yellow gum. Chromatography over silica gel by gradient elution between methylene chloride and 25% ether–methylene chloride gave 62 mg of XXV, mp 183–185°, from benzene–hexane: $[\alpha]^{25}D$ +19.3° (c 0.31, MeOH); ν_{max}^{KBr} 1770 (γ-lactone), 1720 (C=O of α-pyrone), 1640, and 1560 cm^{-1} (C=C, α-pyrone);[20] λ_{max}^{MeOH} 291 nm (ε 5900); 100-MHz nmr ($CDCl_3$) δ 1.08, 1.32 (s, CH_3's at C-10 and C-4), 1.82 (d, H-5, $J_{5,8}$ = 5.5 Hz), 3.60 (s, OMe), 4.60 (dd, H-7, $J_{8,7}$ = 3.5 Hz, $J_{7,14}$ = 2.0 Hz), 4.70 (dd, H-6, $J_{5,6}$ = 5.5 Hz, $J_{6,7}$ = 3.5 Hz), 6.00 (d, H-11, $J_{11,14}$ = 1.0 Hz), 7.62 (dd, H-14, $J_{11,14}$ = 1.0 Hz, $J_{7,14}$ = 2.0 Hz); mass spectrum *m/e* 304 ($C_{17}H_{20}O_5$).

Anal. Calcd for $C_{17}H_{20}O_5$: C, 67.09; H, 6.62. Found: C, 67.33; H, 6.47.

(20) K. Nakanishi, "Infrared Absorption Spectroscopy," Holden-Day, San Francisco, Calif., 1962, p 52.

Molecular Architecture of the Cephalosporins. Insights into Biological Activity Based on Structural Investigations[1]

Robert M. Sweet[2] and Lawrence F. Dahl

Contribution from the Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706. *Received February 28, 1970*

Abstract: Crystal-structure analyses by single-crystal X-ray diffraction methods have been carried out on two representative examples of cephalosporin antibiotics and an example of a biologically inactive cephalosporin derivative. These compounds are all analogous to the penicillins in that they contain a substituted β-lactam fused to a sulfur-containing ring. In addition, the penicillin and cephalosporin antibiotics seem to employ the same mode of action when they inhibit the synthesis of bacterial cell walls. These structural studies have revealed significant stereochemical information related to the dependence of the proposed mechanism upon the lability of the β-lactam amide bond and upon the conformation of the antibiotic in the region of the β-lactam ring. Not only have comparisons been made among active antibiotics, but their structures have also been contrasted to a similar but biologically inactive compound. The most striking structural feature is the large pyramidal character of the β-lactam nitrogen atom in the penicillin and two active Δ^3-cephalosporin antibiotics in contrast to the nearly planar lactam nitrogen in the inactive Δ^2-cephalosporin. The ease of base hydrolysis of the lactam amide bond in these antibiotics correlates with biological activity. This lability is rationalized as being due to decreased amide resonance in the antibiotic β-lactam relative to that in free β-lactams and in the biologically inactive Δ^2-cephalosporin. The presence of this decreased electron delocalization is inferred from C–N and C–O bond length differences and from lactam carbonyl stretching frequency variations among these compounds. It is caused in the penicillins by the observed nonplanarity of the lactam nitrogen atom due to ring fusion and in the cephalosporins by this effect plus electron delocalization due to enamine resonance outside the lactam ring. An analysis of the orientation of the carboxyl groups relative to the β-lactam ring in the molecules studied and in the penicillins indicates that, because of the large variation found, the stereochemical requirements placed on this region by the necessity that these molecules be recognized by the proper enzyme may not be very restrictive. The two Δ^3-cephalosporin antibiotics studied were cephaloridine·HCl·H_2O and cephaloglycine. The derivative investigated of the biologically inactive Δ^2-cephalosporin isomer was phenoxymethyl-Δ^2-desacetoxyl cephalosporin. Cephaloridine·HCl·H_2O, $C_{19}H_{17}O_4N_3S_2$·HCl·H_2O, crystallizes in orthorhombic space group $P2_12_12_1$. Each unit cell contains four formula species and has dimensions a = 11.019 ± 0.003, b = 17.398 ± 0.006, and c = 11.006 ± 0.004 Å. Crystals of cephaloglycine, solvated with one molecule each of acetic acid and water for every cephaloglycine molecule contain four $C_{18}H_{19}O_6N_3S \cdot HO_2CCH_3 \cdot H_2O$ species in a monoclinic unit cell of symmetry C2 and dimensions a = 22.081 ± 0.002, b = 10.296 ± 0.001, c = 11.368 ± 0.001 Å, and β = 108.464 ± 0.004°. The unsolvated Δ^2-cephalosporin, $C_{16}H_{16}O_5N_2S$, packs two molecules to a monoclinic unit cell of symmetry $P2_1$ and dimensions a = 12.922 ± 0.006, b = 5.014 ± 0.003, c = 13.712 ± 0.005 Å, and β = 109.867 ± 0.003°.

A considerable amount of work has been done recently to elucidate the structure and synthesis of bacterial cell walls and to propose the mechanism by which the penicillin (Figure 1a)[3] and cephalosporin (Figure 1b)[3] antibiotics inhibit this synthesis.[4-9] Experiments showed that one of the final steps in bacterial cell wall production is the three-dimensional cross-linking of peptidoglycan strands.[4,5] The enzyme peptidoglycan transpeptidase cleaves the C-terminal D-alanine residue from a short peptide chain which terminates with D-ala-D-ala and replaces it with a particular free amino group fastened to an adjacent peptidoglycan strand. Workers first demonstrated that the cell walls of *S. aureus* grown in the presence of penicillin G contained a larger amount of D-alanine[4,5] and had more

(1) (a) Previous paper reporting preliminary results from this work: R. M. Sweet and L. F. Dahl, *Biochem. Biophys. Res. Commun.*, **34**, 14 (1969); (b) presented in part at the Eighth International Congress of the International Union of Crystallography, Buffalo, N. Y., August 8, 1969; see *Acta Crystallogr., A*, **25**, part S3, S201 (1969).

(2) This manuscript is based in part on a dissertation submitted by R. M. Sweet to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the Ph.D. degree, Jan 1970.

(3) The numbering systems used throughout this article are consistent with those shown in Figure 1.

(4) D. J. Tipper and J. L. Strominger, *Proc. Nat. Acad. Sci.*, **54**, 1133 (1965), and references cited therein.

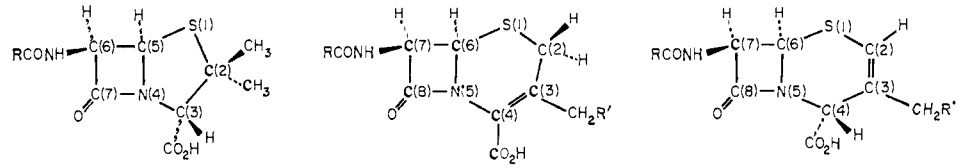
(5) E. M. Wise, Jr., and J. T. Park, *ibid.*, **54**, 75 (1965), and references cited therein.

(6) K. Izaki, M. Matsuhashi, and J. L. Strominger, *ibid.*, **55**, 656 (1966).

(7) J. L. Strominger, K. Izaki, M. Matsuhashi, and D. J. Tipper, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **26**, 9 (1967).

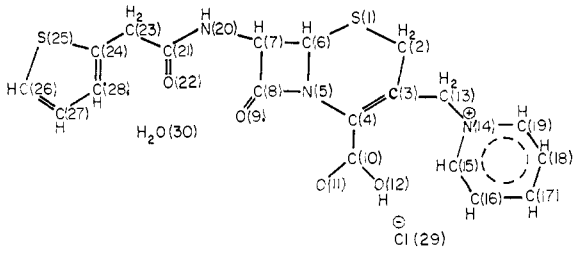
(8) K. Izaki, M. Matsuhashi, and J. L. Strominger, *J. Biol. Chem.*, **243**, 3180 (1968).

(9) D. J. Tipper and J. L. Strominger, *J. Biol. Chem.*, **243**, 3169 (1968).



(a) penicillins (b) Δ^3-cephalosporins (c) Δ^2-cephalosporins

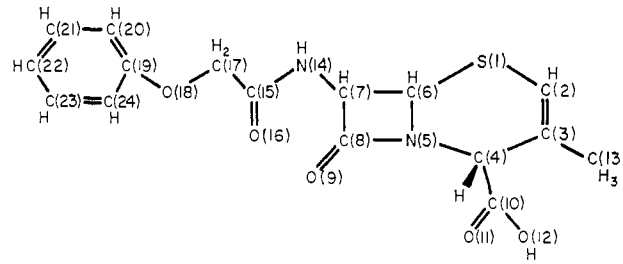
Figure 1. Formulas of the molecular nuclei of the penicillins, the biologically active Δ^3-cephalosporins, and the biologically inactive Δ^2-cephalosporins.



Cephaloridine hydrochloride monohydrate

$C_{19}H_{17}O_4N_3S_2 \cdot HCl \cdot H_2O$

Figure 2. Molecular formula of cephaloridine hydrochloride monohydrate.



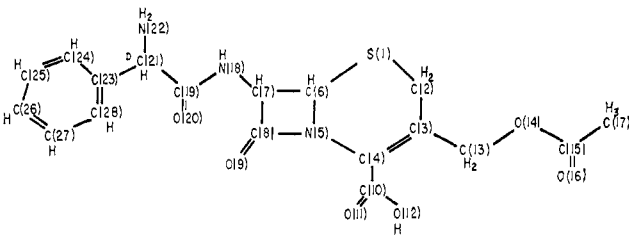
Phenoxymethyl-Δ^2-desacetoxyl cephalosporin

$C_{16}H_{16}O_5N_2S$

Figure 3. Molecular formula of phenoxymethyl-Δ^2-desacetoxyl cephalosporin.

of the proper free amino groups[5] to indicate that less of this transpeptidation occurs than in cells grown in its absence. Direct evidence of the inhibiting effect the pencillin and cephalosporin antibiotics have on this transpeptidation was shown by Strominger and coworkers[6-8] who demonstrated that, in a cell-free, particulate enzyme preparation from *E. coli* acting on monomeric precursors of the bacterial cell wall, an insoluble product was formed along with release of half of the total D-alanine. Conversely, in the presence either of one of various penicillins or of the cephalosporin antibiotic, cephalothin, a soluble product is formed with no release of D-alanine. Finally, Tipper and Strominger[9] demonstrated by pulse-labeling experiments in cultures of *S. aureus* that this transpeptidation is, in fact, the terminal step in the synthesis of the cell wall and that this step is inhibited by several penicillins and cephalothin. Earlier, Tipper and Strominger[4] suggested that penicillin has a conformation equivalent to one which can be adopted by D-ala-D-ala. The transpeptidase recognizes the penicillin molecule as its substrate and becomes irreversibly acylated by the antibiotic when the amide link in the β-lactam ring is cleaved. Thus, further cross-linking by that particular enzyme molecule is prevented. The growing bacterial cell wall eventually loses the structural strength necessary to contain the endoplasm, and the bacterium bursts.

A particularly good review of the relationship between chemistry and biological activity in the cephalosporins and penicillins, which also documents the development of these compounds as useful antibiotics, has been published recently by Abraham.[10] As antibiotics, the cephalosporins are well established as chemotherapeutic agents. In particular, cephalothin (Figure 1b, where R is $-CH_2$-thiophene and R′ is -OAc) and cephaloridine (Figure 1b where R is $-CH_2$-thiophene and R′ is -pyridyl) enjoy wide clinical use. Furthermore, cephaloglycine (Figure 1b where R is -D-$CH(NH_2)$-C_6H_5 and R′ is -OAc) has the property uncommon among cephalosporins of being absorbable through the gut. Cephalosporins are active against many strains of gram-negative and gram-positive bacteria and against some strains of penicillin resistant bacteria, and are not generally allergenic to most penicillin-sensitive subjects.[10]



Cephaloglycine

$C_{18}H_{19}O_6N_3S$

Figure 4. Molecular formula of cephaloglycine.

Early in our studies the curious fact that the Δ^2 isomers of cephalosporins are inactive biologically was noted in the literature.[11] The molecular formula of the basc skeleton of the Δ^2-cephalosporins is shown in Figure 1c.

This paper reports the determination by single-crystal X-ray diffraction methods of detailed and precise molecular parameters for crystalline cephaloridine hydrochloride monohydrate (Figure 2), a cephalosporin antibiotic, and for phenoxymethyl-Δ^2-desacetoxyl cephalosporin (Figure 3), a cephalosporin isomer derivative with negligible antibacterial activity.[12] In addition, approximate molecular parameters have been deter-

(10) E. P. Abraham, *Top. Pharm. Sci.*, 1, 1 (1968).

(11) S. Eardly, G. I. Gregory, M. E. Hall, and A. G. Long, Abstracts, 19th International Congress of UPAC, London, 1963, Sect. A8-6, p 308.

(12) M. Gorman, Lilly Research Laboratories, Indianapolis, Ind., private communication to R. M. Sweet, 1969.

mined for another active cephalosporin, cephaloglycine (Figure 4). The purpose here is not only to compare active antibiotics but also to contrast molecular parameters of active cephalosporins and penicillins with those of a similar but biologically inactive compound. Only in this way can chemical and biological activity be studied in structural terms with any hope for a successful explanation. A novel method of application of the symbolic addition procedure for direct solution of the phase problem in X-ray diffraction crystallography is presented together with an empirical technique by which the weighting scheme for crystallographic least squares can be improved.

Experimental Section

Single-Crystal X-Ray Data. All of the crystals used in these investigations were most kindly furnished by Dr. R. Pfeiffer of Eli Lilly and Co., Indianapolis, Ind. Data for all three compounds were collected on the same four-circle Datex automated General Electric diffractometer. After each crystal was aligned by optical and X-ray techniques,[13] a suitable number of representative diffraction maxima were carefully centered.[14] The lattice constants and orientation parameters used to calculate goniostat settings for data collection were obtained by a least-squares procedure which fits these parameters to all three goniostat angles (χ, ϕ, 2θ) of each setting reflection.[15] All intensity data were collected at a take-off angle of 2.0° by the θ–2θ scan technique[13] with symmetric 2θ scans at a 2.0°/min rate over a range of 2.0° for cephaloridine and cephaloglycine and 1.4° and 1.6° for the Δ^2-cephalosporin. (Stationary-crystal)–(stationary-counter) background counts of 15 sec were taken at the beginning and end of each scan. A counteraperture of 2 mm diameter was placed 31 mm from the crystal. Nickel-filtered Cu Kα radiation was employed with a scintillation detector followed by a pulse-height analyzer adjusted to accept approximately 90% of the Cu Kα pulse distribution.

At least three symmetrically equivalent sets of intensity data were collected for $2\theta \leq 115$ and 125°, respectively, for cephaloridine and the Δ^2-cephalosporin. In both cases observable reflections were shown from X-ray photographs to be available beyond these angular limits, but they could not be measured on the diffractometer due to our particular machine limitations. In order to check electronic and crystal stability, the intensities of three representative standard reflections were measured every 80 reflections. No significant fluctuations in these sets of three standards were observed during the data collection on these two compounds. Several symmetrically equivalent sets of data were collected on each of three different crystals of cephaloglycine; the intensities from the second two crystals were measured 9 months after those of the first. In each data set the intensities of the standard reflections, sampled in a fashion similar to that used with the other two compounds, showed a systematic decrease, for which the intensity data were corrected. The intensities then were corrected for background and Lorentz-polarization effects and reduced to structure amplitudes; variances were calculated according to the following relations: $I = S - BT/t$, $\sigma_I = (S + B(T/t)^2 + (0.05I)^2)^{1/2}$, $F = (I/Lp)^{1/2}$, and $\sigma_F = \sigma_I \partial F/\partial I = \sigma_I/2FLp$, where S is the total count accumulated during the scans of time T, B is the total accumulated background count sampled for time t, I is the integrated peak intensity, Lp is the normal Lorentz-polarization correction, and F is the observed structure factor. A given reflection was considered observed if I was greater than $2\sigma(I)$. For cephaloridine, of the 1665 independent reflections sampled, 1547 were observed. For the Δ^2-cephalosporin, 1269 of the 1415 reflections sampled were observed. In the case of cephaloglycine, the data were of considerably poorer quality, and somewhat less than 1000 of the 1600 reflections sampled were considered suitable for use in least squares.

No corrections were made for absorption, extinction, or anomalous dispersion effects. For cephaloridine the particular crystal used in diffractometer data collection was block-shaped, 0.2 mm on a side, mounted with the spindle axis parallel to c. The linear absorption coefficient, μ, for this material of 37.0 cm^{-1} for Cu Kα radiation results in absorption correction factors which vary only from 1.85 to 1.95; hence, absorption corrections were not applied to the data. Examination of the twenty largest observed and calculated structure factors at the end of refinement showed that extinction effects were minimal. No anomalous dispersion corrections of the scattering factors were made since the real and imaginary dispersion corrections for Cu Kα radiation are small (*i.e.*, $\Delta f' = 0.3$ and $\Delta f'' = 0.6$ for sulfur; $\Delta f' = 0.3$ and $\Delta f'' = 0.7$ for chlorine).[16] For the Δ^2-cephalosporin, the crystal used for intensity measurement was a needle, 0.4 mm long and 0.04 mm × 0.10 mm in the other directions, elongated along the crystallographic b axis. It was mounted with the spindle and needle axes parallel. Since the linear absorption coefficient for this crystal with Cu Kα radiation of 1.93 cm^{-1} resulted in a variation of the absorption correction factors from only 1.04 to 1.14, no absorption corrections were applied to the data. An examination of the ten largest observed and calculated structure factors at the end of least-squares refinement revealed no indication of extinction effects. The poor quality of the data from the cephaloglycine crystals did not warrant consideration of any of these small effects. The atomic scattering factors used for all atoms are those based on Hartree–Fock–Slater calculations as compiled by Hanson and coworkers.[17]

Crystal Data. (a) Cephaloridine Hydrochloride Monohydrate. The measured lattice constants and estimated standard deviations for the orthorhombic unit cell of cephaloridine hydrochloride monohydrate (Figure 2, $C_{19}H_{17}O_4N_3S_2 \cdot HCl \cdot H_2O$) are $a = 11.019(3)$, $b = 17.398(6)$, and $c = 11.006(4)$ A,[18] based on an assumed Cu Kα wavelength of 1.5418 Å. These values are a weighted average of several determinations which were made because the crystal was found to shift slightly in its mount and was realigned periodically. For Z equal to four formula units per cell, the calculated density of these crystals is 1.479 g/cm^3 which compares well with the observed density of 1.475(8) g/cm^3 measured by flotation in mixed liquids. The total number of electrons per cell, $F(000)$, is 968.

Weissenberg photographs taken about two different axes revealed orthorhombic Laue symmetry of D_{2h}–2/m2/m2/m. Systematic absences of h, k, and l odd for $\{h00\}$, $\{0k0\}$, and $\{00l\}$ data, respectively, indicate that the probable space group is uniquely $P2_12_12_1$. The origin in this space group was chosen to give equivalent general positions at x, y, z; $\frac{1}{2} + x, \frac{1}{2} - y, -z$; $-x, \frac{1}{2} + y, \frac{1}{2} - z$; and $\frac{1}{2} - x, -y, \frac{1}{2} + z$.

(b) Cephaloglycine Acetic Acid Hydrate. The lattice constants and estimated standard deviations measured from a reasonably fresh crystal of cephaloglycine (Figure 4, $C_{18}H_{19}O_6N_3S$) acetic acid hydrate were $a = 22.081(2)$, $b = 10.296(1)$, $c = 11.368(1)$ Å, and $\beta = 108.464(4)°$. Weissenberg and precession photographs together with the fact that the compound is optically active and nonracemic indicated that the probable noncentrosymmetric space group is C2; the calculated density based on four molecules per cell is 1.31 g/cm^3. The number of electrons per cell, $F(000)$, is 1012. The cell origin was chosen such that the fourfold equivalent general positions are: x, y, z; $-x, y, -z$; $\frac{1}{2} + x, \frac{1}{2} + y, z$; $\frac{1}{2} - x, \frac{1}{2} + y, -z$.

(c) Phenoxymethyl-Δ^2-desacetoxyl Cephalosporin. It was necessary to align a single crystal of phenoxymethyl-Δ^2-desacetoxyl cephalosporin (Figure 3, $C_{16}H_{16}O_5N_2S$) twice on the diffractometer. The 18 setting reflections in the first case and the 12 in the second case came from the octants from which intensity data were collected. The cell parameters, which are a weighted average of the two sets of parameters determined independently, are $a = 12.922(6)$, $b = 5.014(3)$, and $c = 13.712(5)$ Å; and $\beta = 109.867(3)°$. The standard deviation which appears for each parameter is the statistical rms precision of the weighted average. The observed density of 1.39(1) g/cm^3, measured by flotation in mixed liquids, compares well with the density of 1.38 g/cm^3 calculated from the known volume with an assumed occupancy of $Z = 2$ molecules per unit cell. $F(000)$ is 364.

(13) T. C. Furnas, Jr., "Single Crystal Orienter Instruction Manual," General Electric Company, Milwaukee, Wis., 1966.

(14) A setting reflection was considered aligned if at $2\theta_{max}$ the reflection was centered in the receiving aperture. The $2\theta_{max}$ was defined to be the mean of the two 2θ settings at which the observed radiation intensity was half of the intensity registered at the peak.

(15) Alan S. Foust, Ph.D. Thesis, University of Wisconsin, Madison, Wis., 1970.

(16) "International Tables for X-Ray Crystallography," Vol. III, The Kynoch Press, Birmingham, 1962, p 214.

(17) H. P. Hanson, F. Herman, J. D. Lea, and S. Skillman, *Acta Crystallogr.*, 17, 1040 (1964).

(18) Standard deviations of the last significant figures are given consistently in parentheses throughout this paper.

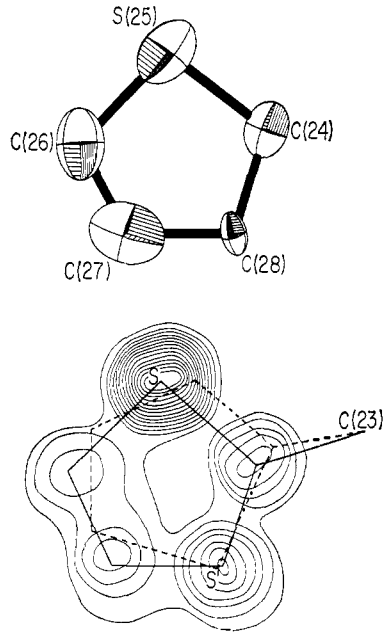


Figure 5. (Top) Ellipsoids representing 50% probability from the least-squares refinement of cephaloridine hydrochloride monohydrate with an anisotropic thermal model for the thiophene ring. The view shown is perpendicular to the plane of the ring. (Bottom) Electron density difference map in the plane of the above thiophene ring phased on all atoms in the structure except those of this ring. Contours are at intervals of 0.5 e/Å^3 beginning at 0.5 e/Å^3. Superimposed on the difference map are the ring positions resulting from the rigid-body refinement. The solid line represents the principal thiophene ring, containing sulfur atom S, with a multiplier of 0.85; the dashed line represents the disordered thiophene, with sulfur atom S′, which has a multiplier of 0.15.

Weissenberg and precession photographs showed monoclinic C_{2h}–(2/m) Laue symmetry; the observed condition that $k = 2n$ for $\{0k0\}$ data indicated either space group $P2_1$ or $P2_1/m$. Since the molecules of this compound are optically active and nonracemic, the probable space group is the noncentrosymmetric $P2_1$; the general twofold set of positions for this space group is: $x, y, z; -x, {}^1/_2 + y, -z$.

Determination of the Structures. (a) Cephaloridine Hydrochloride Monohydrate. The structure was solved by the heavy atom method from 2244 visually estimated film data which were collected from sets of equiinclination Weissenberg photographs taken about each of two different axes with Ni-filtered Cu Kα radiation. Positions of two of the three nearly equivalent heavy atoms, which later turned out to be S(1) and Cl(29), were determined from the three-dimensional Patterson function. A Fourier map, phased on these two atoms, indicated the initial coordinates of the third heavy atom, S(25), with the help of the Patterson function. Subsequent Fourier maps, coupled with one cycle of intermediate least-squares refinement, revealed the positions of the remaining nonhydrogen atoms in the molecule and the oxygen atom of the water of hydration. Four cycles of full-matrix isotropic least-squares refinement[19] based on film data reduced the conventional unweighted R factor to $R_1 = 0.13$.[20] At this point the availability of a diffractometer made it possible for better data to be obtained. During subsequent refinement, only the diffractometer data were used. Before each further least-squares cycle, the idealized positions of hydrogen atoms were calculated.[21] These hydrogen atoms were included in the structure factor calculations but neither their positions nor their temperature parameters, set arbitrarily at an isotropic value of 3.0 Å^2, were allowed to be shifted by the least squares. Three more isotropic least-squares cycles were run followed by four cycles in which the six nonhydrogen atoms in the pyridine ring were refined isotropically and all other nonhydrogen atoms were refined anisotropically. During all least-squares refinement, the weight applied to each observation equaled the squared reciprocal of its standard deviation.

Toward the end of refinement a semi-empirical method was used to improve the weighting scheme because the standard deviation of an average observation of unit weight[22] (the goodness-of-fit parameter) had the undesirably high value of 8. This treatment resulted in the final goodness-of-fit parameter near 1.0. In this method, the observed values of $||F_o| - |F_c||/\sigma(F_o)$ were fit by a least-squares polynomial in F_o. This function was then used as a corrective multiplier for the initial $\sigma(F_o)$. This procedure yields a standard deviation for each reflection which indicates not only the random errors in that observation as determined by its counting statistics but also systematic errors which may be functions of F. At the end of the eleventh least-squares cycle, the residuals were $R_1 = 0.051$ and $R_2 = 0.058$,[23] and the goodness-of-fit parameter was 0.84.

At the termination of this anisotropic refinement, bond lengths and angles within the cephaloridine molecule were completely consistent with what one might expect, with but one exception. The C(24)–C(28) double bond in the thiophene ring was 0.1 Å longer than the 1.37 Å expected,[24] and the semi-major axes of the thermal ellipsoid for C(28) were anomalously short. An electron-density Fourier difference map, phased on all the atoms in the cell except those in this thiophene ring, was not inconsistent with the refined anisotropic model; the peaks at each of the atoms had shapes equivalent to the refined thermal ellipsoids, while the peak at C(28) showed a higher electron density than the densities of any of the other thiophene ring peaks attributable to carbon atoms. Figure 5 shows the thermal ellipsoids which represent the anisotropic model of the thiophene ring together with the electron density calculated in the plane of this ring from the above-mentioned Fourier difference map. The most likely explanation of this anomalous model for the thiophene ring is that a twofold disorder exists wherein the disordered ring is rotated 180° about an axis passing through atom C(24) and a point approximately midway between atoms C(26) and C(27).

Idealized rigid-body thiophene rings[24] placed in these approximate orientations were refined by least squares. For the first cycle the thiophene ring in the orientation of the previously refined ring, which will be referred to as the principal thiophene, was given an accommodation factor of 0.8. The twofold related ring, called the disordered thiophene, was given 0.2 (*i.e.*, 1–0.8) as an accommodation factor. An overall temperature factor and the six positional and orientation parameters were varied for each of the two thiophene rings. The shifts in temperature factors indicated that more appropriate accommodation factors would be 0.85 and 0.15. During the two subsequent cycles, individual atomic isotropic temperature factors in the principal thiophene ring, a group temperature factor for the disordered ring, and all positional and orientation parameters for both rings were allowed to shift. Two final least-squares cycles were run in which these rigid group parameters together with the same atomic parameters for the other part of the structure used in the earlier anisotropic refinement were varied. Average shift-to-error ratios in the final cycle were 1.2 for the rigid groups and 0.4 for the rest of the structure. Bond lengths which resulted from this refinement showed average shifts from the previous model of one standard deviation; only three bond lengths changed as much as two standard deviations (Table II). The residuals at the end of this refinement were $R_1 = 0.057$ and $R_2 = 0.066$. The final positions of the two disordered orientations of the thiophene ring are superimposed on the difference electron density map in Figure 5. The interesting crystallographic observa-

(19) 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. The function minimized was $\Sigma w_i \Delta F_i^2$.

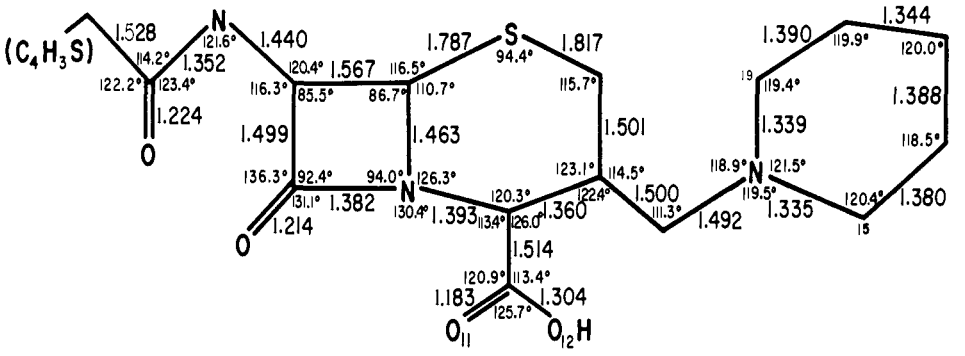
(20) $R_1 = (\Sigma||F_o| - |F_c||)/(\Sigma|F_o|)$.

(21) J. C. Calabrese, "PROGRAM MIRAGE," University of Wisconsin, Madison, Wis., 1970. The hydrogen atoms were placed in idealized positions to complete the polyhedron about the connected atom at interatomic distances proper for the type of bond (1.07 to 1.10 Å for C–H and N–H bonds).

(22) $[\Sigma w_i(|F_o| - |F_c|)^2/(n_{obsd} - n_{param})]^{1/2}$.

(23) $R_2 = [(\Sigma w_i(|F_o| - |F_c|)^2/(\Sigma w_i F_o^2)]^{1/2}$.

(24) The thiophene ring parameters used in the rigid-body model are those averaged from two independent, precise structural determinations (R. A. Bonham and F. A. Momany, *J. Phys. Chem.*, 67, 2474 (1963)); B. Bak, D. Christensen, L. Hansen-Nygaard, and J. Rastrup-Andersen, *J. Mol. Spectrosc.*, 7, 58 (1961), in the gaseous state of "free" thiophene which has C_{2v}–2 mm point group symmetry. These averaged parameters are: S–C(1) = 1.714 Å, C(1)–C(2) = 1.370 Å, C(2)–C(2′) = 1.421 Å, ∠ C(2)–S–C(2′) = 92°11′, ∠ S–C(1)–C(2) = 111°26′, and ∠ C(1)–C(2)–C(2′) = 112°28.5′.



Cephaloridine

Figure 6. Bond lengths and angles which are means from the two refinements of cephaloridine hydrochloride monohydrate.

tion is that although the disordered isotropic model for the thiophene ring seems to be quite satisfactory, it may not represent the disposition of electron density in this region as accurately as the initial anisotropic model, as indicated by the R values of the respective refinements.

The coordinates and thermal parameters as obtained from the output of both least-squares refinements appear in Table I.[25] Atomic distances and angles are presented in Table II and in Figure 6 along with estimated standard deviations which contain the effect of errors in the lattice parameters as calculated by the Busing–Martin–Levy program.[26]

(b) Phenoxymethyl-Δ^2-desacetoxyl Cephalosporin. This structure was solved by the symbolic addition procedure coupled with tangent-formula refinement.[27] Wilson plot statistics and calculated normalized structure factors[28] with statistical averages of $\langle E \rangle = 0.854$, $\langle E^2 \rangle = 1.000$ (rescaled), and $\langle E^2 - 1 \rangle = 0.798$ were reasonably consistent with those expected from an acentric crystal structure.[29]

The strategy employed in the solution of this structure in polar space group $P2_1$ was to select phases for three reflections (including an $h1l$ one)[30] to specify the origin and then to choose other reflections with phases represented by symbols which not only had a large number of interactions themselves but also allowed the reflections which specified the origin to contribute to as large a number of additional phases as possible. The set of starting phases for symbolic addition which finally led to the correct structure is as follows: $|E(-3,1,2)| = 2.82$, $\phi = 0$; $|E(8,0,7)| = 2.08$, $\phi = 0$; $|E(11,0,1)| = 1.93$, $\phi = 0$; $|E(-1,1,1)| = 2.31$, $\phi = W(0)$; $|E(-7,3,2)| = 2.14$, $\phi = X(0)$; $|E(-10,2,2)| = 2.59$, $\phi = Y(-\pi/2)$; $|E(2,1,1)| = 2.67$, $\phi = Z(\pi)$. The computer program MAGIA[31] was used to automate this attempt and previous unsuccessful attempts at symbolic addition. Sixty-three symbolic phases were determined. The largest variance[26] of an accepted phase was 0.43 radian squared. At the end of the symbolic addition run, a list of symbol equivalences was compiled from the occasions on which two or more different phase indications appeared for a single reflection. The variance of a single equivalence was taken to be the sum of the variances of the two contributing phases. Weights were assigned to each equivalence equal to the reciprocal of the variance of that particular equivalence. Then the weights for all occurrences of a single symbol equivalence were summed to give a net weight for each equivalence relation. This process resulted in a collection of eight symbol equivalence relations with their accompanying weights. The numeric solution to these eight equations containing a total of four unknown symbolic phases was not obvious, so an empirical approach was taken. Each equivalence relation was written in the form of a linear combination of symbols set equal to zero. Each of the symbols was then assigned values which varied serially over the whole circle in units of $\pi/4$. These values were plugged into the various relations to calculate a residue for each relation. Then these residues were multiplied by the weight for that particular relation and summed to give a net residue for each set of values for the symbols. The summed, weighted residues were tabulated by computer (there were some four thousand of them) and examined by hand. The one set of phases for which the residue was zero was a trivial result with all phases real. Both the set of phases which yielded the correct structure and the phases which represented the enantiomorph of that structure had residues of three on our arbitrary scale. The next highest residue was 9.1. The values assigned the symbols were: $W = 0$, $X = 0$, $Y = \pi/2$, and $Z = \pi$.

In retrospect, in the attempt to find the best set of phase values for the symbols a more mathematically sound treatment of the equivalence relations would be to use a method more nearly akin to least squares. This can be accomplished by a squaring of the residue from each equivalence relation before one multiplies by the weight for that particular relation and sums over all relations. When this procedure was tried on the present set of equivalences, the result was the same. The lowest least-squares residue, that of the set of phases accepted as correct, was 4.8. The next highest residue was 7.7.

The phase values determined by this method were substituted for the symbolic phases which resulted from symbolic addition. A total of seven cycles of reiterative tangent-formula refinement was carried out by the application of the program TANGFORM[32] on all E's greater than 1.5. These 163 phases were then kept fixed while the tangent formula was used to calculate phases for the additional 146 E factors between 1.5 and 1.2. The phase of the particular reflection $-3,1,2$, which was used to specify the origin in the y direction, was not allowed to shift during this refinement. The electron-density map calculated with the phased E factors used as coefficients revealed all atoms in the molecule except the methyl carbon, C(13). The thirteen largest peaks were correct; nineteen of the largest 23 peaks were correct. The weakest peak which appeared to be correct was the 49th largest.

Five cycles of full-matrix isotropic least-squares refinement were carried out on this structure followed by four cycles of anisotropic refinement.[19] To fix the origin in the b direction the y coordinate of the sulfur atom was constrained to the position at which it appeared on the E map. At the end of isotropic refinement, the pre-

(25) Observed and calculated structure factors derived from the least-squares refinement of cephaloridine hydrochloride monohydrate and phenoxymethyl-Δ^2-desacetoxyl cephalosporin are deposited as Document No. NAPS-01011 with the ASIS National Auxiliary Publication Services, C/O CCM Information Corp., 909 3rd Ave., New York, N. Y. 10022. A copy may be secured by citing the document number and remitting $2.00 for microfiche or $5.00 for photocopies. Advance payment is required. Make checks or money orders payable to ASIS-NAPS.

(26) 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.

(27) J. Karle and H. Hauptman, *Acta Crystallogr.*, **9**, 635 (1956); J. Karle and I. L. Karle, *ibid.*, **21**, 849 (1966).

(28) R. B. K. Dewar and A. L. Stone, "FAME and MAGIC, Fortran Computer Programs for Use in the Symbolic Addition Method," University of Chicago, Chicago, Ill., 1966; *cf.*, E. B. Fleischer, R. B. K. Dewar, and A. L. Stone, Abstracts of Papers, American Crystallographic Association Winter Meeting, Atlanta, Ga., 1967, p 20.

(29) H. Hauptman and J. Karle, *Acta Crystallogr.*, **6**, 136 (1953); J. Karle and H. Hauptman, *ibid.*, 131 (1953).

(30) H. Hauptman and J. Karle, *ibid.*, **9**, 45 (1956).

(31) R. B. K. Dewar, "MAGIA, Fortran Computer Program for Use in the Symbolic Addition Method with Acentric Crystal Structures," Illinois Institute of Technology, 1969.

(32) S. A. Brenner and P. H. Gum, "The Tangent Formula Program for the X-Ray Analysis of Noncentrosymmetric Crystals," Naval Research Laboratory, Washington, D. C., 1968.

Table I

A. Final Atomic Positional Parameters with Their Standard Deviations for the Anisotropic Refinement of Cephaloridine Hydrochloride Monohydrate

	$x(10^4\sigma)$	$y(10^4\sigma)$	$z(10^4\sigma)$
S(1)	0.4272 (1)	0.2654 (1)	0.1557 (2)
C(2)	0.4263 (6)	0.3143 (3)	0.3014 (6)
C(3)	0.3396 (5)	0.3806 (3)	0.3118 (6)
C(4)	0.2390 (5)	0.3876 (3)	0.2414 (6)
N(5)	0.2066 (4)	0.3294 (3)	0.1618 (5)
C(6)	0.2661 (5)	0.2550 (3)	0.1492 (6)
C(7)	0.2085 (5)	0.2517 (4)	0.0189 (6)
C(8)	0.1723 (6)	0.3331 (4)	0.0404 (6)
O(9)	0.1356 (5)	0.3863 (3)	−0.0208 (5)
C(10)	0.1458 (6)	0.4517 (4)	0.2506 (6)
O(11)	0.0404 (4)	0.4374 (3)	0.2500 (6)
O(12)	0.1932 (4)	0.5200 (2)	0.2583 (5)
C(13)	0.3632 (6)	0.4327 (4)	0.4181 (6)
N(14)	0.4808 (5)	0.4742 (3)	0.4040 (5)
C(15)	0.5064 (7)	0.5091 (4)	0.2983 (7)
C(16)	0.6136 (7)	0.5490 (4)	0.2838 (8)
C(17)	0.6952 (7)	0.5504 (5)	0.3792 (8)
C(18)	0.6647 (8)	0.5189 (5)	0.4870 (8)
C(19)	0.5566 (7)	0.4784 (4)	0.4988 (7)
N(20)	0.2832 (5)	0.2376 (3)	−0.0862 (5)
C(21)	0.2559 (6)	0.1821 (4)	−0.1682 (6)
O(22)	0.1777 (4)	0.1329 (2)	−0.1507 (4)
C(23)	0.3217 (6)	0.1895 (4)	−0.2892 (6)
C(24)	0.2414 (6)	0.2336 (4)	−0.3766 (6)
S(25)	0.1989 (2)	0.1960 (1)	−0.5101 (2)
C(26)	0.1120 (7)	0.2723 (6)	−0.5470 (7)
C(27)	0.1149 (8)	0.3253 (5)	−0.4583 (9)
C(28)	0.1858 (5)	0.3094 (3)	−0.3557 (5)
C1(29)	−0.0061 (1)	0.1408 (1)	0.2103 (2)
O(30)	0.0169 (7)	0.0235 (4)	−0.0224 (6)

B. Final Positional Parameters for the Hydrogen Atoms of Cephaloridine Hydrochloride Monohydrate Which Were Found on the Difference Map Phased on the Above Atomic Parameters

	x	y	z
H1(2)	0.512	0.332	0.317
H2(2)	0.406	0.278	0.358
H(6)	0.240	0.219	0.183
H(7)	0.135	0.220	0.029
H(12)	0.166	0.570	0.296
H1(13)	0.371	0.406	0.490
H2(13)	0.300	0.475	0.427
H(15)	0.433	0.519	0.246
H(16)	0.610	0.559	0.200
H(17)	0.775	0.579	0.340
H(18)	0.675	0.508	0.546
H(19)	0.531	0.450	0.573
H(20)	0.342	0.279	−0.108
H1(23)	0.402	0.219	−0.296
H2(23)	0.327	0.139	−0.338
H(26)	0.062	0.266	−0.640
H(27)	0.058	0.380	−0.464
H(28)	0.183	0.340	−0.310

C. Final Thermal Parameters[a] with Their Standard Deviations[b] from the Anisotropic Refinement of Cephaloridine·HCl·H_2O

	$10^4\beta_{11}$	$10^4\beta_{22}$	$10^4\beta_{33}$	$10^4\beta_{12}$	$10^4\beta_{13}$	$10^4\beta_{23}$
S(1)	44 (1)	29 (1)	64 (2)	5 (1)	−4 (1)	−10 (1)
C(2)	53 (6)	24 (2)	74 (7)	2 (3)	−8 (6)	−14 (4)
C(3)	58 (6)	20 (2)	60 (6)	−5 (3)	12 (6)	−1 (3)
C(4)	48 (6)	19 (2)	57 (6)	−4 (3)	−3 (5)	−3 (3)
N(5)	48 (4)	20 (2)	52 (5)	0 (2)	−8 (4)	0 (3)
C(6)	47 (7)	16 (2)	62 (6)	1 (3)	−5 (5)	−1 (3)
C(7)	53 (6)	24 (2)	55 (6)	−4 (3)	3 (5)	3 (3)
C(8)	51 (6)	20 (2)	76 (7)	0 (3)	−4 (6)	0 (4)
O(9)	130 (6)	34 (2)	89 (6)	16 (3)	−34 (5)	14 (3)
C(10)	51 (7)	33 (3)	80 (7)	5 (4)	−4 (6)	−0 (4)
O(11)	51 (5)	33 (2)	196 (9)	3 (2)	8 (5)	−7 (3)
O(12)	72 (4)	17 (2)	139 (6)	−6 (2)	3 (5)	−8 (3)
C(13)	47 (6)	32 (3)	61 (6)	−4 (3)	6 (6)	−12 (4)
N(14)	3.0 (1)[c]					
C(15)	3.9 (1)[c]					
C(16)	4.6 (2)[c]					
C(17)	5.1 (2)[c]					
C(18)	5.6 (2)[c]					
C(19)	4.0 (2)[c]					
N(20)	63 (5)	26 (2)	66 (5)	−11 (3)	0 (5)	−2 (3)
C(21)	65 (7)	25 (3)	65 (6)	10 (4)	−16 (6)	−6 (4)
O(22)	77 (4)	22 (2)	93 (5)	−10 (2)	0 (4)	−2 (3)
C(23)	66 (6)	34 (3)	65 (6)	−2 (4)	13 (6)	−2 (4)
C(24)	60 (6)	34 (3)	46 (6)	−18 (4)	8 (5)	10 (4)
S(25)	115 (2)	55 (1)	79 (2)	−14 (1)	3 (2)	−10 (1)
C(26)	80 (8)	58 (4)	79 (8)	−10 (5)	−5 (7)	24 (5)
C(27)	109 (9)	40 (4)	148 (12)	−11 (5)	43 (9)	13 (6)
C(28)	26 (5)	19 (2)	44 (5)	−3 (3)	−15 (5)	10 (3)
C1(29)	67 (2)	23 (1)	96 (2)	−5 (1)	10 (2)	1 (1)
O(30)	247 (11)	78 (4)	126 (8)	−55 (6)	38 (8)	−31 (5)

D. Final Atomic Positional Parameters with Their Standard Deviations from the Anisotropic Refinement of Cephaloridine·HCl·H_2O with Rigid-Body, Disordered Thiophene Rings

	$x(10^4\sigma)$	$y(10^4\sigma)$	$z(10^4\sigma)$
S(1)	0.4273 (1)	0.2652 (1)	0.1557 (2)
C(2)	0.4255 (6)	0.3141 (4)	0.3018 (7)
C(3)	0.3394 (5)	0.3805 (3)	0.3126 (6)
C(4)	0.2387 (5)	0.3882 (3)	0.2422 (6)
N(5)	0.2060 (4)	0.3299 (3)	0.1608 (5)
C(6)	0.2660 (5)	0.2550 (3)	0.1479 (6)
C(7)	0.2086 (6)	0.2508 (4)	0.0182 (6)
C(8)	0.1724 (6)	0.3332 (4)	0.0408 (6)
O(9)	0.1359 (5)	0.3866 (3)	−0.0203 (5)
C(10)	0.1450 (6)	0.4513 (4)	0.2505 (7)
O(11)	0.0404 (4)	0.4376 (3)	0.2487 (6)
O(12)	0.1932 (4)	0.5199 (2)	0.2584 (5)
C(13)	0.3637 (6)	0.4328 (4)	0.4179 (6)
N(14)	0.4812 (5)	0.4745 (3)	0.4030 (5)
C(15)	0.5058 (6)	0.5093 (4)	0.2985 (7)
C(16)	0.6135 (7)	0.5486 (4)	0.2840 (8)
C(17)	0.6953 (8)	0.5504 (5)	0.3807 (8)
C(18)	0.6652 (8)	0.5185 (5)	0.4872 (8)
C(19)	0.5567 (7)	0.4785 (4)	0.4982 (7)
N(20)	0.2842 (5)	0.2369 (3)	−0.0860 (5)
C(21)	0.2563 (6)	0.1816 (4)	−0.1673 (6)
O(22)	0.1788 (4)	0.1330 (2)	−0.1501 (4)
C(23)	0.3241 (6)	0.1894 (4)	−0.2884 (7)
C1(29)	−0.0062 (2)	0.1408 (1)	0.2102 (2)
O(30)	0.0166 (7)	0.0233 (4)	−0.0224 (6)

Rigid body parameters. Crystallographic coordinates and isotropic thermal parameters, principal thiophene, multiplier = 0.85

	x	y	z	$B(10\sigma)$
C(1-24)	0.239	0.236	−0.375	3.4 (2)
S(1-25)	0.198	0.196	−0.511	4.2 (1)
C(1-26)	0.110	0.274	−0.548	4.1 (2)
C(1-27)	0.113	0.329	−0.459	4.4 (2)
C(1-28)	0.187	0.307	−0.359	5.5 (4)
H(1-28)	0.201	0.343	−0.279	5.0
H(1-27)	0.064	0.383	−0.462	5.0
H(1-26)	0.061	0.275	−0.634	5.0
Disordered thiophene, multiplier = 0.15, group B = −1.2 (4)				
C(2-24)	0.255	0.226	−0.382	3.0
S(2-25)	0.183	0.311	−0.355	4.0
C(2-26)	0.107	0.307	−0.491	4.0
C(2-27)	0.137	0.242	−0.555	4.0
C(2-28)	0.222	0.195	−0.492	4.0
H(2-28)	0.256	0.141	−0.529	5.0
H(2-27)	0.100	0.227	−0.644	5.0
H(2-26)	0.045	0.353	−0.517	5.0

Rigid-body positional and orientation parameters[d] with standard deviations[b] from least-squares refinement

x	y	z	ϕ(rad)	θ(rad)	ρ(rad)
Principal thiophene					
0.2392 (7)	0.2361 (4)	−0.3747 (4)	−3.139 (4)	−3.128 (3)	3.132 (5)
Disordered thiophene					
0.255 (3)	0.226 (1)	−0.382 (2)	−3.02 (2)	−3.04 (1)	3.09 (2)

Table I (*Continued*)

E. Final Thermal Parameters[a] with Standard Deviations[b] from the Anisotropic Refinement of Cephaloridine·HCl·H_2O with Rigid-Body Disordered Thiophene Rings

	$10^4\beta_{11}$	$10^4\beta_{22}$	$10^4\beta_{33}$	$10^4\beta_{12}$	$10^4\beta_{13}$	$10^4\beta_{23}$
S(1)	44 (2)	30 (1)	64 (2)	6 (1)	−4 (1)	−11 (1)
C(2)	57 (6)	23 (2)	73 (7)	0 (3)	−13 (6)	−12 (4)
C(3)	51 (6)	22 (2)	58 (6)	−2 (3)	6 (6)	0 (3)
C(4)	52 (6)	18 (2)	56 (6)	−6 (3)	5 (6)	−1 (3)
N(5)	50 (5)	21 (2)	51 (5)	3 (2)	−9 (5)	−1 (3)
C(6)	52 (6)	17 (2)	69 (6)	3 (3)	−3 (5)	0 (3)
C(7)	55 (6)	23 (2)	60 (6)	−1 (3)	0 (5)	6 (4)
C(8)	53 (6)	22 (3)	76 (7)	1 (3)	−8 (6)	−1 (4)
O(9)	137 (6)	32 (2)	98 (6)	15 (3)	−38 (5)	13 (3)
C(10)	53 (7)	34 (3)	78 (8)	6 (4)	10 (6)	−3 (4)
O(11)	51 (5)	33 (2)	186 (9)	2 (2)	2 (5)	−9 (4)
O(12)	67 (4)	18 (2)	140 (6)	−7 (2)	11 (5)	−12 (3)
C(13)	48 (6)	36 (3)	62 (7)	−3 (4)	1 (6)	−9 (4)
N(14)	3.0 (1)[c]					
C(15)	3.7 (2)[c]					
C(16)	4.7 (2)[c]					
C(17)	5.2 (2)[c]					
C(18)	5.3 (2)[c]					
C(19)	3.9 (2)[c]					
N(20)	61 (5)	27 (2)	60 (5)	−13 (3)	2 (5)	−2 (3)
C(21)	57 (6)	21 (2)	66 (7)	19 (4)	−14 (6)	−2 (4)
O(22)	76 (5)	23 (2)	89 (5)	−6 (2)	−3 (5)	−2 (3)
C(23)	69 (6)	31 (3)	63 (7)	10 (4)	2 (6)	2 (4)
C1(29)	66 (2)	24 (1)	93 (2)	−6 (1)	11 (2)	1 (1)
O(30)	240 (12)	75 (4)	124 (8)	−51 (6)	27 (8)	−28 (5)

[a] Anisotropic thermal parameters were used with the form exp $[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl)]$. [b] Standard deviations of the least significant figures are shown in parentheses. [c] The isotropic thermal parameters shown were used for the six atoms of the pyridyl ring. [d] The three angles ϕ, θ, ρ represent the series of Eulerian rotations which bring an orthonormal basis set of vectors (x,y,z) into coincidence with a second orthonormal set (x''',y''',z'''). The starting set (x,y,z) is defined with x along a of the orthorhombic crystallographic basis set, y is along b, and z is along c. The series of Eulerian rotations which take x,y,z, into x''', y''', z''' are: ϕ about z to form x', y', z'; θ about x' to form x'', y'', z''; finally ρ about y'' to form x''', y''', z''', all rotations counterclockwise. The positions of the individual rigid group atoms in the initial orthogonal basis set with dimensions of ångström units are

	x	y	z
C(1-24)	0.000	0.000	0.000
S(1-25)	−0.475	−0.675	−1.502
C(1-26)	−1.440	0.689	−1.884
C(1-27)	−1.395	1.629	−0.888
C(1-28)	−0.566	1.232	0.197
C(2-24)	0.000	0.000	0.000
S(2-25)	−0.586	1.536	0.485
C(2-26)	−1.495	1.717	−0.958
C(2-27)	−1.349	0.630	−1.780
C(2-28)	−0.489	−0.357	−1.229

viously defined discrepancy factors were $R_1 = 0.149$ and $R_2 = 0.165$. During anisotropic refinement, isotropic hydrogen atoms were placed in idealized positions[21] for the structure factor calculations but were not shifted by the least squares. After cycle seven, the observed values of $||F_c| - |F_o||/\sigma(F)$ were fit to a fifth-order polynomial in F_o by least-squares methods similar to the procedure used with cephaloridine. This polynomial, $\Delta F/\sigma(F) = 0.132 + (0.131)F_o - [0.146(10^{-2})]F_o^2 + [0.673(10^{-5})]F_o^3 - [0.130(10^{-7})]F_o^4 + [0.831(10^{-11})]F_o^5$, was used as a function of each F_o to multiply its own σ. At the end of anisotropic refinement this treatment had resulted in residuals of $R_1 = 0.056$ and $R_2 = 0.064$ and in a goodness-of-fit parameter equal to 1.25. A final calculation of $\Delta F/\sigma(F)$ as a polynomial in F_o revealed a reasonably flat curve with an average ordinate of about 1.0.

A difference Fourier electron-density map based on the nonhydrogen atoms in the structure yielded the positions of all of the hydrogen atoms in the structure and had no other outstanding features above 0.2 e/Å^3.

The coordinates and thermal parameters as obtained from the output of the final least-squares cycle appear in Table III.[25] Atomic distances and bond angles are presented in Table IV and in Figure 7 along with estimated standard deviations which contain the effect of errors in the lattice parameters.[28]

(c) **Cephaloglycine Acetic Acid Hydrate.** A long series of attempts was made to solve this structure both by the symbolic addition procedure coupled with tangent-formula refinement and by the heavy-atom method based on data from the first crystal used. To carry out the symbolic addition procedure the phases of a suitable number of reflections must be specified arbitrarily in order to choose the position of the origin. As one may specify phases from only the smallest number of parity groups which will allow the generation of all other parity species in the space group, only two phases were chosen for the C2 space group. Customarily, the phases of a small number of reflections are represented by symbols when the symbolic addition procedure is being applied. During these early attempts, a consistent set of numeric phases to represent these symbols which gave an interpretable E map could not be found. After numerous attempts at solving the structure by symbolic addition, and Patterson map was examined in considerable detail and the position of the sulfur atom was determined. The electron density map phased on this sulfur atom and weighted according to the method of Sim[33] revealed the β-lactam ring in a suitable position along with a few other believable atomic positions. Although this lead was pursued with some vigor, no solution was found.

Because other possibilities seemed to have been exhausted, and because the initial set of data had undergone such severe intensity decay, fresh data were collected. Two crystals were used to assure that minimal decay would occur during the entire data collection. The initial batch of crystals, which was 9 months old by this time, was still judged to be usable as the crystals showed little visible decomposition after having been stored in a well-stoppered vial under refrigeration. The data from each of the two crystals used showed decay, for which a correction was made, of no more than 20%. The mean measured cell parameters were $a = 21.97(3)$, $b = 10.27(1)$, $c = 11.33(1)$ Å, and $\beta = 108.34(1)°$. One should note that the cell-edge lengths from these crystals are uniformly shorter and that the statistical errors are larger than those from the previous crystal which was relatively fresher at the time the parameters were measured.

Finally, symbolic addition coupled with tangent-formula refinement[27] yielded the solution of the structure. The phases and symbols used to begin the symbolic addition, carried out with Dewar's program MAGIA, were: $|E(-3,1,3)| = 3.67$, $\phi = 0$; $|E(4,0,9)| = 2.15$, $\phi = 0$; $|E(-6,6,8)| = 3.46$, $\phi = W$; $|E(-8,4,5)| = 2.19$, $\phi = X$. The only difference between this attempt at the solution of the structure and an earlier one was that here all E factors >1.5, rather than >1.9, were entered into the addition from the beginning. When the symbolic addition was terminated, 59 phases had been determined, six with variances larger than 0.50 radian squared and one with a variance as high as 0.59. Symbolic equivalence relations were compiled in the same fashion as with the Δ^2-cephalosporin. The one relation which occurred with the smallest net variance was $2W - 4X = 0$. The next most reliable equivalence had a variance three times that of this indication. The symbol X was arbitrarily confined to the range 0–π in order to specify the enantiomorph.[30] Two values of X were then assigned, $\pi/3$ and $2\pi/3$, with appropriate values of W to satisfy the symbol equivalence relation. The sets which were tried were as follows: set 1, $W = -\pi/3$; $X = \pi/3$; set 2, $W = 2\pi/3$, $X = \pi/3$; set 3, $W = \pi/3$; $X = 2\pi/3$; set 4, $W = -2\pi/3$, $X = 2\pi/3$. The phases determined by symbolic addition, with the numeric phases listed above substituted for the symbols, were used as the starting point for six or seven cycles of tangent-formula refinement. At least three of these were reiterative cycles run on all E factors 1.50 and greater. Sets 2 and 3 appeared more promising than the other two as they had lower values for the tangent formula R factor.[27] The tangent formula was used to calculate phases for additional E factors down to an E value of 1.25. Although the E map resulting from set 2 was uninterpretable, the E map resulting from set 3 showed a large peak at approximately 0.11, y, 0.29, which is consistent with the Patterson function mentioned earlier. The five atoms in the β-lactam group were also apparent. Other atoms were eventually identified through the use of successive Fourier electron-density maps weighted by Sim's method. As it later turned out, 20 of the 28 atoms in the molecule and the oxygen atom, O(29) were actually

(33) G. A. Sim, *Acta Crystallogr.*, **13**, 511 (1960).

Table II

A. Intramolecular Lengths and Angles with Standard Deviations[a] from the Two Refinements of Cephaloridine·HCl·H_2O

	Bond lengths, Å		
	Anisotropic thiophene	Disordered, rigid-body thiophene	Mean[b]
S(1)–C(2)	1.815 (7)	1.819	1.817
S(1)–C(6)	1.787 (6)	1.788	1.787
C(2)–C(3)	1.502 (8)	1.499	1.501
C(3)–C(4)	1.359 (8)	1.361	1.360
C(3)–C(13)	1.502 (9)	1.497	1.500
C(4)–N(5)	1.386 (7)	1.401	1.393
C(4)–C(10)	1.518 (9)	1.509	1.514
N(5)–C(6)	1.458 (7)	1.469	1.463
N(5)–C(8)	1.390 (8)	1.373	1.382
C(6)–C(7)	1.570 (9)	1.564	1.567
C(7)–C(8)	1.490 (10)	1.509	1.499
C(7)–N(20)	1.441 (8)	1.438	1.440
C(8)–O(9)	1.214 (8)	1.215	1.214
C(10)–O(11)	1.189 (8)	1.177	1.183
C(10)–O(12)	1.300 (8)	1.309	1.304
C(13)–N(14)	1.491 (8)	1.493	1.492
N(14)–C(15)	1.342 (9)	1.328	1.335
N(14)–C(19)	1.339 (9)	1.340	1.339
C(15)–C(16)	1.380 (10)	1.379	1.380
C(16)–C(17)	1.382 (11)	1.395	1.388
C(17)–C(18)	1.349 (11)	1.339	1.344
C(18)–C(19)	1.390 (11)	1.389	1.390
N(20)–C(21)	1.354 (8)	1.350	1.352
C(21)–O(22)	1.230 (8)	1.217	1.224
C(21)–C(23)	1.522 (10)	1.534	1.528
C(23)–C(24)	1.515 (10)		
C(24)–S(25)	1.675 (7)		
C(24)–C(28)	1.473 (9)		
S(25)–C(26)	1.686 (9)		
C(26)–C(27)	1.342 (12)		
C(27)–C(28)	1.401 (11)		
C(23)–C(1-24)		1.561	
C(23)–C(2-24)		1.431	

	Bond angles, °		
	Anisotropic thiophene	Disordered, rigid-body thiophene	Mean[b]
C(2)–S(1)–C(6)	94.4 (3)	94.5	94.4
S(1)–C(2)–C(3)	115.6 (5)	115.9	115.7
C(2)–C(3)–C(4)	123.0 (6)	123.2	123.1
C(2)–C(3)–C(13)	114.4 (5)	114.6	114.5
C(4)–C(3)–C(13)	122.1 (6)	121.8	122.4
C(3)–C(4)–N(5)	120.3 (5)	120.2	120.3
C(3)–C(4)–C(10)	125.4 (6)	126.6	126.0
N(5)–C(4)–C(10)	113.9 (5)	112.9	113.4
C(4)–N(5)–C(6)	126.4 (5)	126.1	126.3
C(4)–N(5)–C(8)	130.1 (5)	130.8	130.4
C(6)–N(5)–C(8)	94.1 (5)	93.8	94.0

	Bond angles, °		
	Anisotropic thiophene	Disordered, rigid-body thiophene	Mean[b]
S(1)–C(6)–N(5)	110.6 (4)	110.8	110.7
S(1)–C(6)–C(7)	116.2 (4)	116.8	116.5
N(5)–C(6)–C(7)	86.4 (4)	87.0	86.7
C(6)–C(7)–C(8)	85.9 (5)	85.0	85.5
C(6)–C(7)–N(20)	120.6 (5)	120.1	120.4
C(8)–C(7)–N(20)	116.3 (6)	116.4	116.3
N(5)–C(8)–C(7)	92.1 (5)	92.7	92.4
N(5)–C(8)–O(9)	131.2 (6)	130.9	131.1
C(7)–C(8)–O(9)	136.4 (6)	136.2	136.3
C(4)–C(10)–O(11)	120.4 (6)	121.5	120.9
C(4)–C(10)–O(12)	113.8 (6)	112.9	113.4
O(11)–C(10)–O(12)	125.8 (6)	125.6	125.7
C(3)–C(13)–N(14)	111.2 (5)	111.4	111.3
C(13)–N(14)–C(15)	119.5 (6)	119.6	119.5
C(13)–N(14)–C(19)	119.2 (6)	118.6	118.9
C(15)–N(14)–C(19)	121.3 (6)	121.8	121.5
N(14)–C(15)–C(16)	120.5 (7)	120.2	120.4
C(15)–C(16)–C(17)	118.5 (7)	118.6	118.5
C(16)–C(17)–C(18)	120.0 (8)	119.9	120.0
C(17)–C(18)–C(19)	120.0 (8)	119.8	119.9
N(14)–C(19)–C(18)	119.4 (7)	119.5	119.4
C(7)–N(20)–C(21)	122.0 (5)	121.0	121.6
N(20)–C(21)–O(22)	123.2 (6)	123.6	123.4
N(20)–C(21)–C(23)	114.6 (6)	113.7	114.2
O(22)–C(21)–C(23)	121.9 (6)	122.6	122.2
C(21)–C(23)–C(24)	108.7 (5)		
C(23)–C(24)–S(25)	121.5 (5)		
C(23)–C(24)–C(28)	126.7 (6)		
S(25)–C(24)–C(28)	111.7 (5)		
C(24)–S(25)–C(26)	93.6 (4)		
S(25)–C(26)–C(27)	110.6 (6)		
C(26)–C(27)–C(28)	117.7 (8)		
C(24)–C(28)–C(27)	106.4 (6)		

Disordered, rigid-body thiophene, °		Disordered, rigid-body thiophene, °	
C(21)–C(23)–C(1-1)	106.4	C(23)–C(1-1)–C(1-5)	129.9
C(21)–C(23)–C(2-1)	114.0	C(23)–C(2-1)–S(2-2)	120.3
C(23)–C(1-1)–S(1-2)	118.7	C(23)–C(2-1)–C(2-5)	127.6

B. Intermolecular Hydrogen Bonds[a,c]

	Anisotropic thiophene, Å	Rigid-body thiophene, Å	Mean,[b] Å
O(22)···H0(30)	2.960 (8)	2.968	2.964
O(12)H···C1(29)	2.964 (4)	2.965	2.964
C1(29)···H0(30)	3.285 (7)	3.286	3.285

C. Intermolecular Nonbonding Distances Less than 3.3 Å[a,c]

	Anisotropic thiophene, Å	Rigid-body thiophene, Å	Mean,[b] Å
C(15)···O(30)''	3.092 (10)	3.094	3.093
S(1)···O(22)''	3.279 (5)	3.289	3.284
O(9)···N(14)'''	2.868 (8)	2.866	2.867

[a] The standard deviation of the last significant figures of a value is in parentheses. The values shown from the refinement with anisotropic thiophene were calculated by the Busing, Martin, Levy function and error program.[26] The errors in atomic positional parameters from the final cycle of the refinement with the rigid-body thiophenes were uniformly 4–5% higher. It is assumed, therefore, that errors in bond lengths and angles from the two refinements are equivalent. [b] Means were rounded toward the anisotropic thiophene values. [c] Symmetry operations are indicated as follows: $' = -x, {}^1/_2 + y, {}^1/_2 - z$; $'' = {}^1/_2 + x, {}^1/_2 - y, -z$; $''' = {}^1/_2 - x, 1 - y, z - {}^1/_2$.

present on this first E map, although they were not necessarily identified.

A rather extensive but fruitless series of attempts to refine this structure was undertaken. Fourier and least-squares methods were applied, based on the initially collected set of data, the set of data collected later, and a combination of the two. The structure was even solved again by Fourier methods, with the sulfur atom as a source of initial phases, to assure that no ambiguity existed in the solution. In no case was it possible to lower the R factor for an isotropic structure factor calculation below 18% or to locate in the unit cell the acetic acid of solvation which was predicted by the chemists who supplied the crystals.[34] Table V contains atomic parameters from the final cycle of least-squares refinement, with an R value of 20%, on a model of the cephaloglycine structure which contained a rigid-body phenyl ring with D_{6h} symmetry and C–C bond length of 1.392 Å. Carbon, nitrogen, and oxygen atoms in the molecule were refined isotropically; the sulfur atom, anisotropically. Atoms 29 and 30 were refined as oxygen atoms. Interatomic distances and bond angles resulting from the output of the last cycle of this refinement appear in Table VI and in Figure 8 along with estimated standard deviations which contain the effect of errors in the lattice parameters.[28]

The equations of planes which were fitted to various sets of atoms by an unweighted least-squares procedure,[35] and the perpendicular

(34) The formulation of the solvate in fresh crystals of cephaloglycine·HOAc·H_2O was determined by titration and nmr measurements; private communication from R. Pfeiffer (Eli Lilly and Co., Indianapolis, Ind.) to R. M. Sweet, 1969.

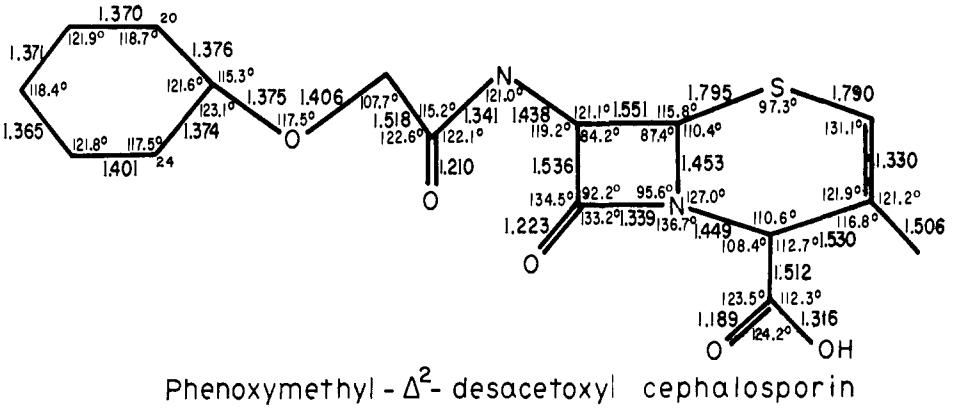


Figure 7. Bond lengths and angles from the anisotropic refinement of phenoxymethyl-Δ^2-desacetoxyl cephalosporin.

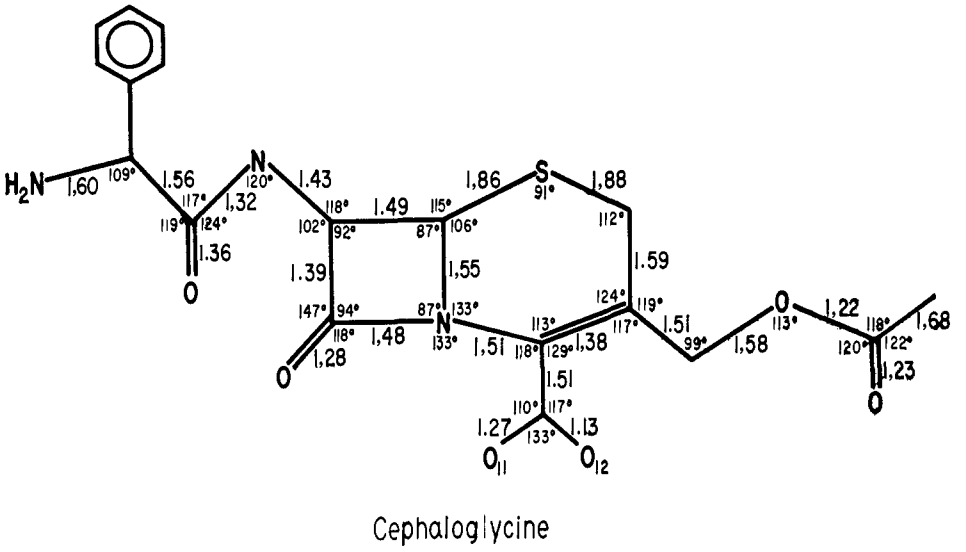


Figure 8. Bond lengths and angles from the rigid-body refinement of cephaloglycine.

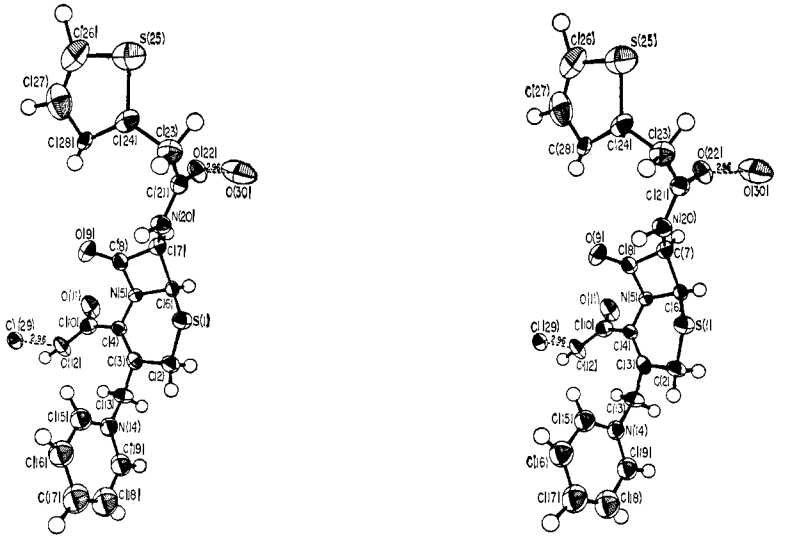


Figure 9. Stereoscopic view of the conformation and thermal ellipsoids representing 50% probability for cephaloridine hydrochloride monohydrate.

displacements of atoms from these planes are given for cephaloridine, phenoxymethyl-Δ^2-desacetoxyl cephalosporin, and cephaloglycine in Table VII.

(35) D. L. Smith, "A Least-Squares Planes Program for the CDC 1604 Computer," Ph.D. Thesis (Appendix IV), University of Wisconsin (Madison), 1962.

Results and Discussion

Description of the Structures. (a) Cephaloridine Hydrochloride Monohydrate. The molecular conformation of the cephaloridine molecule is shown in Figure 9. The most significant structural features of the

Table III

A. Final Atomic Parameters with Standard Deviations from the Anisotropic Refinement of Phenoxymethyl-Δ^2-desacetoxyl Cephalosporin (PDC)

	$x(10^4\sigma)$	$y(10^3\sigma)$	$z(10^4\sigma)$
S(1)	0.9159 (1)	0.7228	0.0639 (2)
C(2)	0.8346 (6)	0.890 (2)	−0.0520 (6)
C(3)	0.7294 (5)	0.964 (2)	−0.0853 (5)
C(4)	0.6551 (4)	0.916 (1)	−0.0207 (4)
N(5)	0.7181 (3)	0.808 (1)	0.0801 (3)
C(6)	0.8042 (5)	0.608 (2)	0.1028 (5)
C(7)	0.8192 (4)	0.645 (2)	0.2191 (5)
C(8)	0.7338 (4)	0.869 (1)	0.1793 (5)
O(9)	0.6950 (3)	1.039 (1)	0.2208 (3)
C(10)	0.5616 (4)	0.726 (2)	−0.0725 (4)
O(11)	0.5567 (3)	0.505 (1)	−0.0429 (4)
O(12)	0.4860 (4)	0.835 (1)	−0.1528 (3)
C(13)	0.6802 (7)	1.116 (2)	−0.1851 (6)
N(14)	0.9250 (4)	0.708 (1)	0.2940 (4)
C(15)	0.9887 (5)	0.517 (2)	0.3532 (5)
O(16)	0.9600 (4)	0.286 (1)	0.3473 (4)
C(17)	1.0960 (4)	0.614 (2)	0.4317 (5)
O(18)	1.1441 (3)	0.793 (1)	0.3807 (3)
C(19)	1.2324 (5)	0.941 (1)	0.4409 (5)
C(20)	1.2756 (5)	1.116 (2)	0.3874 (5)
C(21)	1.3622 (6)	1.274 (2)	0.4424 (6)
C(22)	1.4068 (6)	1.263 (2)	0.5484 (6)
C(23)	1.3641 (6)	1.084 (2)	0.5996 (5)
C(24)	1.2754 (5)	0.919 (2)	0.5470 (5)

B. Final Thermal Parameters[a] with Standard Deviations[b] from the Anisotropic Refinement of PDC

	$10^4\beta_{11}$	$10^3\beta_{22}$	$10^4\beta_{33}$	$10^4\beta_{12}$	$10^4\beta_{13}$	$10^4\beta_{23}$
S(1)	51 (1)	114 (2)	120 (2)	48 (4)	34 (1)	−19 (5)
C(2)	77 (6)	87 (5)	95 (5)	−74 (16)	41 (5)	−45 (16)
C(3)	64 (5)	50 (4)	93 (5)	−53 (11)	41 (4)	−50 (12)
C(4)	54 (4)	31 (2)	61 (4)	−7 (9)	17 (3)	0 (9)
N(5)	50 (3)	34 (2)	62 (3)	1 (8)	14 (3)	−17 (8)
C(6)	63 (4)	40 (3)	92 (5)	24 (11)	21 (4)	−29 (11)
C(7)	57 (4)	37 (3)	84 (5)	8 (10)	9 (4)	−6 (10)
C(8)	51 (4)	28 (3)	73 (4)	−25 (9)	13 (4)	11 (10)
O(9)	68 (3)	47 (2)	67 (3)	29 (8)	13 (2)	−19 (7)
C(10)	56 (4)	31 (3)	66 (4)	11 (10)	23 (4)	13 (10)
O(11)	67 (3)	35 (2)	100 (3)	−2 (8)	14 (3)	29 (8)
O(12)	86 (4)	54 (3)	72 (3)	−41 (9)	−4 (3)	43 (8)
C(13)	138 (8)	91 (6)	75 (5)	3 (20)	50 (5)	84 (16)
N(14)	59 (4)	29 (2)	91 (4)	−6 (8)	−4 (3)	6 (9)
C(15)	63 (4)	27 (3)	81 (5)	13 (10)	18 (4)	9 (10)
O(16)	79 (4)	34 (2)	121 (4)	2 (8)	3 (3)	19 (9)
C(17)	55 (4)	44 (3)	78 (4)	2 (11)	3 (4)	32 (11)
O(18)	71 (3)	47 (2)	71 (3)	−37 (8)	2 (3)	12 (7)
C(19)	60 (4)	35 (3)	67 (4)	16 (10)	12 (4)	15 (10)
C(20)	75 (5)	60 (4)	86 (5)	−29 (14)	24 (4)	22 (14)
C(21)	93 (6)	53 (4)	95 (6)	−42 (14)	31 (5)	2 (14)
C(22)	74 (5)	54 (4)	108 (6)	−35 (14)	24 (5)	7 (15)
C(23)	81 (5)	67 (5)	77 (5)	−13 (15)	1 (4)	7 (14)
C(24)	68 (5)	57 (4)	68 (4)	−28 (12)	2 (4)	10 (12)

C. Hydrogen Atom Positions from the Difference Map Phased on the Atomic Parameters for PDC at the End of Anisotropic Refinement

	x	y	z
H(2)	0.885	0.970	−0.108
H(4)	0.635	1.087	−0.006
H(6)	0.776	0.433	0.063
H(7)	0.787	0.499	0.260
H(12)	0.415	0.723	−0.196
H1(13)	0.632	1.275	−0.195
H2(13)	0.741	1.208	−0.216
H3(13)	0.626	1.025	−0.221
H(14)	0.934	0.893	0.289
H1(17)	1.086	0.741	0.488
H2(17)	1.143	0.444	0.463
H(20)	1.229	1.074	0.298
H(21)	1.400	1.425	0.403
H(22)	1.473	1.347	0.589
H(23)	1.384	1.062	0.670
H(24)	1.243	0.739	0.535

[a] Thermal parameters have the form $\exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl)]$. [b] Standard deviations of least significant figures are shown in parentheses.

Table IV

A. Intramolecular Lengths and Angles with Standard Deviations[a] for the Final Anisotropic Model of Phenoxymethyl-Δ^2-desacetoxyl Cephalosporin

Bond lengths, Å

S(1)–C(2)	1.790 (9)	C(10)–O(11)	1.189 (8)
C(1)–C(6)	1.795 (7)	C(10)–O(12)	1.316 (7)
C(2)–C(3)	1.330 (10)	N(14)–C(15)	1.341 (8)
C(3)–C(4)	1.530 (8)	C(15)–O(16)	1.210 (8)
C(3)–C(13)	1.506 (10)	C(15)–C(17)	1.518 (8)
C(4)–N(5)	1.449 (7)	C(17)–O(18)	1.406 (8)
C(4)–C(10)	1.512 (8)	O(18)–C(19)	1.375 (7)
N(5)–C(6)	1.453 (7)	C(19)–C(20)	1.376 (9)
N(5)–C(8)	1.339 (7)	C(19)–C(24)	1.374 (9)
C(6)–C(7)	1.551 (9)	C(20)–C(21)	1.370 (10)
C(7)–C(8)	1.536 (8)	C(21)–C(22)	1.371 (10)
C(7)–N(14)	1.438 (7)	C(22)–C(23)	1.365 (10)
C(8)–O(9)	1.223 (7)	C(23)–C(24)	1.401 (10)

Bond angles, °

S(1)–C(2)–C(3)	131.1 (6)	C(8)–C(7)–N(14)	119.2 (5)
C(2)–S(1)–C(6)	97.3 (3)	N(5)–C(8)–C(7)	92.2 (5)
C(2)–C(3)–C(4)	121.9 (6)	N(5)–C(8)–O(9)	133.2 (6)
C(2)–C(3)–C(13)	121.2 (6)	C(7)–C(8)–O(9)	134.5 (6)
C(4)–C(3)–C(13)	116.8 (6)	C(7)–N(14)–C(15)	121.0 (5)
C(3)–C(4)–N(5)	110.6 (5)	N(14)–C(15)–O(16)	122.1 (6)
C(3)–C(4)–C(10)	112.7 (5)	N(14)–C(15)–C(17)	115.2 (6)
N(5)–C(4)–C(10)	108.4 (4)	O(16)–C(15)–C(17)	122.6 (6)
C(4)–C(10)–O(11)	123.5 (5)	C(15)–C(17)–O(18)	107.6 (5)
C(4)–C(10)–O(12)	112.3 (5)	C(17)–O(18)–C(19)	117.5 (4)
O(11)–C(10)–O(12)	124.2 (6)	O(18)–C(19)–C(20)	115.3 (6)
C(4)–N(5)–C(6)	127.0 (5)	O(18)–C(19)–C(24)	123.0 (6)
C(6)–N(5)–C(8)	95.6 (4)	C(20)–C(19)–C(24)	121.6 (6)
C(4)–N(5)–C(8)	136.7 (5)	C(19)–C(20)–C(21)	118.7 (6)
N(5)–C(6)–S(1)	110.4 (4)	C(20)–C(21)–C(22)	121.9 (7)
S(1)–C(6)–C(7)	115.8 (4)	C(21)–C(22)–C(23)	118.4 (7)
N(5)–C(6)–C(7)	87.4 (4)	C(22)–C(23)–C(24)	121.8 (6)
C(6)–C(7)–C(8)	84.2 (5)	C(23)–C(24)–C(19)	117.5 (7)
C(6)–C(7)–N(14)	121.1 (5)		

B. Intermolecular Hydrogen Bonds[b]

O(16)···HN(14)′ 2.986 (8) Å O(9)···H0(12)″ 2.657 (7) Å

[a] Standard deviations of least significant figures are shown in parentheses. The values shown were calculated by the Busing, Martin, Levy function and error program.[28] [b] Symmetry operations are indicated as follows: ′ = $x, y - 1, z$; ″ = $1 - x, \frac{1}{2} + y, -z$.

cephaloridine molecule which relate to its biological activity are those which contribute to the chemistry of the β-lactam amide bond. In particular, the lack of planarity of the lactam nitrogen atom and the presence of unsaturation in the dihydrothiazine ring α,β to this nitrogen together decrease the amide resonance which otherwise presumably occurs in this bond.

Several unweighted least-squares planes calculations have been made on the nonrigid body refined model of cephaloridine in an attempt to describe the molecule in more geometrical terms. The results of these calculations are shown in Table VII. The most significant molecular parameter resulting from them is the fact that the nitrogen atom in the β-lactam ring, N(5), lies 0.24 Å from the plane of its three substituents, C(4), C(6), and C(8). Other results document the slight nonplanar character of the β-lactam ring, the atoms involved in the double bond of the thiazine ring, and the amide group exocyclic to the β-lactam. In addition a high degree of planarity is observed in the carboxylate group, the pyridyl ring, and the anisotropically refined thiophene ring.

Bond lengths and angles for the two refinements are shown in Table II. Bond lengths and angles which are mean values from the two refinements appear in Figure

Table V

A. Atomic Parameters with Standard Deviations from Isotropic Rigid-Body Least-Squares Refinement for Cephaloglycine

	$x(10^3\sigma)$	$y(10^3\sigma)$	$z(10^3\sigma)$	$B(10\sigma)$
S(1)	0.1175 (0.5)	0.4441 (−)	0.2827 (0.8)	*a*
C(2)	0.099 (2)	0.520 (4)	0.418 (4)	4.9 (11)
C(3)	0.132 (2)	0.445 (5)	0.544 (3)	3.1 (9)
C(4)	0.194 (2)	0.401 (4)	0.580 (4)	4.7 (10)
N(5)	0.226 (1)	0.419 (4)	0.480 (3)	4.0 (8)
C(6)	0.204 (2)	0.484 (4)	0.351 (3)	1.8 (8)
C(7)	0.249 (2)	0.395 (4)	0.317 (4)	2.6 (9)
C(8)	0.267 (2)	0.335 (6)	0.432 (5)	5.9 (12)
O(9)	0.304 (1)	0.254 (3)	0.507 (3)	4.6 (7)
C(10)	0.230 (2)	0.325 (4)	0.694 (3)	2.5 (8)
O(11)	0.285 (1)	0.375 (3)	0.744 (3)	4.5 (7)
O(12)	0.203 (1)	0.244 (3)	0.722 (2)	2.3 (6)
C(13)	0.095 (1)	0.422 (4)	0.632 (3)	2.2 (8)
O(14)	0.069 (1)	0.563 (3)	0.639 (2)	3.6 (6)
C(15)	0.105 (2)	0.629 (4)	0.723 (4)	2.0 (8)
O(16)	0.157 (1)	0.585 (4)	0.785 (3)	6.0 (8)
C(17)	0.082 (2)	0.781 (6)	0.741 (5)	7.2 (14)
N(18)	0.222 (1)	0.289 (3)	0.235 (3)	2.6 (7)
C(19)	0.235 (2)	0.276 (4)	0.129 (3)	2.7 (9)
O(20)	0.266 (1)	0.367 (3)	0.084 (2)	3.3 (6)
C(21)	0.208 (2)	0.152 (4)	0.052 (4)	3.8 (10)
N(22)	0.245 (1)	0.132 (3)	−0.049 (2)	1.7 (6)
O(29)	0.163 (1)	0.092 (4)	0.314 (2)	5.9 (8)
O(30)	0.119 (2)	0.103 (5)	0.548 (3)	8.8 (10)
Phenyl Group Idealized Crystallographic Coordinates				
C(23)	0.134	0.169	−0.011	2.7 (9)
C(24)	0.100	0.054	−0.034	10.6 (18)
C(25)	0.035	0.056	−0.101	6.8 (11)
C(26)	0.005	0.174	−0.145	8.5 (17)
C(27)	0.040	0.289	−0.123	5.4 (11)
C(28)	0.105	0.286	−0.056	5.7 (12)

B. Final Rigid-Body Parameters from Least-Squares Refinement. Standard Deviations of Least Significant Figures are in Parentheses[b]

x	*y*	*z*	ϕ(rad)	θ(rad)	ρ(rad)
Phenyl					
0.0698 (9)	0.1712 (25)	−0.0784 (14)	−3.14 (2)	−3.10 (2)	−3.08 (1)

[a] Anisotropic temperature factors for the sulfur atom were used with the form $\exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl)]$. The resulting thermal coefficients, with the standard deviations of the least significant digits given in parentheses, were

	$\beta_{11}10^4$	$\beta_{22}10^4$	$\beta_{33}10^4$	$\beta_{12}10^4$	$\beta_{13}10^4$	$\beta_{23}10^4$
S(1)	20(3)	82(15)	32(11)	−4(6)	3(5)	−17(12)

[b] The three angles ϕ, θ, ρ represent the series of Eulerian rotations which bring an orthonormal basis set of vectors (x,y,z) into coincidence with a second orthonormal set (x''',y''',z'''). The starting set (x,y,z) is defined according to: x along a of the crystallographic basis set, y along b, and z along $a \times b$. The series of Eulerian rotations which take x,y,z into x''',y''',z''' are: ϕ about z to form x',y',z'; θ about x' to form x'',y'',z''; finally ρ about y'' to form x''',y''',z'''; all rotations are counterclockwise. The coordinates of the individual atoms in the basis orthogonal ångström coordinate system with a carbon–carbon bond length of 1.392 Å and D_{6h} symmetry assumed for the group are

C(23)	1.146	−0.049	0.789
C(24)	0.476	−1.224	0.460
C(25)	−0.670	−1.175	−0.329
C(26)	−1.146	0.049	−0.789
C(27)	−0.476	1.224	−0.460
C(28)	0.670	1.175	0.329

Table VI

A. Intermolecular Lengths and Angles with Standard Deviations[a] for the Cephaloglycine Structure After Isotropic Refinement with a Rigid-Body Phenyl Ring

Bond lengths, Å			
S(1)–C(2)	1.88 (4)	C(8)–O(9)	1.28 (5)
S(1)–C(6)	1.86 (3)	C(10)–O(11)	1.27 (4)
C(2)–C(3)	1.59 (6)	C(10)–O(12)	1.13 (4)
C(3)–C(4)	1.38 (5)	C(13)–O(14)	1.58 (5)
C(3)–C(13)	1.51 (4)	O(14)–C(15)	1.22 (4)
C(4)–N(5)	1.51 (5)	C(15)–O(16)	1.23 (4)
C(4)–C(10)	1.51 (5)	C(15)–C(17)	1.68 (7)
N(5)–C(6)	1.55 (5)	N(18)–C(19)	1.32 (4)
N(5)–C(8)	1.48 (5)	C(19)–O(20)	1.36 (4)
C(6)–C(7)	1.49 (5)	C(19)–C(21)	1.56 (6)
C(7)–C(8)	1.39 (6)	C(21)–N(22)	1.60 (4)
C(7)–N(18)	1.43 (5)	C(21)–C(23)	1.57 (d)
Bond angles, °			
C(2)–S(1)–C(6)	91 (2)	N(5)–C(8)–C(7)	94 (4)
S(1)–C(2)–C(3)	112 (3)	N(5)–C(8)–O(9)	118 (4)
C(2)–C(3)–C(4)	124 (3)	C(7)–C(8)–C(9)	147 (5)
C(2)–C(3)–C(13)	119 (3)	C(4)–C(10)–O(11)	110 (3)
C(4)–C(3)–C(13)	117 (3)	C(4)–C(10)–O(12)	117 (4)
C(3)–C(4)–N(5)	113 (4)	O(11)–C(10)–O(12)	133 (4)
C(3)–C(4)–C(10)	129 (4)	C(3)–C(13)–O(14)	99 (3)
N(5)–C(4)–C(10)	118 (3)	C(13)–O(14)–C(15)	113 (3)
C(4)–N(5)–C(6)	133 (3)	O(14)–C(15)–O(16)	120 (4)
C(4)–N(5)–C(8)	133 (4)	O(14)–C(15)–C(17)	118 (4)
C(6)–N(5)–C(8)	87 (3)	O(16)–C(15)–C(17)	122 (4)
S(1)–C(6)–N(5)	106 (2)	C(7)–N(18)–C(19)	120 (3)
S(1)–C(6)–C(7)	115 (3)	N(18)–C(19)–O(20)	124 (4)
N(5)–C(6)–C(7)	87 (3)	N(18)–C(19)–C(21)	117 (3)
C(6)–C(7)–C(8)	92 (3)	O(20)–C(19)–C(21)	119 (3)
C(6)–C(7)–N(18)	118 (3)	C(19)–C(21)–N(22)	109 (3)
C(8)–C(7)–N(18)	102 (3)		

B. Cephaloglycine Intramolecular Hydrogen Bond[b]

O(20)···HN(22)	2.81 (4) Å

C. Cephaloglycine Intermolecular Hydrogen Bonds,[b,c] Å

N(18)H···O(29)	2.72 (4)	O(16)···HN(22)′	3.14 (4)
O(29)···O(30)	3.11 (6)	O(12)···N(22)″	2.72 (4)
O(11)···O(29)′	2.69 (5)	O(20)···HN(22)‴	2.76 (4)
O(12)···O(30)	2.67 (5)		

D. Cephaloglycine Nonbonding Intermolecular Contacts Less than 3.4 Å[b,c]

C(2)···O(9)′	3.16 (5)	C(25)···C(25)′′′′	3.14
C(6)···O(9)′	3.24 (4)	C(26)···C(26)′′′′	3.38
O(16)···C(19)′	3.01 (5)	N(5)···O(29)′	3.32 (4)
O(16)···C(21)′	3.04 (5)		

[a] Standard deviations shown in parentheses for the least significant digits are calculated by the Busing, Martin, Levy function and error program.[26] [b] Standard deviations, shown in parentheses for the least significant digits, are calculated from the diagonal elements of the inverse least-squares matrix. [c] Symmetry operations are indicated as follows: ′ = $\frac{1}{2} - x, \frac{1}{2} + y, 1 - z$; ″ = $x, y, 1 + z$; ‴ = $\frac{1}{2} - x, \frac{1}{2} + y, -z$; ′′′′ = $-x, y, -z$. [d] Distance to an atom on the rigid phenyl group. A reasonable error estimate is 0.06 Å.

6. The pyridyl ring, the carboxyl group, and the exocyclic amide have approximately the expected dimensions. The double bond of length 1.360 (8) Å in the dihydrothiazine ring, C(3)–C(4), is a bit longer, while the single bond adjacent to it, C(4)–N(5), of length 1.393 (7) Å is perhaps a bit shorter than the expected values of 1.33 and 1.47 Å, respectively.[36] This variation may be due to normal enamine resonance, the partial delocalization of the unshared electron pair on N(5) into the olefinic π-orbital system of C(3) and C(4).

The amide link in the β-lactam ring is clearly different from the amide fastened to this ring. The lactam nitrogen atom, N(5), is definitely not planar; it lies 0.24 Å from the plane of its substituents, as mentioned before. Equivalent values for $(CH_3)_3N$[37] and the penicillins (Table VIII) are 0.56 Å and 0.4 Å, respectively. Thus, this lactam nitrogen atom clearly is less pyramidal than the nitrogen atom in a tertiary amine but more so than a normal amide nitrogen atom. In a

(36) L. Pauling, "The Nature of the Chemical Bond," 3rd ed, Cornell University Press, Ithaca, N. Y., 1960, p 224.

(37) L. O. Brockway and H. O. Jenkins, *J. Amer. Chem. Soc.*, **58**, 2036 (1936).

Table VII

A. Equations of Least-Squares Planes for Cephaloridine and Distances in Å of Specified Atoms from These Planes[a]

a. Plane containing C(4), C(6), and C(8)

$0.8628X + 0.2927Y - 0.4121Z - 3.1512 = 0$

C(4)	0	C(8)	0
C(6)	0	N(5)	−0.243

b. Plane containing C(2), C(3), C(4), N(5), and C(6)

$0.5179X + 0.4167Y - 0.7471Z - 2.1863 = 0$

C(2)	0.046	S(1)	0.895
C(3)	−0.053	C(13)	−0.415
C(4)	0.003	C(10)	−0.140
N(5)	0.050	C(8)	0.879
C(6)	−0.047	C(7)	0.673

c. Plane containing C(2), S(1), and C(6)

$-0.0710X + 0.8809Y - 0.4680Z - 2.9308 = 0$

S(1)	0	C(6)	0
C(2)	0		

d. Plane containing N(5), C(6), C(7), and C(8)

$0.8672X + 0.3806Y - 0.3212Z - 3.6472 = 0$

N(5)	−0.063	O(9)	0.280
C(6)	0.056	C(4)	0.350
C(7)	−0.055	S(1)	1.642
C(8)	0.062	N(20)	0.937

e. Plane containing N(5), C(7), C(8), and O(9)

$0.9328X + 0.2792Y - 0.2278Z - 3.3119 = 0$

N(5)	0.007	O(9)	0.011
C(7)	0.007	C(6)	0.287
C(8)	−0.024		

f. Plane containing C(2), C(3), C(4), N(5), C(10), and C(13)

$0.5300X + 0.5312Y - 0.6610Z - 3.1688 = 0$

C(2)	0.033	N(5)	−0.095
C(3)	0.064	C(10)	0.034
C(4)	0.053	C(13)	−0.090

g. Plane containing C(3), C(4), C(2), and C(13)

$0.5488X + 0.5677Y - 0.6136Z - 3.6608 = 0$

C(2)	−0.015	C(4)	−0.017
C(3)	0.046	C(13)	−0.014

h. Plane containing C(4), C(3), N(5), and C(10)

$0.5056X + 0.4867Y - 0.7124Z - 2.6837 = 0$

C(3)	−0.014	N(5)	−0.012
C(4)	0.037	C(10)	−0.012

i. Plane containing C(4), C(10), O(11), and O(12)

$-0.0122X + 0.0780Y - 0.9969Z + 2.1547 = 0$

C(4)	0.000	O(11)	0.001
C(10)	−0.001	O(12)	0.000

j. Plane containing C(7), N(20), C(21), O(22), and C(23)

$-0.7078X + 0.5578Y - 0.4334Z - 0.6280 = 0$

C(7)	0.099	C(23)	0.081
N(20)	−0.120	C(24)	1.552
C(21)	−0.054	C(6)	−0.941
O(22)	−0.006	C(8)	1.068

k. Plane containing N(20), C(21), O(22), and C(23)

$-0.7173X + 0.5969Y - 0.3593Z - 0.5609 = 0$

N(20)	0.009	C(23)	0.008
C(21)	−0.027	C(7)	0.331
O(22)	0.010		

l. Plane containing N(14), C(15), C(16), C(17), C(18), and C(19)

$0.4430X - 0.8406Y - 0.3117Z + 5.9896 = 0$

N(14)	0.016	C(18)	−0.024
C(15)	−0.007	C(19)	−0.001
C(16)	−0.018	C(13)	0.000
C(17)	0.034		

m. Plane containing C(24), S(25), C(26), C(27), and C(28)

$0.7852X + 0.4363Y - 0.4395Z - 5.6794 = 0$

C(24)	0.004	C(27)	0.000
S(25)	−0.003	C(28)	−0.003
C(26)	0.002	C(23)	−0.059

Angles in deg between the normals of planes

b–c,	47.2	g–h,	7.7
d–e,	8.8	h–i,	42.1
d–j,	74.8	j–m,	83.0

B. Equations of Least-Squares Planes for PDC and Distances in Å of Specified Atoms from These Planes[b]

a. Plane containing C(4), C(6), and C(8)

$-0.7322X - 0.6801Y - 0.0379Z + 9.3804 = 0$

C(4)	0	C(8)	0
C(6)	0	N(5)	0.065

b. Plane containing S(1), C(2), C(3), C(4), and C(13)

$-0.1153X - 0.8762Y - 0.4680Z + 4.8785 = 0$

S(1)	−0.013	C(13)	−0.019
C(2)	0.010	N(5)	−0.183
C(3)	0.028	C(6)	0.445
C(4)	−0.006	C(10)	1.252

c. Plane containing S(1), C(2), and C(3)

$-0.1050X - 0.8818Y - 0.4598Z + 4.7857 = 0$

S(1)	0	C(3)	0
C(2)	0		

d. Plane containing C(2), C(3), C(4), and C(13)

$-0.1230X - 0.8709Y - 0.4758Z + 4.9164 = 0$

C(2)	−0.009	C(4)	−0.007
C(3)	0.024	C(13)	−0.007

e. Plane containing N(5), C(6), C(7), and C(8)

$-0.7330X - 0.6788Y - 0.0437Z + 9.3609 = 0$

N(5)	0.037	O(9)	−0.127
C(6)	−0.032	C(4)	−0.019
C(7)	0.030	S(1)	−1.592
C(8)	−0.035	N(14)	−0.972

f. Plane containing N(5), C(7), C(8), and O(9)

$-0.7739X - 0.6333Y - 0.0041Z + 9.4622 = 0$

N(5)	−0.001	O(9)	−0.001
C(7)	−0.001	C(6)	−0.145
C(8)	0.003		

g. Plane containing C(4), C(10), O(11), and O(12)

$0.7316X - 0.3324Y - 0.5953Z - 4.8984 = 0$

C(4)	−0.001	O(11)	−0.002
C(10)	0.004	O(12)	−0.001

h. Plane containing C(7), N(14), C(15), O(16), and C(17)

$0.7152X - 0.1462Y - 0.6835Z - 4.4481 = 0$

C(7)	−0.012	C(17)	−0.014
N(14)	0.010	C(6)	1.290
C(15)	0.020	C(8)	−0.481
O(16)	−0.004	O(18)	0.920

i. Plane containing C(19), C(20), C(21), C(22), C(23), and C(24)

$0.7090X - 0.7006Y - 0.0811Z - 6.0754 = 0$

C(19)	−0.008	C(23)	0.005
C(20)	0.005	C(24)	0.003
C(21)	0.004	O(18)	−0.036
C(22)	−0.008		

Angles in deg between the normals of planes

c–d,	1.5	e–h,	66.7
e–f.	4.2	h–i,	48.3

C. Equations of Least-Squares Planes for Cephaloglycine and Distances in Å of Specified Atoms from these Planes[b]

a. Plane containing C(4), C(6), and C(8)

$-0.5661X - 0.6753Y - 0.4727Z + 6.9893 = 0$

C(4)	0	C(8)	0
C(6)	0	N(5)	−0.22

b. Plane containing C(2), C(3), C(4), N(5), and C(6)

$-0.2371X - 0.8776Y - 0.4167Z + 6.7260 = 0$

C(2)	−0.02	C(6)	0.00
C(3)	0.03	S(1)	1.07
C(4)	−0.02	C(7)	0.70
N(5)	0.01	C(8)	0.72

c. Plane containing S(1), C(2), and C(6)

$-0.0069X + 0.8779Y - 0.4787Z - 2.5442 = 0$

S(1)	0	C(6)	0
C(2)	0		

d. Plane containing N(5), C(6), C(7), C(8), and O(9)

$-0.6638X - 0.6795Y - 0.3125Z + 6.7127 = 0$

N(5)	0.00	O(9)	−0.02
C(6)	−0.01	S(1)	1.60
C(7)	−0.01	C(4)	0.49
C(8)	0.03	N(18)	1.20

e. Plane containing C(2), C(3), C(4), N(5), C(10), and C(13)

$-0.2156X - 0.8687Y - 0.4460Z + 6.8005 = 0$

C(2)	−0.01	N(5)	0.04
C(3)	−0.01	C(10)	0.00
C(4)	−0.05	C(13)	0.03

f. Plane containing C(2), C(3), C(4), and C(13)

$-0.1861X - 0.8718Y - 0.4530Z + 6.8389 = 0$

C(2)	−0.001	C(4)	−0.002
C(3)	0.004	C(13)	−0.001

g. Plane containing C(3), C(4), N(5), and C(10)

$-0.2329X - 0.8701Y - 0.4345Z + 6.7778 = 0$

C(3)	0.02	N(5)	0.01
C(4)	−0.04	C(10)	0.01

Table VII (*Continued*)

h. Plane containing C(4), C(10), O(11), and O(12)

$0.5638X - 0.6233Y - 0.5419Z + 4.7118 = 0$

C(4)	−0.01	O(11)	−0.01
C(10)	0.02	O(12)	−0.01

i. Plane containing C(7), N(18), C(19), O(20), and C(21)

$-0.7536X + 0.4828Y - 0.4460Z + 2.8009 = 0$

C(7)	−0.04	C(6)	1.08
N(18)	0.05	C(8)	−0.88
C(19)	−0.01	N(22)	−0.51
O(20)	0.01	C(23)	1.42
C(21)	−0.02		

j. Plane containing O(14), C(15), O(16), and C(17)

$0.6740X + 0.3471Y - 0.6521Z + 3.0098 = 0$

O(14)	0.01	C(17)	0.01
C(15)	−0.02	C(13)	−0.05
O(16)	0.01		

k. Plane containing C(23), C(24), C(25), C(26), C(27), and C(28)

$0.5123X - 0.1257Y - 0.8496Z - 1.4300 = 0$

C(23)	0	C(27)	0
C(24)	0	C(28)	0
C(25)	0	C(21)	0.16
C(26)	0		

Angles between normals of planes, deg

b–c,	55	f–g,	3
d–i,	72	g–h,	50
e–j,	81	i–k,	86

[a] The equation of the plane is expressed in orthogonal coordinates X, Y, Z which are related to the fractional crystallographic coordinates x, y, z by the transformation: $X = ax$, $Y = by$, $Z = cz$. Unit weights were given by all atoms in the calculations. [b] The equation of the plane is expressed in orthogonal coordinates X, Y, Z which are related to the crystallographic fractional coordinates x, y, z by the transformation: $X = ax + cz \cos \beta$, $Y = by$, $Z = cz \sin \beta$.

lar contacts between nonhydrogen atoms are: 3.09 Å between the carbon atom at position two of the pyridyl ring, C(15), and the oxygen atom of the water hydration, O(30); 3.28 Å between the sulfur atom of the dihydrothiazine, S(1), and the oxygen atom of the exocyclic amide, O(22); and 2.87 Å between the lactam oxygen atom, O(9), and the pyridyl nitrogen atom, N(14). This last distance is very close to the sum of the van der Waals radii for nitrogen and oxygen of 2.9 Å.[38] In addition, this arrangement is equivalent to that seen in pyridine hydrochloride[39] where a chloride ion sits directly over the pyridyl ring above the nitrogen atom at slightly more than van der Waals distance. The fact that the interaction in pyridine-HCl is primarily ionic in nature indicates that there likewise may be some negative charge localized on atom O(9) in solid-state cephaloridine. Three apparent hydrogen bonds exist in this structure (Table II). The first, a $COOH \cdots Cl^-$ interaction of length 2.964 (4) Å, is between the hydroxyl oxygen on the carboxyl group, O(12), and the chloride ion, Cl(29). The other two are from the water of hydration to the carbonyl oxygen of the exocyclic amide and to the chloride ion. These hydrogen-bonded distances are 2.964 (8) Å for $CO(22) \cdots H_2O(30)$ and 3.285 (7) Å for $Cl(29)^- \cdots H_2O(30)$.

(b) Cephaloglycine. The crystal-structure analysis of cephaloglycine was carried out to corroborate the conclusions related to chemical and biological activity which were drawn from the molecular parameters of the cephaloridine nucleus and to examine further the types

Table VIII. Structural Parameters of Some β-Lactam Compounds[a]

	Sum of angles around nitrogen, °	Distance of nitrogen from plane, Å	C=O stretch	C=O bond length, Å	C—N bond length, Å	Active antibiotic
Ampicillin[b]	339 (1)			1.198 (7)	1.369 (7)	Yes
Penicillin G[c]	337 (3)	0.40		1.17 (4)	1.34 (4)	Yes
Penicillins[d]			1780–1770 cm^{-1}			Yes
Cephaloridine[e]	350.7 (8)	0.24		1.214 (8)	1.382 (8)	Yes
Cephaloglycine[e]	353 (6)	0.22		1.28 (5)	1.48 (5)	Yes
Δ^3-Cephalosporins[f]			1776–1764 cm^{-1}			Yes
Δ^2-Cephalosporins V[e]	359.3 (8)	0.065		1.223 (7)	1.339 (7)	No
Δ^2-Cephalosporins[f]			1760–1756 cm^{-1}			No
Unfused β-lactams[d]	360	0	1760–1730 cm^{-1}			No

[a] Compounds are arranged approximately in the order of decreasing rate of base hydrolysis of the lactam amide bond. [b] Private communication from M. N. G. James, Department of Biochemistry, University of Alberta, Edmonton, Canada. [c] G. J. Pitt, *Acta Crystallogr.*, **5**, 770 (1952). [d] L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed, Wiley, New York, N. Y., 1958. [e] This study. [f] G. F. H. Green, J. E. Page, and S. E. Staniforth, *J. Chem. Soc.*, 1595 (1965).

normal amide the nitrogen atom and the three atoms connected to it are coplanar, which implies that its unshared electron pair can be delocalized into the π-orbital system of the adjacent carbonyl. This results in increased double-bond character for the C–N bond and decreased double-bond character for the C–O bond. Thus, the lengthening of the C–N bond in the β-lactam amide relative to that in the amide exocyclic to the β-lactam implies that less amide resonance occurs here. Factors which combine to decrease the likelihood of this amide delocalization are bond angle strain within the four-membered ring, the lack of planarity of the nitrogen atom, and the possibility that the nitrogen atom is involved in enamine delocalization with the adjacent double bond.

The molecular packing of cephaloridine·HCl·H_2O is shown in Figure 10. Close nonbonding intermolecular contacts between nonhydrogen atoms are: 3.09 Å between the carbon atom at position two of the pyridyl ring, C(15), and the oxygen atom of the water hydration, O(30); 3.28 Å between the sulfur atom of the dihydrothiazine, S(1), and the oxygen atom of the exocyclic amide, O(22); and 2.87 Å between the lactam oxygen atom, O(9), and the pyridyl nitrogen atom, N(14). This last distance is very close to the sum of the van der Waals radii for nitrogen and oxygen of 2.9 Å.[38] In addition, this arrangement is equivalent to that seen in pyridine hydrochloride[39] where a chloride ion sits directly over the pyridyl ring above the nitrogen atom at slightly more than van der Waals distance. The fact that the interaction in pyridine-HCl is primarily ionic in nature indicates that there likewise may be some negative charge localized on atom O(9) in solid-state cephaloridine. Three apparent hydrogen bonds exist in this structure (Table II). The first, a $COOH \cdots Cl^-$ interaction of length 2.964 (4) Å, is between the hydroxyl oxygen on the carboxyl group, O(12), and the chloride ion, Cl(29). The other two are from the water of hydration to the carbonyl oxygen of the exocyclic amide and to the chloride ion. These hydrogen-bonded distances are 2.964 (8) Å for $CO(22) \cdots H_2O(30)$ and 3.285 (7) Å for $Cl(29)^- \cdots H_2O(30)$.

(b) Cephaloglycine. The crystal-structure analysis of cephaloglycine was carried out to corroborate the conclusions related to chemical and biological activity which were drawn from the molecular parameters of the cephaloridine nucleus and to examine further the types of packing and hydrogen bonding which occur with these antibiotics. Although the structure could not be refined well, a comparison of structural features between cephaloglycine and cephaloridine is nevertheless meaningful (*vide infra*). In attempting to explain the inability of Fourier or least-squares techniques to refine this structure, one may suggest that the binding of solvate in the crystal lattice of this compound is nebulous, at best. Either gradual removal of this solvate or partial replacement of HOAC in the lattice with H_2O could explain the apparent decrease in cell parameters with time and could account for the evidently poor-quality diffraction data. The cephaloridine and cephaloglycine structures are similar in several very important ways, however. The most significant of

(38) Reference 36, p 260.
(39) P. C. Rerat, *Acta Crystallogr.*, **15**, 427 (1962).

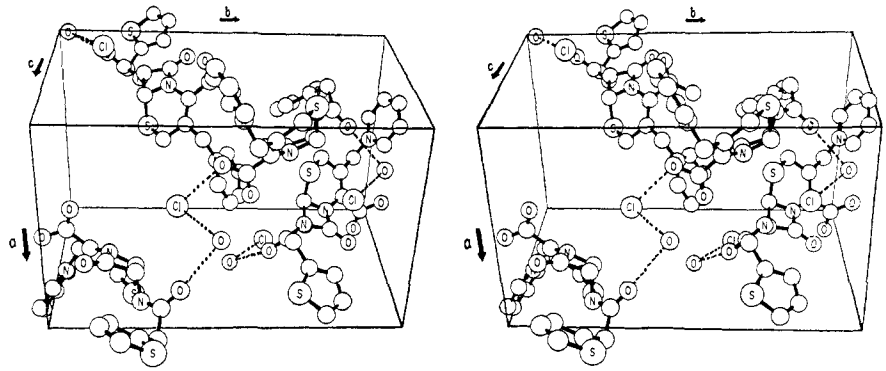


Figure 10. Stereoscopic view of the packing in one complete unit cell of cephaloridine hydrochloride monohydrate. Each of the axes shown runs from 0 to 1.

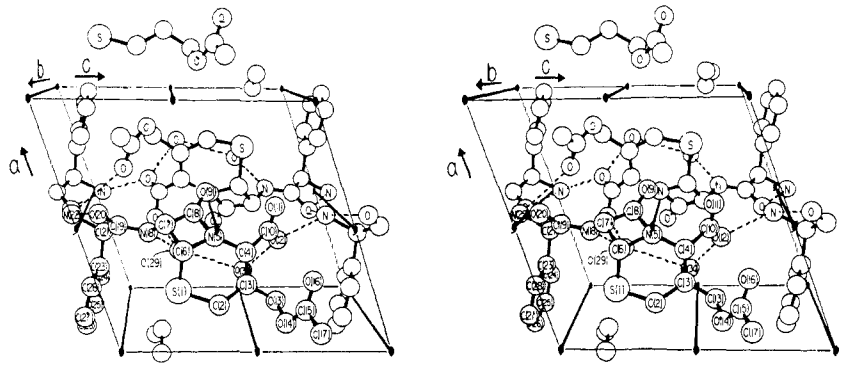


Figure 11. Stereoscopic view of the molecular conformation and packing of cephaloglycine. The cell edges shown are $0 - {}^1/_2$ in a, $1{}^1/_2 - {}^1/_2$ in b, and $0 - 1$ in c.

these is the distinct nonplanar character of the β-lactam nitrogen atom with respect to the three atoms connected to it.

The molecular conformation and packing of cephaloglycine are shown in Figure 11. The suppliers felt that when the crystals were fresh there was one molecule of water and one of acetic acid of crystallization per molecule of cephaloglycine.[34] In addition to the main molecule, two positions of moderately high electron density were discovered in each asymmetric unit of the lattice during this X-ray investigation. These two positions were designated O(29) and O(30), and were refined as oxygen atoms. The fact that each of these atoms is involved in at least two apparent hydrogen bonds of reasonable length indicates the correctness of our assignment of the two peaks as oxygen atoms. In particular, O(29) lies 2.72 (4) Å (Table VI) from the nitrogen atom of the exocyclic peptide, N(18), 2.69 (5) Å from one of the carboxyl atoms, O(11), and 3.11 (6) Å from O(30). Atom O(30), in addition to its proximity to O(29), lies 2.67 (5) Å from one of the carboxyl oxygen atoms, O(12). The amine nitrogen atom, N(22), of the phenylglycine residue is involved in three intermolecular hydrogen bonds. The one to the acetate oxygen atom, O(16), is 3.14 (4) Å, the one to the carboxy oxygen atom, O(12), is 2.72 (4) Å, and the one to the phenylglycyl amide carbonyl oxygen atom, O(20), is 2.76 (4) Å. In addition, an apparent intramolecular hydrogen bond of length 2.81 (4) Å exists between N(22) and the phenylglycine carbonyl oxygen atom, O(20). The N(22)H···O(20) intermolecular hydrogen bonds form a spiral linkage along one of the crystallographic twofold screw axes. Close nonbonded intermolecular contacts are from the lactam oxygen atom, O(9), to the carbon atoms C(2) and C(6) on either side of the sulfur atom in the dihydrothiazine ring at distances of 3.16 (5) and 3.24 (4) Å, respectively, from the acetate carbonyl oxygen atom, O(16), to atoms C(19) and C(21) at distances of 3.01 (5) and 3.04 (5) Å, respectively. Other close contacts also exist between corresponding carbon atoms of two phenyl rings related by the twofold rotation axis with the C(25)···C(25′) distance being 3.14 Å and the C(26)-C(26′) distance being 3.38 Å.

The results of least-squares planes calculations similar to those made on the cephaloridine structure are displayed in Table VII. The nitrogen atom in the β-lactam ring, N(5), has a conformation analogous to that found in cephaloridine. It lies 0.22 Å compared to 0.24 Å in cephaloridine out of the plane of the three atoms connected to it, C(4), C(6), and C(8). Other regions of the molecule are seen to adopt configurations which are much nearer idealized conformations than the errors in atomic positions allow one to expect.

Bond lengths and angles for this compound appear in Table VI and in Figure 8. In general, these values are remarkably near to those which might be expected despite their relatively large standard deviations. Although the imprecision with which molecular parameters are known for this compound makes it impossible for detailed comparisons to be made between it and cephaloridine, the structures are clearly quite similar, hence any conclusions made which relate the structure to the chemical or biological activity in cephaloridine apparently can apply to cephaloglycine. The normal

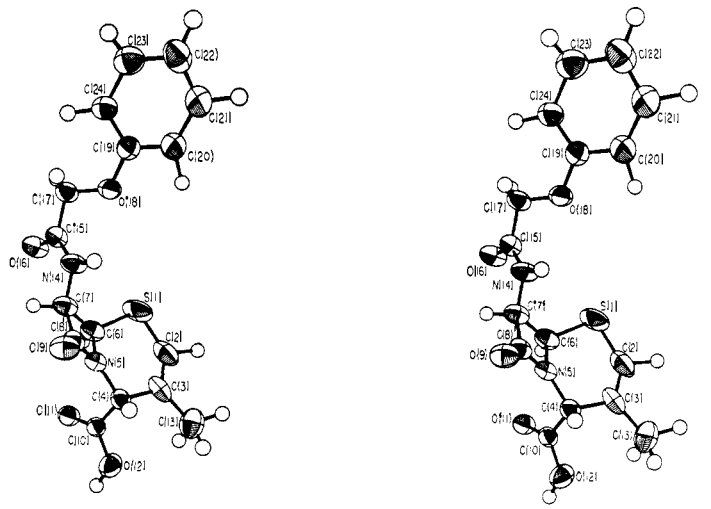


Figure 12. Stereoscopic view of the conformation and thermal ellipsoids representing 50% probability for phenoxymethyl-Δ^2-desacetoxyl cephalosporin.

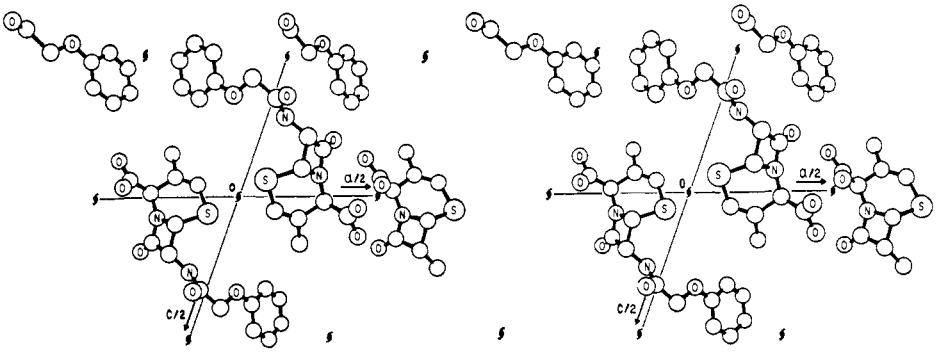


Figure 13. Stereoscopic view of the packing of phenoxymethyl-Δ^2-desacetoxyl cephalosporin. The observer is looking down one complete cell thickness in *b*.

character of the molecular architecture of this molecule, as described by least-squares planes and by bond length and angle calculations, together with the intricate, reasonable, and packing-dependent matrix of hydrogen bonds gives strong support to the correctness of the part of the structure describing the main cephaloglycine molecule. Unfortunately, the existence and true identity of the atoms tentatively designated O(29) and O(30) will necessarily await the collection of new data, perhaps at low temperature, from a truly fresh crystal of this material.

(c) Phenoxymethyl-Δ^2-desacetoxyl Cephalosporin. The molecular conformation of this compound is shown in Figure 12. The results of a series of least-squares planes calculations similar to those made on the other two compounds are also presented in Table VII. The most interesting result of these calculations is that the amide nitrogen atom in the β-lactam ring has approximately the planar trigonal character commonly found in free amides but not found in the penicillins or biologically active cephalosporins. This atom, N(5), lies only 0.06 Å from the plane of its three substituent atoms, C(4), C(6), and C(8). Other results show that the atoms involved in the thiazine double bond, the carboxyl group, the exocyclic amide, and the phenyl ring have the expected degrees of coplanarity and that the atoms of the β-lactam ring are slightly nonplanar.

Bond lengths and angles are shown in Table IV and in Figure 7. All bond lengths but one in both amide linkages, those of the β-lactam and the exocyclic amide, are within 0.02 Å of the values expected for a normal amide group.[40] The one exception is the length of the carbonyl bond in the exocyclic amide between atoms C(15) and O(16) which, at 1.210(8) Å, is 0.03 Å shorter than the mean value tabulated by Marsh and Donohue.[40] Unlike that of cephaloridine, the amide link in the β-lactam ring of this Δ^2-cephalosporin has a stereochemistry similar to that of a normal amide in that the three atoms attached to the nitrogen atom are nearly coplanar with it, although there clearly is bond strain in the four-membered ring. The root-mean-square deviation among the bond lengths in the phenyl ring is 0.01 Å, about the same size as the experimental standard deviation for each of those bond lengths.

The molecular packing of phenoxymethyl-Δ^2-desacetoxyl cephalosporin in the unit cell of symmetry $P2_1$ is shown in Figure 13. The phenyl rings are arranged at an angle of 45° to a nearby twofold screw axis. A peptide-like hydrogen bond, CO(16)···HN(14), of length 2.986 (8) Å from the carbonyl oxygen to the amide nitrogen links the exocyclic amide group in each molecule to the translationally equivalent amide group in the cells adjacent along the *b* axis. A hydrogen bond, COO(12)H···O(9)C, of length 2.657 (7) Å occurs from the carboxyl OH of one molecule to the β-lactam carbonyl oxygen atom of the molecule related

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

to the first one by a screw axis. Other intermolecular contacts are all greater than 3.2 Å.

Discussion of Structure–Activity Relationships. The β-lactam antibiotics, the only known examples of which are the cephalosporins (Figure 1b) and the penicillins (Figure 1a), are extremely valuable chemotherapeutic agents. Pencillins, historically the first antibiotics, have been used to combat bacterial diseases in humans for several decades. The cephalosporins have enjoyed a much briefer period of usefulness. However, they are widely applied in hospital situations when massive doses are indicated of an agent which is active against a wide range of bacterial species. In addition to the fact that the cephalosporin antibiotics are active against many penicillin-resistant bacteria, many patients who experience an allergic reaction to the penicillins have no such reaction to the cephalosporins.[10] Because of these desirable properties, the cephalosporin antibiotics deserve extensive study, both clinically and chemically.

Various workers have established[4-9] that the penicillins and the cephalosporins inhibit the terminal step in bacterial cell wall synthesis which is a peptide cross-linking of peptidoglycan strands catalyzed by the enzyme peptidoglycan transpeptidase. Tipper and Strominger[4] suggested that the penicillins imitate a possible conformation of this enzyme's substrate, D-alanyl-D-alanine. The enzyme mistakes the penicillin molecule for its proper substrate, cleaves the amide bond in the penicillin's β-lactam ring, is thus acylated, and is blocked irreversibly from further activity.

Because of the stereochemical nature of the arguments regarding the biological activity of these compounds, precise molecular parameters from the complete analysis of crystal structures of appropriate derivatives of both types of antibiotic are of great value in the formulation of further explanations for this biological activity. When the studies reported in this paper were begun, the crystal structures of only two β-lactam antibiotics, penicillin G[41] and cephalosporin C,[42] had been reported but not precisely determined. As evidence of the great interest in these antibiotics which has developed recently, crystal structure analyses have been carried out on three related compounds in addition to the three in this study. The compounds investigated in other laboratories are the biologically inactive penicillin V sulfoxide,[43] the commercially available pencillin antibiotic, ampicillin,[44] and the deacetyl cephalosporin C lactone.[45]

Careful investigation of the structures of cephalosporin antibiotics not only makes possible an examination of the various molecular forms which are apparently recognized by the inhibited bacterial enzyme, but it also provides a stereochemical explanation of the apparent lability of the β-lactam ring in a biological environment and the very real lability of these antibiotic β-lactams to base hydrolysis as compared to the stability observed for other β-lactams.

The Δ^2-isomers of the cephalosporins (Figure 1c) are known to be relatively inactive biologically.[11] In light of the following discussion, the contention[46] that this biological inactivity is due to an effect of chemical activity rather than to one of conformation seems quite reasonable. Figure 14 shows the actual solid-state conformation of the molecular nuclei of the penicillins,[41] the biologically active Δ^3-cephalosporins, and the biologically inactive Δ^2-cephalosporins. In each drawing the amide bond of the β-lactam ring, the region which must be closely approached by the enzyme, is toward the observer. One might expect that the orientation of the carboxyl group, which is immediately adjacent to the β-lactam ring, is an important factor in the ability of the enzyme to recognize the antibiotic as its substrate. Although the penicillins and the Δ^3-cephalosporins might both be recognized by this enzyme, the drawings clearly indicate, however, that the conformations of this carboxyl group are quite different. In addition, the inactive Δ^2-cephalosporin possesses a conformation in this region very similar to that of the penicillins. This suggests that the gross conformational requirements placed on the recognition by the enzyme of the antibiotic as its substrate are not very restrictive. The conformation of the Δ^2-cephalosporin differs little more from that of the active cephalosporins or penicillins than the active cephalosporins differ from the penicillins. These structural results indicate that there must be a basic chemical difference between the Δ^3- and Δ^2-cephalosporins to account for the difference in biological activity. The detailed structural study which has been carried out on one of these Δ^2 isomers yields information about this difference.

As representative examples of the Δ^3-cephalosporins, the crystal structures of cephaloridine hydrochloride monohydrate (Figure 2) and cephaloglycine acetic acid hydrate (Figure 4) have been solved. Two models of the cephaloridine structure have been refined. The cephaloglycine structure has been refined only to an R value of approximately 20% because the data were of poor quality. The example of a Δ^2-cephalosporin for which a crystal structure was solved and refined was phenoxymethyl-Δ^2-desacetoxyl cephalosporin (Figure 3). These particular compounds and crystalline forms were chosen because they were the ones for which crystals were available.

The most striking difference between the structures of the molecular nuclei of the Δ^3-cephalosporins and of the Δ^2-cephalosporins relates to the geometry of the β-lactam ring. Table VIII contains a list of structural properties of several β-lactams as well as pertinent spectral and biological properties. Although the bridgehead nitrogen atom in the β-lactam ring of the penicillins and Δ^3-cephalosporins is very definitely pyramidal, that of the biologically inactive Δ^2-cephalosporin is very nearly planar. In addition, the β-lactam amide bond in the Δ^2-cephalosporin is shorter by two and four standard deviations than those of ampicillin and cephaloridine, respectively. When one attempts to assess the reliability of these molecular parameters,

(41) D. Crowfoot, C. W. Bunn, B. W. Rogers-Low, and A. Turner-Jones in "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, p 310; G. J. Pitt, *Acta Crystallogr.*, **5**, 770 (1952).

(42) D. C. Hodgkin and E. N. Maslen, *Biochem. J.*, 79, 383 (1961).

(43) R. D. G. Cooper, P. V. De Marco, J. C. Cheng, and N. D. Jones, *J. Amer. Chem. Soc.*, **91**, 1408 (1969).

(44) M. N. G. James, D. Hall, and D. C. Hodgkin, *Nature* (London), 220, 168 (1968). Molecular parameters were obtained by private communication with Dr. James.

(45) Unpublished results of D. C. Hodgkin and coworkers, Department of Chemical Crystallography, University of Oxford, Oxford, England. Molecular parameters were not available.

(46) E. Van Heyningen and L. K. Ahern, *J. Med. Chem.*, **11**, 933 (1968).

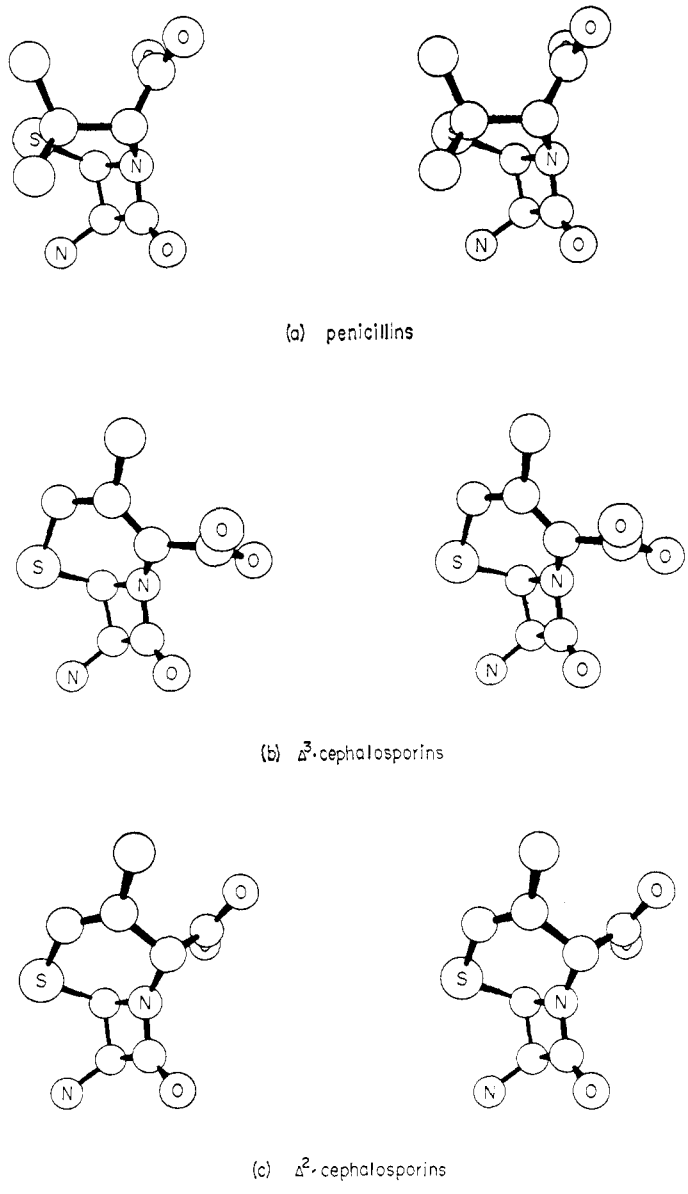
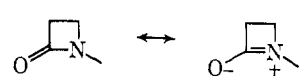


Figure 14. Stereoscopic views of the solid-state conformations of the molecular nuclei of the penicillins, the biologically active Δ^3-cephalosporins, and the biologically inactive Δ^2-cephalosporins.

the extreme similarity of equivalent parts of the cephaloridine and Δ^2-cephalosporin molecules should be noted: the exocyclic amide groups have very similar dimensions as do the carboxyl groups (refer to Figures 6 and 7).

In addition to the correlation of biological activity with the degree of nonplanarity in the β-lactam nitrogen atom, other properties which seem to correlate well with these properties are lactam C=O stretching frequency and ease of base hydrolysis of the β-lactam amide bond. Referring to Table VIII, one notes that as the biological activity increases, the lactam C=O stretching frequency increases, the lactam nitrogen planarity decreases, and the ease of basic hydrolysis of the lactam amide bond increases.

As the biological activity of these compounds seems to correlate with the ease of base hydrolysis of the β-lactam and as the proposed biological mechanism for these antibiotics involves the breaking of the bond which cleaves under hydrolysis, a discussion of structural features which might affect the lability of this bond is necessary in order to understand further the biological activity of these compounds. To begin, let us follow arguments used earlier by Woodward[47] and suggest that in a β-lactam not fused to another ring, normal amide resonance (I) can occur which tends to shorten the



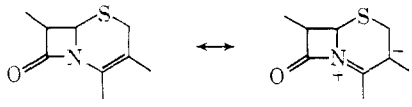
I

C-N and lengthen the C-O bonds. The only real requirement for maximization of this type of charge delocalization is that the three atoms connected to the nitrogen atom be coplanar with it so that the unshared electron pair of the nitrogen atom can be involved in π bonding with the adjacent carbonyl carbon atom. In the penicillins, however, the nitrogen atom cannot

(47) R. B. Woodward in "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, p 440.

be planar for steric reasons. The infrared data certainly indicate that the shift in C=O stretching frequency from unfused β-lactams to the penicillins is toward less of this electron delocalization. This absorption band increases in frequency from about 1750 to 1780 cm^{-1} as the planarity of the nitrogen atom decreases, which indicates an accompanying increase in double bond character between the carbon and oxygen atoms. In addition, one notes the expected increase in C-N bond length which follows the increase in C-O absorption frequency. Loss by the antibiotic lactams of this amide resonance certainly favors the formation of the tetrahedral intermediate which most likely forms at the carbonyl carbon atom during nucleophilic attack at the C-N bond.

In the cephalosporins, however, another factor in addition to the lack of planarity of the amide nitrogen may add to the lability of the β-lactam amide bond. In the cephalosporins, the possibility exists for normal enamine resonance, the delocalization of the nitrogen atom's unshared electron pair into the adjacent olefinic π-orbital system (II). To the extent that this delocal-

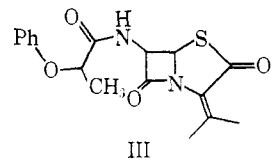


II

ization exists, the unshared electron pair of the nitrogen atom must be involved in some π bonding even though the orbitals which contain it must possess s-orbital character because the atoms connected to the nitrogen atom are not coplanar with it. Thus, one might expect that in spite of the nonplanar nitrogen atom in the cephalosporin β-lactam, this atom's unshared electron pair could be involved to some degree in amide as well as enamine resonance. In the case of cephaloridine the shift in bond lengths, which indicates the existence of this electron delocalization in the N—C=C system, is observed as mentioned earlier. An increased contribution of one type of resonance would clearly decrease the contribution of the other type. Thus, one might suggest that enamine resonance plus the lack of planarity of the nitrogen atom combine to decrease the amide resonance in the lactam amide bond of the cephalosporins which otherwise occurs to a much larger extent in an unfused β-lactam without the α,β unsaturation present in cephalosporins.

The β-lactam nitrogen atom in the Δ^2-cephalosporin is very nearly planar, and there are no possibilities for electron delocalization outside the lactam ring. This implies that the β-lactam amide bond of the Δ^2-cephalosporin is more like a free amide than that of the Δ^3-cephalosporins. Thus, one could predict, and it is observed, that on going from the Δ^2- to the Δ^3-cephalosporins the C=O stretching frequency increases, the C=O bond length decreases, and the C-N bond length increases.

Discussion of the activity of these antibiotics is not complete without mention of the newly discovered compound, anhydro-α-phenoxyethylpenicillin (III).[48] Because the nitrogen atom in the β-lactam must be nonplanar (similar to what is found in the penicillins) and be-



III

cause unsaturation exists α,β to the nitrogen atom equivalent to that in the cephalosporins, one might expect even less amide resonance in the lactam ring. This hypothesis is supported by the fact that an increase in double bond character in the lactam carbonyl of the anhydropenicillin from that in the penicillins is observed, as indicated by the shift in the infrared carbonyl band absorption from 1775 cm^{-1} for penicillins to 1820 cm^{-1} for the anhydropenicillin. However, a significantly increased stability relative to that for the active penicillins is in fact observed for this compound in that it is recovered unchanged after refluxing in various neutral solvents or from a melt.[48] This stability is completely in contrast with the known instability of the active cephalosporin systems and the penicillins. One can hope that further structural and chemical studies will ameliorate this unexpected and unexplained difference in chemical activities.

In conclusion, structural comparisons have been made among the penicillins, the cephalosporins, and a biologically inactive Δ^2-cephalosporin isomer each possessing a β-lactam ring. The penicillin and Δ^3-cephalosporin antibiotics are stereochemically similar, not in their detailed conformations or dimensions, but because each contains a N-CO bond in its β-lactam ring with less normal amide character than that in an unfused β-lactam ring or in the fused β-lactam ring of a Δ^2-cephalosporin system. The latter system has less ring strain to force the lactam nitrogen atom to a nonplanar configuration and in addition has no unsaturation α,β to the lactam nitrogen. The β-lactam N-CO amide bond is observed to lengthen on going from the inactive Δ^2- to the active Δ^3-cephalosporin, which is consistent with the increase in C=O stretching frequency accompanying the decreased amide character.

The assertion that these structure-activity relationships based on observed solid-state geometries are valid is based on the premise that the fused ring conformations, found in the crystalline state for the penicillins and these cephalosporin derivatives, remain unchanged upon their dissolution. Prime evidence in support of this basic assumption comes from the recent structural investigation of penicillin V sulfoxide.[43] X-Ray diffraction and nuclear Overhauser effect studies showed that the fused ring system in this compound adopts the same detailed conformation both in the solid state and in solution. The fused ring system in the penicillin sulfoxide molecule is very similar to that in the penicillins and in the Δ^3- and Δ^2-cephalosporin derivatives. Thus, most likely these latter ring systems are also sufficiently rigid to dictate the same configuration both in the solid state and in solution.

Acknowledgments. The authors wish to express their real appreciation to Drs. Noel Jones and Ralph Pfeiffer of Eli Lilly and Company, Indianapolis, Ind., for having most generously supplied crystals for these investigations and for their helpful advice and interest in this work. We are also indebted to Professor Howard

(48) S. Wolfe, J. C. Godfrey, C. T. Holdrege, and Y. G. Perron, *Can. J. Chem.*, 46, 2549 (1968).

Whitlock of the University of Wisconsin (Madison) for enlightening discussions. This research was financially supported by the National Institutes of Health (Grant No. AI07795). The use of the CDC 1604 and 3600 computers and the UNIVAC 1108 computer at the University of Wisconsin Computing Center was made available through partial support of National Science Foundation and Wisconsin Alumni Research Foundation through the University Research Committee. One of us (R. M. S.) acknowledges the support of the National Institutes of Health through Graduate Research Fellowship No. 3-FO1-GM-39444-02.

Polynucleotides. VIII.[1] A New Method for the Synthesis of Protected Deoxyribooligonucleotides with 5′-Phosphate

Eiko Ohtsuka, Masaru Ubasawa, and Morio Ikehara

Contribution from the Faculty of Pharmaceutical Sciences, Osaka University, Toyonaka, Osaka, Japan. *Received February 16, 1970*

Abstract: Phosphoroanilidate was used to protect the 5′-terminal phosphate in the synthesis of derivatives of deoxyribooligonucleotides. Three deoxyribotrinucleotides containing acid labile N-benzoyldeoxyadenosine were synthesized. The synthetic step involved the condensation of thymidine 5′-phosphoranilidate and N-benzoyl-3′-O-acetyldeoxyadenosine 5′-phosphate using dicyclohexylcarbodiimide, and selective removal of the 3′-O-acetyl group to yield the protected dinucleotide (III, Scheme I) in a yield of 58%. For the synthesis of trinucleotides, III was condensed with N-benzoyl-3′-O-acetyldeoxyadenosine 5′-phosphate, N-anisoyl-3′-O-acetyldeoxycytidine 5′-phosphate, and N,3′-O-diisobutyryldeoxyguanosine 5′-phosphate. Treatment with isoamyl nitrite and subsequent removal of the 3′-O-acetyl group gave IVa, IVb, and IVc, respectively. Yields in the synthesis of the trinucleotides were 30–36%.

Nucleoside 5′-phosphates protected at the phosphate group have been used as key intermediates in the synthesis of deoxyribopolynucleotides.[2] β-Cyanoethyl ester is one of the most common protecting groups. Since it is cleaved by treatment with alkali,[3,4] reprotection of the 5′-phosphate is required after removal of the 3′-O-acetyl group.[5] Other useful protecting groups include the trichloroethyl ester, which is cleaved by reduction,[6] and the S-ethylphosphorothioates,[7] which are stable in alkali and are removed by mild oxidation. Blackburn described the synthesis of pTpTpT[8] on a phosphoramidate resin. The acid treatment[9] used to release the product, however, is not compatible with purine deoxyribonucleotides.

In this paper we report the use of aromatic phosphoramidates as protecting groups for the 5′-phosphate group in the stepwise synthesis of deoxyribooligonucleotides. Aromatic amidates of protected ribonucleoside 3′-phosphate were previously shown to be stable in alkali and to be subject to selective removal with isoamyl nitrite.[10] A new general procedure for the synthesis of oligonucleotide blocks is outlined in Scheme I. Since the glycosyl bond of N-benzoyldeoxyadenosine is extremely labile under the condition in which those of N-benzoyladenosine and deoxyadenosine were stable,[11] the synthesis of trinucleotides containing N-benzoyldeoxyadenosine was chosen to test the stability of the glycosyl linkage during the treatment with isoamyl nitrite.

Thymidine 5′-phosphoranilidate (I) was prepared by a method similar to that described for the synthesis of adenosine 5′-phosphoro-*p*-anisidate.[12] Anilidate of the 5′-phosphate could be removed with isoamyl nitrite in a 1:1 mixture of acetic acid and pyridine or a 1:2 mixture of acetic acid and triethylamine. Although the latter mixture seems to give a slower rate, it might be safer to use a trialkylamine for acid labile deoxypurine nucleotides. Thymidine 5′-phosphoranilidate (I) and N-benzoyl-3′-O-acetyldeoxyadenosine 5′-phosphate (IIa) were allowed to react with dicyclohexylcarbodiimide (DCC). Triisopropylbenzenesulfonyl chloride (TPS) was also used as the condensing reagent in a preliminary experiment. After 4 days an aliquot was treated with ammonia. PhNHpTpA was found on paper chromatogram as almost the sole product (see Table I for R_f values of the protected and unprotected compound). The 3′-O-acetyl group was removed selectively by treatment with strong alkali to give III. The stability of the amidate in this treatment was checked by paper chromatography and paper elec-

(1) Paper VII: M. Ikehara, I. Tazawa, and T. Fukui, *Biochemistry*, **8**, 736 (1969).
(2) H. G. Khorana, *Pure Appl. Chem.*, **17**, 349 (1968).
(3) G. M. Tener, *J. Amer. Chem. Soc.*, **83**, 159 (1961).
(4) G. Weimann, H. Schaller, and H. G. Khorana, *ibid.*, **85**, 3835 (1963).
(5) E. Ohtsuka and H. G. Khorana, *ibid.*, **89**, 2185 (1967).
(6) A. Franke, F. Eckstein, K. Scheit, and F. Cramer, *Chem. Ber.*, **101**, 944 (1968).
(7) A. F. Cook, M. J. Holman, and A. L. Nussbaum, *J. Amer. Chem. Soc.*, **91**, 6479 (1969).
(8) The abbreviation of nucleotides in this paper follows that described in *J. Biol. Chem.*, **241**, 527 (1966), and E. Ohtsuka, M. W. Moon, and H. G. Khorana, *J. Amer. Chem. Soc.*, **87**, 2956 (1965). Other abbreviations used were PhNHpTpA, 5′-phosphoranilidothymidilyl-(3′–5′)-deoxyadenosine; d-pG^{1b}-OIb, N,3′-O-diisobutyryldeoxyguanosine 5′-phosphate.
(9) G. M. Blackburn, M. J. Brown, and M. R. Harris, *J. Chem. Soc.*, 2438 (1967).
(10) E. Ohtsuka, K. Murao, M. Ubasawa, and M. Ikehara, *J. Amer. Chem. Soc.*, **91**, 1537 (1969).
(11) M. W. Moon, S. Nishimura, and H. G. Khorana, *Biochemistry*, **5**, 937 (1966).
(12) J. G. Moffatt and H. G. Khorana, *J. Amer. Chem. Soc.*, **83**, 649 (1961).