and the distance of 1.415 (7) Å found for the β-lactam N(4)–C(7) bond, but is nearly identical with the observed N–C bond lengths of 1.32 Å (av) found for normal amides. Furthermore, the C(6)–N(13)–C(14) bond angle of 121° is characteristic of trigonal hybridization for the N atom, which implies that there must be considerable delocalization of the lone electron pair on N(13). In accord with all of these observations, a least-squares plane calculated for N(13), C(6), C(14), O(15), and C(16) shows none of these atoms perpendicularly displaced from the mean plane by more than 0.011 Å.

The average C–C distance in the isotropically refined phenyl ring is 1.378 (3) Å, slightly shorter than the expected value of 1.398 Å.[43] The intra-ring bond angles vary from 118.9 (6)° to 121.4 (6)°, while the maximum deviation from a mean least-squares plane is 0.011 Å.

The crystal packing[38] of phenoxymethylanhydropenicillin·C_2H_5OH is shown in Figure 3. The role of the ethanol molecule of crystallization appears to be twofold.[44] It not only fills what might otherwise be a large hole in the lattice (thereby stabilizing the crystal), but moreover it is involved in hydrogen bonding as evidenced by the ethanolic oxygen atom O(26) being located at 2.79 Å from the carbonyl oxygen atom O(15) of the peptide linkage in the side chain; the hydrogen atom attached to O(26) is oriented such that it lies 1.8 Å from O(15), which is appropriate for a normal hydrogen bond.

Stereochemical Relationship with Other β-Lactams. In Table IV some parameters of interest for several β-lactam systems are listed. First noted is the fact that the β-lactam carbonyl stretching frequency of the anhydropenicillins is significantly higher than those of the other β-lactam systems, implying that the C(7)–O(8) bond has the greatest double bond character in the anhydropenicillins. Hence, there must be a further reduction in the associated enamide resonance of these compounds (over that of the active penicillins) resulting in a longer N–C distance. The observed N–C bond lengths (Table IV) for anhydropenicillin and ampicillin

(41) J. Karle and I. Karle, *J. Amer. Chem. Soc.*, 89, 805 (1967).
(42) H. Ringertz, *Acta Crystallogr.*, 20, 397 (1966).
(43) V. Schomaker and L. Pauling, *J. Amer. Chem. Soc.*, 61, 1769 (1939).
(44) Attempts to crystallize phenoxymethylanhydropenicillin without the ethanol molecule of crystallization were unsuccessful, the only products being amorphous. Furthermore, the amorphous anhydropenicillin decomposes upon standing, while the ethanol-solvated sample is air stable over a period of months.

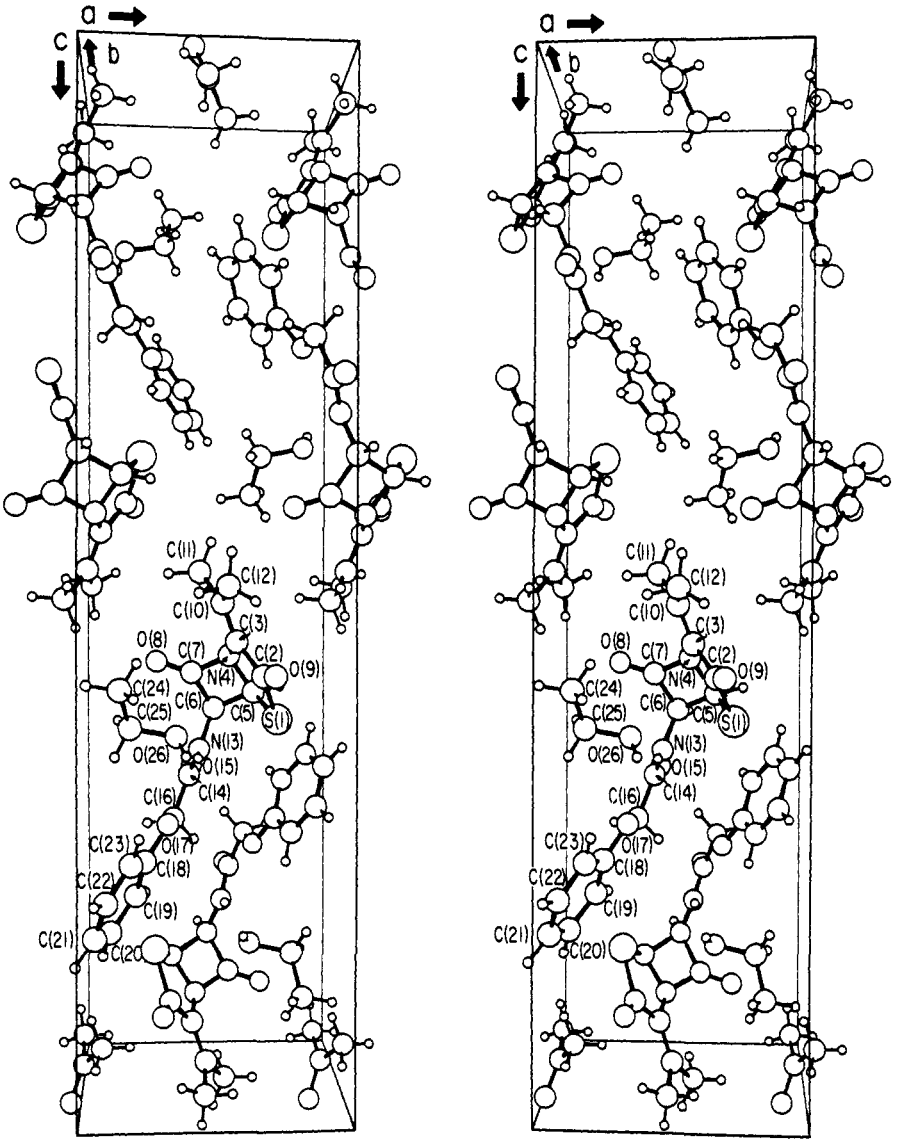
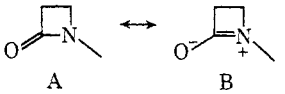


Figure 3. Stereoscopic view of the packing in one unit cell of phenoxymethylanhydropenicillin.

are in accord with these predictions with a significant Δ/σ ratio of 5.6. Unfortunately, the precision of the other earlier structural determinations of the penicillins listed in Table IV does not allow for any meaningful individual comparisons.

Relationship between Structure and Activity of Anhydropenicillin. According to the arguments first postulated by Woodward,[13] normal unfused β-lactams are capable of existing in two canonical forms. Any



A B

effect which alters the resonance condition and reduces the contribution of the B form will tend to destabalize the system, making it more susceptible to nucleophilic attack. Hydrolysis of the amide most probably proceeds *via* a tetrahedral intermediate whose rate of formation should be increased as the above equilibrium is shifted to the left.[45] The high reactivity of the β-lactams of the penicillins can be ascribed, at least partly, to the nonplanarity of the β-lactam nitrogen, which tends to restrict this resonance. Sweet and Dahl[2] correlated the chemical and biological activity of the penicillins and cephalosporins with the degree of nonplanarity of the β-lactam nitrogen atom. They noted that as the degree of lactam nitrogen nonplanarity increases, the lactam carbonyl stretching frequency increases and the ease of basic hydrolysis of the lactam amide bond increases. An examination of the structural similarities of anhydropenicillin with the other penicillins indicates that, in accord with the arguments presented, the molecule should be even more chemically labile than the penicillins (antibacterial activity, however, is not expected for the anhydropenicillins due to the absence of the carboxylate group). Thus, the relative chemical stability of the anhydropenicillins must indicate that the nonplanar character of the β-lactam nitrogen atom is a *necessary but not sufficient* condition for chemical activity.

Wolfe and coworkers[15] previously postulated that the anhydropenicillins are stabilized by delocalization of the lone electron pair of N(4) into the adjacent π orbital of the olefinic side chain. Our structural data indi-

(45) M. L. Bender, *Chem. Rev.*, **60**, 53 (1960).

Table IV. Molecular Parameters of Appropriate β-Lactam Compounds

	C=O stretch, cm^{-1}	Distance of nitrogen from plane, Å	C(7)–O(8) bond length, Å	N(4)–C(7) bond length, Å	C(3)–N(4) bond length, Å
Anhydropenicillins[a]	1810				
Phenoxymethylanhydropenicillin[b]		0.41	1.183 (7)	1.415 (7)	1.431 (7)
Penicillin[c]	1770–1790				
Ampicillin[d]		0.38	1.98 (7)	1.360 (7)	1.463 (7)
Penicillin V[e]		0.40	1.207 (18)	1.451 (18)	1.460 (18)
Penicillin G[f]		0.40	1.172 (35)	1.343 (35)	1.485 (35)
Δ^3-Cephalosporins[g,h]	1764–1776				
Cephaloridine hydrochloride[i]		0.24	1.214 (8)	1.382 (8)	1.393 (7)
Δ^2-Cephalosporins[g,h]	1756–1760				
Phenoxymethylcephalosporin[i]		0.06	1.223 (7)	1.339 (7)	1.449 (7)
Unfused β-lactams[j]	1730–1760				

[a] Reference 15. [b] This study. [c] Reference 11. [d] Private communication from M. N. G. James, Department of Biochemistry, University of Alberta, Edmonton, Canada. [e] S. Abrahamsson, D. C. Hodgkin, and E. N. Maslen, *Biochem. J.*, **86**, 514 (1963). [f] G. J. Pitt, *Acta Crystallogr.*, **5**, 770 (1952). [g] The numbering of the atoms in the cephalosporins is slightly different than in the penicillins and anhydropenicillins; *i.e.*, C(3), N(4), C(7), and C(8) become C(4), N(5), C(8), and C(9), respectively. [h] G. F. H. Green, J. E. Page, and S. E. Staniforth, *J. Chem. Soc.*, 1595 (1965). [i] Reference 2. [j] L. J. Bellamy, "The Infared Spectra of Complex Molecules," 2nd ed, Wiley, New York, N. Y., 1958.

cate, however, that there is relatively little of this type of delocalization, at least in the ground electronic state.[46] In contrast, in the chemically active cephaloridine, the bond lengths indicate a discernible delocalization of the unshared electron pair of the β-lactam nitrogen atom. Thus, the relative stability of the anhydropenicillins cannot properly be ascribed to this type of delocalization. These results indicate that there must be other major effects contributing to the rate of base hydrolysis of the β-lactam system which are not obvious from a solid-state structural investigation. The problem has become one that apparently must be attacked by kinetic studies in order to elucidate the roles which the reactive functional groups play in the chemical and biological behavior of these compounds.

(46) These structural results are only valid for the ground electronic state of phenoxymethylanhydropenicillin. The possibility exists that there may be considerable delocalization of the unshared electron pair of N(4) into the olefinic side chain when the molecule is in a transition state; *i.e.*, after the molecule has undergone nucleophilic attack but before the β-lactam bond is broken, the unshared pair may become involved in π-bonding with the isopropylidene group and thus stabilize the system.[15] Although the question of why the Δ^3-cephalosporins (which should be capable of similar electronic interaction) are reactive is not resolved by this explanation, it is noteworthy that the Δ^3-desacetoxycephalosporins are less reactive than the Δ^3-cephalosporins, which would indicate that the presence of the acetate group influences the degree of electronic interaction of the β-lactam nitrogen with the unsaturated dihydrothiazine ring.

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