The Crystal and Molecular Structure of the Barium Salt of an Antibiotic Containing a High Proportion of Oxygen[1]

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Abstract: The molecular structure of an antibiotic X-537A, $C_{34}H_{54}O_8$, from an unidentified streptomyces has been determined by the X-ray structure analysis of a barium salt. Two molecules of the barium salt monohydrate, $Ba(C_{34}H_{53}O_8)_2 \cdot H_2O$, crystallize in the space group $P2_1$ with $a = 14.59$, $b = 17.95$, $c = 13.99$ Å, and $\beta = 105°17'$. The structure has been refined to an R factor of 0.11 on 4753 structure amplitudes measured by film methods. The two crystallographically independent antibiotic molecules wrap themselves around the barium, with eight oxygen atoms and the water molecule of crystallization in the range 2.6–3.1 Å from the metal ion.

Eighteen years ago, three hitherto unknown, crystalline antibiotics were obtained from three unidentified streptomyces, designated X-206, X-464, and X-537A.[3] All three antibiotics are optically active organic acids, containing a high proportion of oxygen. These antibiotics are capable of forming a variety of salts with Ag^+, Na^+, K^+, and Ba^{2+}.[3] More recently, another antibiotic, monensic acid, was isolated from *Streptomyces cinnamonensis*, and showed similar high oxygen content and capability to form metal salts.[4] The structure of monensic acid has been determined by a combination of chemical studies and an X-ray structural analysis of the monosilver salt.[4]

The antibiotic X-537A produced by X-537A has the molecular formula $C_{34}H_{54}O_8$.[5] Nmr studies indicate that, while mainly aliphatic, it has two protons in a region indicative of a tetrasubstituted aromatic group, and that there is a methyl group ($\delta = 2.18$) which is either bound to an aromatic ring or to a C=C double bond.[6] There is no evidence for a methoxyl group;[6] monensic acid contains one methoxyl group.[4] Mass spectra also provided evidence for the presence of an aromatic ring or of a structure which could readily form such a ring.[6] The infrared spectra of the piperidine salt and the free acid both have a band at 1700 cm^{-1}, suggestive of a saturated ketone.[6]

In view of the limited structural evidence available, an X-ray structure analysis of a suitable metal salt was proposed as the most convenient means of structure determination of the antibiotic from X-537A. Furthermore, the conformation of the antibiotic when bound to a metal will be of interest when compared to that of monensic acid. Since the inception of this study, two independent X-ray studies of a related antibiotic, nigericin, have appeared[7,8] and chemical studies have shown that nigericin and X-464 are identical.[9] A preliminary account of this crystal structure has been published.[10]

Experimental Section

Of several metal salts examined, the crystals of the barium salt, $Ba(C_{34}H_{53}O_8)_2 \cdot H_2O$, obtained from a water–ethanol mixture, were most suitable for X-ray work. As the supply of the recrystallized material was extremely limited, the proportion of Ba^{2+} present was checked by atomic absorption spectroscopy, 8.78% (measured by Dr. A. Stempel and colleagues at Hoffmann-La Roche, Inc.) (calcd, 10.29%); crystal data: $Ba(C_{34}H_{53}O_8)_2 \cdot H_2O$, $M = 1334.94$, monoclinic, $a = 14.59$ (4), $b = 17.95$ (5), $c = 13.99$ (4) Å, $\beta = 105°17'$ (15'), $V = 3534.3 \times 10^{-24}$ cm^3, $\rho_{measd} = 1.22$ g cm^{-3} (aqueous zinc chloride), $Z = 2$, $\rho_{calcd} = 1.255$ g cm^{-3}, $F(000) = 1416$, systematic absences $0k0$, when $k = 2n + 1$; space group $P2_1$, μ (Cu Kα) = 49 cm^{-1}.

The crystals are colorless, transparent plates lying on well-developed {001} faces. The {110} faces are the only other developed faces of the crystal. The unique axis corresponds to the major diagonal of the rhombic face of the plate. Cell dimensions were obtained from precession photographs, using Mo Kα radiation ($\lambda = 0.7107$ Å). A crystal, mounted about the b axis, and having the major and minor diagonals of the plate 0.7 and 0.6 mm, and the perpendicular to the rhombic face 0.3 mm, was used to obtain the levels of data $h0l$ to $h14l$ on an equiinclination Weissenberg camera at 4° (Cu Kα, $\lambda = 1.5418$ Å).[11] The data were estimated visually and corrected for Lorentz, polarization, and spot shape effects. No correction was made for absorption. A total of 4753 independent, nonzero structure amplitudes was obtained.

Structure Determination. As there are two Ba^{2+} ions in the unit cell, and the space group is $P2_1$, all Fourier maps with phases calculated on the basis of the positions of the Ba^{2+} ions will contain a false center of symmetry. In view of this pseudosymmetry, the size of the structural problem (85 C + O atoms to be located in the crystal asymmetric unit), and the paucity of chemical information known on the antibiotic, we realized that the structure determination would not be a routine matter. The first Fourier map clearly showed a benzene ring with at least three substituents and one substantially puckered six-membered ring. A second Fourier map, calculated with the additional contributions of the nine identified atoms of the substituted benzene fragment, did little to destroy the pseudosymmetry. Attempts to assess, on the basis of structure factor calculations and the resulting R factors, which of the two images of the six-membered ring fragment corresponded to the position chosen for the benzene ring, proved indecisive; R on barium alone was 0.26, and the effect of putting six or seven carbon atoms into such a calculation was very small. At this stage, however, it was clear that the antibiotic molecules were clustered around the cation in a manner reminiscent of monensic acid,[4] and that a number of peaks within 3 Å of the cation probably corresponded to coordinating oxygen atoms. After extensive examination of

(1) Work supported by U. S. Public Health Service, Grant No. GM 12470 and by a grant from the Hoffmann-La Roche Foundation.

(2) Alfred P. Sloan Research Fellow.

(3) J. Berger, A. I. Rachlin, W. E. Scott, L. H. Sternbach, and M. W. Goldberg, *J. Amer. Chem. Soc.*, 73, 5295 (1951).

(4) A. Agtarap, J. W. Chamberlin, M. Pinkerton, and L. K. Steinrauf, *ibid.*, 89, 5737 (1967).

(5) In reference 3, this formula was given as $C_{34}H_{52}O_8$.

(6) A. Stempel and J. W. Westley, personal communication, 1968.

(7) L. K. Steinrauf, M. Pinkerton, and J. W. Chamberlin, *Biochem. Biophys. Res. Commun.*, 33, 29 (1968).

(8) T. Kubota, S. Matsutani, M. Shiro, and H. Koyama, *Chem. Commun.*, 1541 (1968); M. Shiro and H. Koyama, *J. Chem. Soc. B*, 243 (1970).

(9) A. Stempel, J. W. Westley, and W. Benz, *J. Antibiot.* (Tokyo), 22, 384 (1969).

(10) S. M. Johnson, J. Herrin, S. J. Liu, and I. C. Paul, *Chem. Commun.*, 72 (1970).

(11) While the crystal used to collect data was rather larger than desirable, it was clearly the best among the very few crystals of this complex available to us as regards having well-developed faces and no satellites.

this map, and rejection of a number of plausible structural fragments on the basis of bond length and angle calculations (these fragments can be seen in retrospect to have arisen from the close approach of two or three genuine atoms to the pseudosites for two or three others), we were able to identify the main skeleton of the two crystallographically independent antibiotic molecules. The site of an atom was only accepted as genuine when it could be identified on both antibiotic molecules. A structure factor calculation including 68 atoms together with the Ba^{2+} ion produced an R factor of 0.20 and allowed the computation of a Fourier map which had no pseudosymmetry. Two further rounds of structure factor and Fourier calculations were necessary to determine the positions of all 85 carbon and oxygen atoms.

The next major problem involved the identification of the eight oxygen atoms and the location of the one double bond in addition to the benzene ring, the carboxyl group, and the two other rings found in the molecule, as required by the molecular formula. In the unprimed antibiotic molecule[12] six oxygen atoms (O_{15}, O_{20}, O_{26}, O_{31}, O_{33}, and O_{40}) could be identified by virtue of coordination to Ba^{2+}, and the identity of a seventh (O_{27}) was deduced from its presence in the carboxyl group. The mode of coordination of the two antibiotic molecules to Ba^{2+} is quite distinct, and the coordination of the primed antibiotic molecule allowed us to verify some of these choices. Some assignments could also be verified by reasonable hydrogen bonding schemes, both intramolecular and with the water molecule which coordinates to the Ba^{2+} ion. The identity of the eighth oxygen atom could not be established on the basis of metal coordination or hydrogen bonding. We considered the unidentified double bond to represent the saturated ketone believed present in the molecule;[6] the geometry of the carbon chain suggested O_{33} as most likely to be the ketonic oxygen atom.

Full-matrix least-squares refinement[13] of the positional and isotropic thermal parameters was introduced at this stage. The quantity minimized was $\Sigma w|F_{obsd} - F_{calcd}|^2$ and the weighting scheme had $\sqrt{w} = |F_{obsd}|/40.0$ when $|F_{obsd}| \leq 40.0$, and $\sqrt{w} = 40.0/|F_{obsd}|$ when $|F_{obsd}| > 40.0$. As the number of variable parameters was too great for the capacity of our computer, we divided the atoms in the asymmetric unit into three groups and refined these in turn. One group contained atoms 8–24 and atoms 30–42, the second group contained the corresponding atoms in the primed molecule, and the third group consisted of the atoms 1–7, 25–29, 1′–7′, 25–29′, and O_w; the Ba^{2+} ion was included in all refinements. The resulting temperature factors from one complete "cycle" of refinement with all atoms, other than the Ba^{2+} ion, included with carbon scattering factors, confirmed our previous assignments of oxygen atoms, and provided compelling evidence that the one-atom substituent on the phenyl ring *ortho* to the carboxyl group was the eighth oxygen atom.[14] Calculated bond lengths and angles substantiated this assignment and also favored O_{33} as the ketonic oxygen atom. With the appropriate atomic scattering factors included for all atoms, $R = 0.13$. Anisotropic temperature factors were introduced for the Ba^{2+} ion. Two further cycles of refinement (dividing the molecule as previously described) gave a final R factor of 0.11 on all observed reflections.

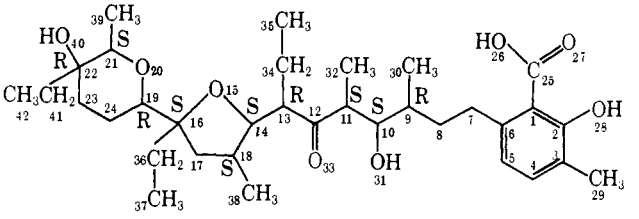
The exact location of C_{42} is somewhat uncertain. The position chosen originally (corresponding to C_{42a} in Table I) gave very reasonable values for the C_{41}–C_{42} bond length and the C_{22}–C_{41}–C_{42} bond angle, but seemed unacceptably close (3.40 and 3.55 Å) to the terminal carbon atoms of two ethyl groups in adjacent complexes. Furthermore, at the conclusion of the refinement, the $B\theta$ value for C_{42} was 11.7 Å^2, by far the highest such value in the complex. Inspection of a difference map revealed two other possible sites for C_{42}. One of these (C_{42b}) was refined, along with Ba^{2+} and the other atoms comprising the six-membered ring and substituents, on roughly one-third of the data. The temperature factor became 14.5 Å^2, and the C_{41}–C_{42b} length was 1.26 Å, and the C_{22}–C_{41}–C_{42b} angle was 128°. The intracomplex C_{42b}–$C_{42'}$ contact was 3.27 Å, which is very short. A third possible site (C_{42c}) was also refined as described above. The isotropic temperature factor was 12.5 Å^2, the C_{41}–C_{42c} distance was 1.42 Å, and the C_{22}–C_{41}–C_{42c} angle was 82°. The intracomplex C_{42c}–$C_{42'}$ contact was 2.74 Å. This position was thus rejected. None of these possibilities seems entirely reasonable and the most plausible conclusion appears to be that the position of this atom is distributed on a near statistical basis between sites C_{42a} and C_{42b}. In all diagrams, we use the C_{42a} position.

The largest positive peak (except for a few very near the Ba^{2+} ion) on a difference map lay at $x = 0.49$, $y = 0.31$, $z = 0.28$; this peak is too close to a hydrophobic region of the molecule (2.45 Å) to represent a molecule of solvation. There was no convincing evidence for any of the other positive peaks on this map to represent sites of significant occupancy for solvent molecules.

The atomic scattering curves for C and O were taken from the compilation by Ibers,[16a] that for Ba^{2+} from the calculations of Cromer and Waber,[16b] with correction for the real component of anomalous dispersion.[16c] The final atomic coordinates and temperature parameters are listed in Table I and the final list of h, k, l, $|F_{obsd}|$ and $|F_{calcd}|$ has been deposited with the ASIS agency.[17]

Results and Discussion

The molecular structure and stereochemistry of the antibiotic X-537 A is shown to be I. The atom numbering adopted in this study is shown in I. If one considers



I

the antibiotic as a derivative of salicylic acid, 3-methyl-6-{7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl}salicylic acid is the systematic name for the molecule. The molecule has been shown[15] to have the absolute stereochemistry shown in Figure 1. The stereochemistry at the ten asymmetric centers is designated R or S according to the sequence rule of Cahn and Ingold.[18] These assignments are shown in I. A view of the molecular structure looking down b is shown in Figure 1. Figures 2 and 3 show stereoscopic views of the molecular structure viewed along a and b. The individual bond lengths and angles within the antibiotic molecules have been deposited with ASIS agency.[17] The values obtained agree with generally accepted values[19] within the accuracy of the analysis.

The two antibiotic anions coordinate to the Ba^{2+} ion in different ways. A schematic view of the coordination around barium is shown in Figure 4. In the unprimed molecule, O_{26}, O_{31}, O_{33}, O_{15}, O_{20}, and O_{40} lie in the range 2.71–3.08 Å from the Ba^{2+} ion (Table II). In the primed molecule, $O_{26'}$ and $O_{40'}$ are, respectively,

(12) We designate the two antibiotic molecules "primed" and "unprimed," see Figure 2.

(13) We used a revised version of the program written by P. K. Gantzel, R. A. Sparks, and K. N. Trueblood, "I. U. C. World List of Crystallographic Computer Programs," International Union of Crystallography, 1962, No. 384.

(14) This assignment was then supported by a comparison of uv and potentiometric data with model salicyclic acid derivatives, J. W. Westley and A. Stempel, personal communication, 1968. See also ref 15.

(15) J. W. Westley, R. H. Evans, Jr., T. Williams, and A. Stempel, *Chem. Commun.*, 71 (1970).

(16) (a) J. A. Ibers in "International Tables for X-ray Crystallography," the Kynoch Press, Birmingham, England, Vol. III, 1962, pp 201–209; (b) D. T. Cromer and J. T. Waber, *Acta Cryst.*, **18**, 104 (1965); (c) For Ba^{2+} (Cu Kα), $\Delta f' = -2.1$, $\Delta f'' = 8.9$, ref 16a, pp 213–216.

(17) The final values of h, k, l, $|F_{obsd}|$, and $|F_{calcd}|$ and the individual bond lengths and angles are available (order document No. NAPS-00839) from ASIS National Auxiliary Publications Service, c/o CCM Information Corp., 909 3rd Ave., New York, N. Y. 10022; remit $2.00 for microfilm or $5.00 for photocopies.

(18) R. S. Cahn and C. K. Ingold, *J. Chem. Soc.*, 612 (1955); see also R. S. Cahn, *J. Chem. Educ.*, **41**, 116 (1964).

(19) Reference 16a, pp 255–285.

Table I. Final Atomic and Thermal Parameters with Estimated Standard Deviations

	x	y	z	B_θ		x	y	z	B_θ
Ba	0.84366 (7)	0.49979[b]	0.69376 (7)	a	C_{42c}	0.823	0.500	0.380	12.5
C_1	0.806 (1)	0.239 (1)	0.548 (1)	2.3 (4)	$C_{1'}$	0.726 (2)	0.735 (2)	0.655 (2)	3.7 (5)
C_2	0.761 (2)	0.208 (1)	0.457 (2)	2.6 (4)	$C_{2'}$	0.726 (2)	0.770 (1)	0.565 (2)	3.5 (4)
C_3	0.712 (2)	0.140 (1)	0.451 (2)	2.9 (4)	$C_{3'}$	0.751 (2)	0.844 (2)	0.567 (2)	4.6 (6)
C_4	0.719 (2)	0.105 (2)	0.538 (2)	3.7 (5)	$C_{4'}$	0.766 (2)	0.879 (2)	0.661 (3)	5.5 (8)
C_5	0.769 (2)	0.128 (1)	0.625 (2)	2.9 (4)	$C_{5'}$	0.752 (2)	0.847 (2)	0.735 (3)	6.0 (8)
C_6	0.812 (2)	0.196 (1)	0.633 (2)	3.2 (4)	$C_{6'}$	0.733 (2)	0.775 (2)	0.739 (2)	4.3 (5)
C_7	0.864 (2)	0.228 (1)	0.736 (2)	2.9 (4)	$C_{7'}$	0.723 (2)	0.741 (2)	0.837 (2)	3.8 (5)
C_8	0.971 (2)	0.231 (2)	0.747 (2)	3.8 (6)	$C_{8'}$	0.618 (2)	0.724 (2)	0.827 (2)	3.9 (5)
C_9	1.030 (2)	0.269 (1)	0.848 (2)	3.3 (5)	$C_{9'}$	0.602 (2)	0.680 (2)	0.918 (2)	3.6 (5)
C_{10}	0.993 (1)	0.347 (1)	0.850 (1)	2.5 (4)	$C_{10'}$	0.644 (2)	0.604 (1)	0.922 (2)	3.5 (4)
C_{11}	1.041 (2)	0.391 (2)	0.941 (2)	3.3 (4)	$C_{11'}$	0.621 (2)	0.559 (2)	1.002 (2)	3.7 (6)
C_{12}	0.998 (2)	0.469 (1)	0.933 (2)	3.2 (4)	$C_{12'}$	0.662 (2)	0.478 (1)	0.998 (2)	3.0 (5)
C_{13}	1.060 (2)	0.530 (1)	0.977 (2)	3.2 (4)	$C_{13'}$	0.598 (2)	0.410 (2)	1.003 (2)	4.0 (5)
C_{14}	1.098 (2)	0.557 (1)	0.892 (2)	3.5 (5)	$C_{14'}$	0.528 (2)	0.401 (2)	0.899 (2)	3.5 (5)
O_{15}	1.020 (1)	0.577 (1)	0.808 (1)	2.9 (3)	$O_{15'}$	0.582 (1)	0.363 (1)	0.839 (1)	4.3 (4)
C_{16}	1.048 (2)	0.642 (1)	0.759 (2)	2.6 (4)	$C_{16'}$	0.519 (2)	0.318 (2)	0.768 (2)	3.7 (5)
C_{17}	1.153 (2)	0.658 (2)	0.810 (2)	5.1 (6)	$C_{17'}$	0.420 (3)	0.326 (2)	0.797 (3)	6.0 (8)
C_{18}	1.165 (2)	0.625 (2)	0.911 (2)	4.2 (6)	$C_{18'}$	0.452 (2)	0.350 (2)	0.903 (2)	5.0 (7)
C_{19}	1.029 (2)	0.628 (2)	0.648 (2)	3.5 (5)	$C_{19'}$	0.509 (2)	0.348 (2)	0.661 (2)	3.4 (5)
O_{20}	0.927 (1)	0.619 (1)	0.611 (1)	2.7 (3)	$O_{20'}$	0.600 (1)	0.335 (1)	0.643 (1)	3.7 (3)
C_{21}	0.899 (2)	0.611 (2)	0.502 (2)	3.6 (5)	$C_{21'}$	0.603 (2)	0.350 (1)	0.545 (2)	3.3 (4)
C_{22}	0.939 (2)	0.538 (2)	0.473 (2)	3.6 (5)	$C_{22'}$	0.578 (2)	0.432 (1)	0.518 (2)	3.1 (4)
C_{23}	1.045 (2)	0.536 (2)	0.512 (2)	4.2 (5)	$C_{23'}$	0.487 (2)	0.455 (2)	0.544 (2)	3.7 (5)
C_{24}	1.075 (2)	0.555 (2)	0.626 (2)	3.7 (5)	$C_{24'}$	0.491 (2)	0.431 (2)	0.651 (2)	3.6 (5)
C_{25}	0.852 (1)	0.314 (1)	0.549 (2)	2.7 (4)	$C_{25'}$	0.712 (2)	0.652 (2)	0.650 (2)	3.2 (5)
O_{26}	0.864 (1)	0.355 (1)	0.625 (1)	2.9 (3)	$O_{26'}$	0.739 (1)	0.613 (1)	0.722 (1)	2.9 (3)
O_{27}	0.865 (1)	0.338 (1)	0.471 (1)	3.9 (3)	$O_{27'}$	0.678 (1)	0.625 (1)	0.563 (1)	5.9 (4)
O_{28}	0.756 (1)	0.242 (1)	0.372 (1)	4.4 (4)	$O_{28'}$	0.709 (1)	0.737 (1)	0.480 (1)	4.9 (4)
C_{29}	0.656 (2)	0.113 (2)	0.353 (2)	4.7 (6)	$C_{29'}$	0.761 (2)	0.881 (2)	0.471 (2)	5.6 (7)
C_{30}	1.140 (2)	0.263 (2)	0.854 (2)	5.1 (6)	$C_{30'}$	0.497 (3)	0.677 (2)	0.910 (3)	6.7 (8)
O_{31}	1.010 (1)	0.392 (1)	0.772 (1)	3.0 (3)	$O_{31'}$	0.608 (1)	0.565 (1)	0.830 (1)	4.3 (4)
C_{32}	1.025 (2)	0.351 (2)	1.035 (2)	4.9 (6)	$C_{32'}$	0.664 (3)	0.589 (3)	1.110 (3)	8.5 (1.1)
O_{33}	0.915 (1)	0.475 (1)	0.898 (1)	3.3 (4)	$O_{33'}$	0.746 (1)	0.469 (1)	0.991 (1)	5.1 (4)
C_{34}	1.005 (2)	0.592 (2)	1.019 (2)	4.1 (6)	$C_{34'}$	0.657 (2)	0.340 (2)	1.044 (2)	5.3 (6)
C_{35}	0.972 (2)	0.557 (2)	1.106 (2)	6.0 (7)	$C_{35'}$	0.693 (3)	0.350 (3)	1.160 (3)	8.4 (1.0)
C_{36}	0.985 (2)	0.704 (2)	0.778 (2)	4.3 (6)	$C_{36'}$	0.563 (2)	0.240 (2)	0.792 (3)	6.3 (7)
C_{37}	1.003 (2)	0.780 (2)	0.725 (3)	5.1 (7)	$C_{37'}$	0.508 (3)	0.184 (3)	0.711 (4)	8.3 (1.2)
C_{38}	1.270 (3)	0.600 (2)	0.970 (3)	6.6 (8)	$C_{38'}$	0.366 (3)	0.389 (3)	0.931 (3)	7.3 (9)
C_{39}	0.920 (2)	0.681 (2)	0.454 (2)	4.4 (5)	$C_{39'}$	0.540 (2)	0.295 (2)	0.465 (2)	6.0 (7)
O_{40}	0.896 (1)	0.483 (1)	0.522 (1)	3.7 (3)	$O_{40'}$	0.656 (1)	0.473 (1)	0.576 (1)	3.4 (3)
C_{41}	0.905 (2)	0.530 (2)	0.357 (2)	5.1 (7)	$C_{41'}$	0.564 (3)	0.457 (2)	0.407 (3)	7.8 (9)
C_{42a}	0.942 (5)	0.459 (3)	0.316 (4)	11.7 (1.5)	$C_{42'}$	0.648 (4)	0.444 (3)	0.377 (4)	10.6 (1.4)
C_{42b}	0.837	0.491	0.307	14.5	O_{43} (w)	0.753 (1)	0.398 (1)	0.784 (1)	3.6 (3)

[a] The anisotropic thermal parameters for Ba^{2+} expressed as $\exp(-[b_{11}h^2 + b_{22}k^2 + b_{33}l^2 + b_{12}hk + b_{13}hl + b_{23}kl])$: b_{11}, 287 (4); b_{22}, 135 (4); b_{33}, 208 (4); b_{12}, −31 (17); b_{13}, 137 (6); b_{23}, 56 (16, all × 10^5)). [b] The y coordinate of the barium ion was held constant to determine the origin in the polar space group, $P2_1$.

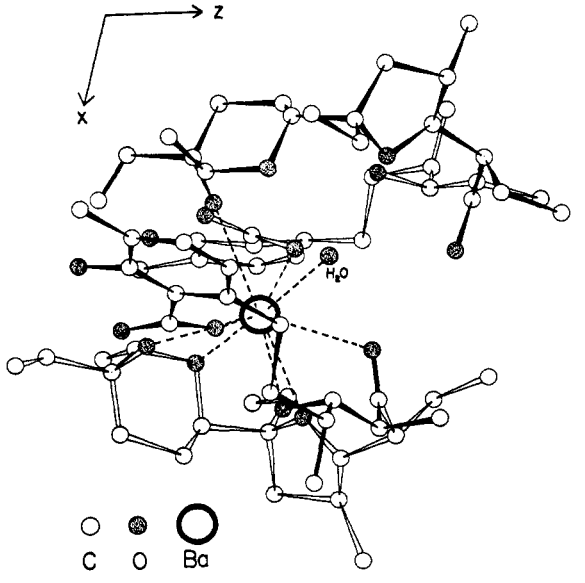


Figure 1. View of the structure of $Ba(C_{34}H_{53}O_8)_2 \cdot H_2O$ along the y axis.

2.64 and 2.84 Å from the metal ion, while the water molecule (O_w) is 2.74 Å distant. The Ba^{2+}---$O_{27'}$ distance (3.45 Å) is the only other metal ion–oxygen distance less than 4 Å. It is appropriate, therefore, to consider the barium ion as 9-coordinate. Metal coordination to oxygen atoms in pyranose rings has been found in the cyclohexaamylose–potassium acetate complex.[20] While, as will be discussed below, the conformations of the two antibiotic anions are quite similar, there is a major difference in their position relative to the metal ion. A "pocket" is created for the water molecule in the primed antibiotic molecule, involving $O_{31'}$, $O_{33'}$, $O_{15'}$, $O_{20'}$, and $O_{40'}$. The two antibiotic molecules are held together by virtue of coordination to the metal ion and by the water molecule possibly forming a hydrogen bond to oxygen atoms in each molecule; there is no intracomplex intermolecular H-bonding. (We reserve the term "intramolecular" for effects within an antibiotic anion.) A stereoscopic view of the barium coordination is shown in Figure 5.

(20) A. Hybl, R. E. Rundle, and D. E. Williams, *J. Amer. Chem. Soc.*, 87, 2779 (1965).

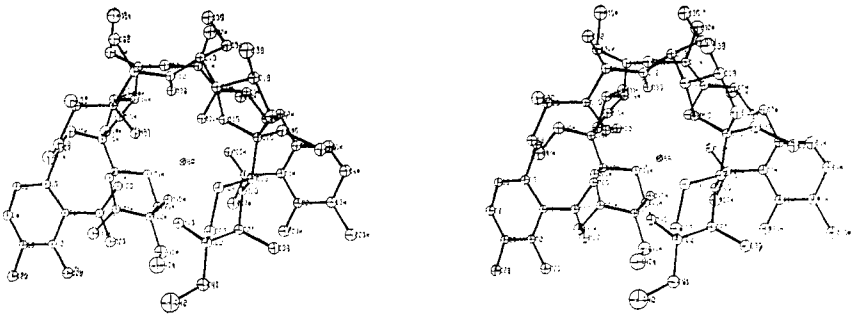


Figure 2. Stereoscopic picture of the complex viewed along the x axis. Atoms indicated by primes in text are represented by asterisks in this and the other stereo drawings.

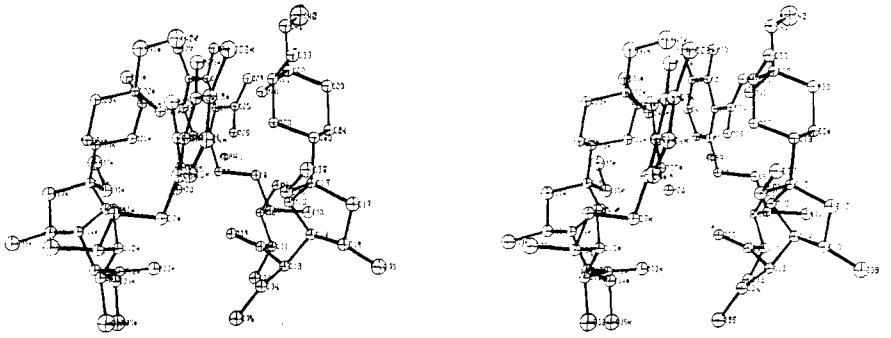


Figure 3. Stereoscopic picture of the complex viewed along the y axis.

Table II. Various Intracomplex and Intramolecular Distances

Ba^{2+}---O distances			
Ba---O_{26}	2.81 (2)	Ba---$O_{26'}$	2.64 (2)
Ba---O_{31}	3.08 (2)	Ba---$O_{27'}$	3.45 (2)
Ba---O_{33}	2.80 (2)	Ba---$O_{40'}$	2.84 (2)
Ba---O_{15}	2.98 (2)	Ba---O_w	2.74 (2)
Ba---O_{20}	2.86 (2)		
Ba---O_{40}	2.71 (1)		
O---O distances			
O_{27}---O_{28}	2.49 (3)[a]	$O_{27'}$---O_{28}	2.41 (3)[a]
O_{27}---O_{40}	2.72 (3)[a]	$O_{27'}$---$O_{40'}$	2.77 (3)[a]
O_{26}---O_{40}	2.82 (2)	$O_{26'}$---O_{40}	3.27 (2)
O_{26}---O_{31}	2.62 (2)[a]	$O_{26'}$---$O_{31'}$	2.87 (2)[a]
O_{31}---O_{33}	2.92 (2)	$O_{31'}$---$O_{33'}$	3.11 (3)
O_{31}---O_{15}	3.35 (3)	$O_{31'}$---$O_{15'}$	3.66 (3)
O_{33}---O_{15}	2.87 (2)	$O_{33'}$---$O_{15'}$	3.35 (3)
O_{15}---O_{20}	2.84 (2)	$O_{15'}$---$O_{20'}$	2.87 (2)
O_{20}---O_{40}	2.72 (2)	$O_{20'}$---O_{40}	2.84 (2)
O_{20}---$O_{26'}$	3.49 (2)	O_{40}---$O_{40'}$	3.78 (2)
O_{20}---$O_{27'}$	3.51 (2)		
O_{33}---$O_{33'}$	3.09 (3)		
O_{33}---$O_{26'}$	3.94 (2)		
O_{26}---$O_{40'}$	3.61 (2)		
Water---O distances			
O_w---O_{26}	3.17 (2)	O_w---$O_{33'}$	3.20 (3)[a]
O_w---O_{31}	3.81 (2)	O_w---$O_{15'}$	2.87 (2)
O_w---O_{33}	2.84 (2)	O_w---$O_{20'}$	2.80 (2)[a]
O_w---$O_{26'}$	3.95 (2)	O_w---$O_{40'}$	3.17 (2)
O_w---$O_{31'}$	3.81 (3)		

[a] Denotes probable hydrogen bond (see text).

Previously reported Ba^{2+}---O distances for ninefold coordination lie in the range 2.67–3.14 Å.[19,21]

There are three hydroxyl groups in each antibiotic anion, and these all seem to be involved in intramolecular hydrogen bonding. The hydroxyl group on the phenyl ring *ortho* to the carboxylate group forms a hydrogen

(21) Ranges of Ba^{2+}---O distances found in various structures have been tabulated by M. Nardelli and G. Fava, *Acta Cryst.*, **15**, 477 (1962).

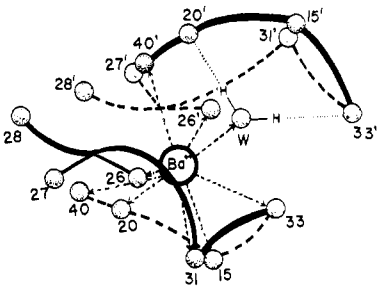


Figure 4. Schematic drawing of the coordination of barium. Only the oxygen atoms of the antibiotic molecules are shown.

bond of the type found in salicylic acid.[22] In salicylic acid, the carboxyl group is almost coplanar (1.1°) with the phenyl ring. In the two molecules in the present investigation, there is a rotation of the carboxyl group from the plane of the phenyl ring by 24–25°. These large angles of rotation are probably due to nonbonded interactions between the carboxyl group and the carbon substituent (*i.e.*, C_7 and bonded atoms) at the other *ortho* position. A rotation of the carboxyl group from the plane of the phenyl ring by 18° was found in the case of *o*-bromobenzoic acid.[23] In the unprimed anion, O_{26}, O_{27}, and O_{28} lie −0.58, 0.28, and 0.09 Å from the best plane through the phenyl ring, while in the primed anion the corresponding distances are −0.68, 0.15, and 0.12 Å. Rotation of the carboxyl group places O_{27} and O_{28} on the same side of the ring, and minimizes the C_7---O_{26} overcrowding by placing O_{26} at least 0.50 Å from the plane of the ring; the two C_7---O_{26} distances are 2.76 and 2.85 Å. The nonbonded interactions

(22) M. Sundaralingam and L. H. Jensen, *Acta Cryst.*, **18**, 1053 (1965); W. Cochran, *ibid.*, **6**, 260 (1953).

(23) G. Ferguson and G. A. Sim, *ibid.*, **15**, 346 (1962).

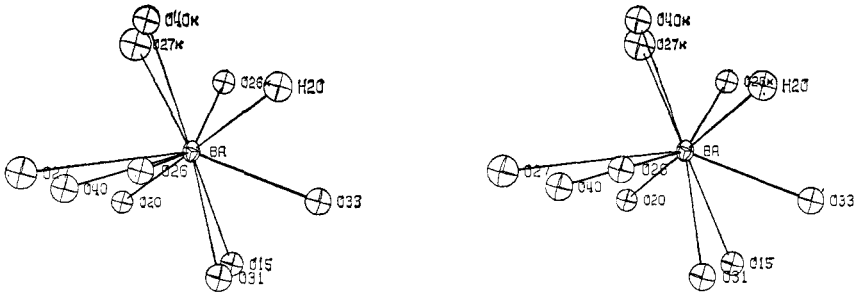


Figure 5. Stereoscopic view of the coordination to Ba^{2+} although O_{27} and $O_{27'}$ are not directly coordinated to Ba^{2+}, they are shown in this picture.

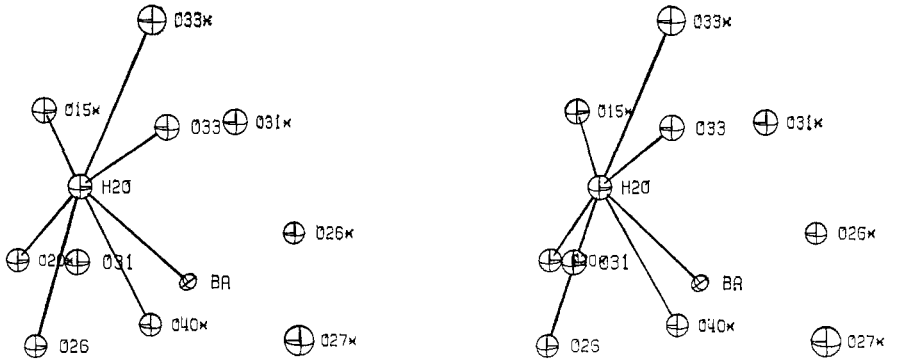


Figure 6. Stereoscopic view of the immediate environment of the water molecule. O_w---O distances <3.5 Å are shown by solid lines.

cause a contraction of the $C_2C_1C_{25}$ and $C_1C_{25}O_{27}$ angles and result in O_{27}---O_{28} distances of 2.49 and 2.41 Å, as compared to 2.62 Å in salicylic acid.[22] The shorter O---O distance compared to that in salicylic acid implies a stronger hydrogen bond. Such a trend in 3,6-disubstituted salicylic acids had been proposed on the basis of an infrared study of the C=O and OH stretching frequencies.[24] The C_2O_{28}---O_{27} angles are 88 and 87° while the corresponding angle in salicylic acid is 84.9°.[22] The anionic nature of the carboxyl group in the present molecule would also lead one to anticipate a stronger hydrogen bond than in salicylic acid.

The hydroxyl group $O_{31}H$ is almost certainly involved in intramolecular hydrogen bonding to O_{26} in each molecule. The O_{31}---O_{26} and $O_{31'}$---$O_{26'}$ distances are 2.62 and 2.87 Å, respectively, and the $C_{10}O_{31}$---O_{26} and $C_{10'}O_{31'}$---$O_{26'}$ angles are each 101°. The only other geometrically possible hydrogen acceptor for O_{31} would be O_{15}, yet the O_{15}---O_{31} distances in the two molecules are 3.35 and 3.65 Å.

The final intramolecular hydrogen bond involves $O_{40}H$ and the carboxyl group. In the unprimed molecule, the O_{40}---O_{27} and O_{40}---O_{26} distances are 2.72 and 2.82 Å, respectively, while the $C_{22}O_{40}$---O_{27} and $C_{22}O_{40}$---O_{26} angles are 126 and 163°. Thus, O_{27} is the more probable hydrogen acceptor. In the primed molecule, the $O_{40'}$---$O_{27'}$ distance is 2.77 Å, while the $O_{40'}$---$O_{26'}$ length is 3.27 Å. The $C_{22'}O_{40'}$---$O_{27'}$ angle is 124°.

The hydrogen bonds between O_{31} and O_{26} and between O_{40} and O_{27} constrain both antibiotic molecules in a circular conformation, and the high degree of rotational flexibility in this molecule permits most of the remaining oxygen atoms to be directed inward toward the center of the circle. A somewhat similar circular conformation imposed by "head-to-tail" hydrogen bonding is found for the monensic acid anion in the Ag^+ salt studied by X-ray methods.[4] Inspection of a figure presented for the Ag^+ salt of nigericin[8] also suggests a similar conformation stabilized by intramolecular hydrogen bonding.

The water molecule (O_w) is surrounded by six oxygen atoms (O_{26}, O_{33}, $O_{15'}$, $O_{20'}$, $O_{40'}$, and $O_{33'}$) with O_w---O distances less than 3.5 Å (Figure 6 and Table II); the shortest distances are O_w---O_{33}, O_w---$O_{15'}$, and O_w---$O_{20'}$, which are 2.84, 2.87, and 2.80 Å, respectively. The most reasonable hydrogen bonding scheme appears to be either $O_{15'}$---HO_wH---O_{33} (OO_wO angle 128°) or $O_{20'}$---HO_wH---$O_{33'}$ (angle 126°). The first possibility involves shorter O---O_w distances (2.87 and 2.84 Å), and would represent an additional force bringing the two antibiotic molecules together, although the occurrence of hydrogen bonds along the edges of metal ion coordination polyhedra is considered unlikely by several authors.[25,26] There seems little doubt, however, that an exception to this rule occurs in the case of the $O_{31}H$---O_{26} hydrogen bond. Furthermore, the Ba^{2+}---O_w---O_{33} angle is only 60°, which would represent a considerable distortion from the geometries normally found for water molecules which both coordinate to metal ions and form hydrogen bonds to other oxygen atoms.[27,28] The alternative scheme involves an O_w---$O_{33'}$ distance of 3.20 Å, which would correspond to a relatively weak hydrogen bond.[26] This latter scheme

(24) C. J. W. Brooks, G. Eglinton, and J. F. Morman, *J. Chem. Soc.*, 661 (1961).

(25) D. H. Templeton, *Acta Cryst.*, **13**, 684 (1960).
(26) J. R. Clark, *Rev. Pure Appl. Chem.*, **13**, 50 (1963).
(27) W. C. Hamilton in "Structural Chemistry and Molecular Biology," A. Rich and N. Davidson, Ed., W. H. Freeman and Co., San Francisco, Calif., 1968, pp 466–483.
(28) R. Chidambaram, A. Sequeira, and S. K. Sikka, *J. Chem. Phys.*, **41**, 3616 (1964).

involves angles Ba^{2+}---O_w---$O_{20'}$, Ba^{2+}---O_w---$O_{33'}$, and $O_{20'}$---O_w---$O_{33'}$ of 110, 107, and 126°, respectively. These angles suggest a slightly closer approach to a planar–trigonal coordination for water than to a tetrahedral coordination.[27] O_w lies 0.71 Å out of the plane defined by Ba^{2+}, $O_{20'}$, and $O_{33'}$. The distances between atoms not involved in hydrogen bonding would be consistent with the intermolecular contact radii for various bonded states of oxygen given by Bondi.[29] Oxygen atoms in furanose rings do not usually form hydrogen bonds,[30-32] although they are often involved in short contacts,[30-33] which may imply a residual interaction.[31] This property of furanose rings probably results from some double bond character in the CO bonds associated with the $O_2C<$ or $O(N)C<$ group of atoms and would not therefore apply to the tetrahydrofuran ring in the present structure.

An arrangement involving bifurcated hydrogen bonds[34] for both hydrogen atoms of the water molecule is also possible; one H atom would interact with $O_{15'}$ and $O_{20'}$, while the other interacts with $O_{33'}$ and O_{33}. The bifurcated assignment would have the desirable effect of removing a hydrogen atom from the edge of the coordination polyhedron of barium. While we are unable to make a definite assignment of the hydrogen bonding involving water, we have indicated the $O_{20'}$---O_w---$O_{33'}$ scheme in Figure 4.

In view of the differences in coordination to Ba^{2+}, the conformations of the two antibiotic molecules are remarkably similar. Some of the torsional angles along the bonds comprising the main chain of the molecule are listed in Table III. Only in the neighborhood of the five-membered ring do these angles differ by more than 10°. The difference in orientation of this ring is probably dictated by the direct coordination to Ba^{2+} in the unprimed molecule as compared to the interposition of the water molecule in the case of the primed molecule. The conformation of the five-membered rings in the two molecules can be described approximately as an "envelope,"[35] with C_{18} and $C_{18'}$ lying 0.50 and 0.66 Å, respectively, out of the best plane through the other four atoms of each ring. In a recent survey of the conformations of the furanose rings in various crystals, Sundaralingam[36] finds envelope conformations with one of the two carbon atoms not adjacent to oxygen being twisted out of the ring by between 0.53 and 0.66 Å. The details of the ring conformations are best described by the torsion angles around the ring as indicated in Table IV, where a comparison is made with the conformation of a somewhat similarly substituted tetrahydrofuran ring is ophiobolin methoxybromide,[37] and with the furanose ring in sucrose.[38]

(29) A. Bondi, *J. Phys. Chem.*, **68**, 441 (1964).
(30) M. Sundaralingam, *Biopolymers*, **6**, 189 (1968).
(31) E. Shefter, M. Barlow, R. A. Sparks, and K. N. Trueblood, *Acta Cryst.*, **B25**, 895 (1969).
(32) K. N. Trueblood, P. Horn, and V. Luzzati, *ibid.*, **14**, 965 (1961).
(33) E. Shefter and K. N. Trueblood, *ibid.*, **18**, 1067 (1965).
(34) See, for example, J. Donohue in "Structural Chemistry and Molecular Biology," A. Rich and N. Davidson, Ed., W. H. Freeman and Co., San Francisco, Calif., 1968, pp 443–465.
(35) E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, "Conformational Analysis," Interscience Publishers, New York, N. Y., 1965, pp 200–206.
(36) M. Sundaralingam, *J. Amer. Chem. Soc.*, **87**, 599 (1965).
(37) M. Morisaki, S. Nozoe, and Y. Iitaka, *Acta Cryst.*, **B24**, 1293 (1968).
(38) These values were taken from the results of G. M. Brown and H. A. Levy, *Science*, **141**, 921 (1963), as quoted by Sundaralingam in ref 36, wherein a number of other five-membered furanose rings are also described.

Table III. Torsion Angles (τ) which Describe the Conformation of the Main Chain of the Two Molecules[a]

A–B–C–D	τ, deg (unprimed)	τ, deg (primed)
C_1–C_6–C_7–C_8	−70	−74
C_6–C_7–C_8–C_9	176	172
C_7–C_8–C_9–C_{10}	−60	−67
C_8–C_9–C_{10}–C_{11}	180	−174
C_9–C_{10}–C_{11}–C_{12}	179	177
C_{10}–C_{11}–C_{12}–C_{13}	−148	−133
C_{11}–C_{12}–C_{13}–C_{14}	92	78
C_{12}–C_{13}–C_{14}–O_{15}	56	80
C_{12}–C_{13}–C_{14}–C_{18}	178	−167
C_{13}–C_{14}–C_{18}–C_{17}	−155	−158
C_{14}–C_{18}–C_{17}–C_{16}	34	40
C_{18}–C_{17}–C_{16}–O_{15}	−26	−22
C_{17}–C_{16}–O_{15}–C_{14}	6	−6
C_{16}–O_{15}–C_{14}–C_{18}	15	32
O_{15}–C_{14}–C_{18}–C_{17}	−31	−45
C_{18}–C_{17}–C_{16}–C_{19}	−148	−140
C_{17}–C_{16}–C_{19}–O_{20}	−176	−178
C_{16}–C_{19}–O_{20}–C_{21}	176	172
C_{19}–O_{20}–C_{21}–C_{22}	65	60
O_{20}–C_{21}–C_{22}–C_{23}	−57	−50
C_{21}–C_{22}–C_{23}–C_{24}	51	49
C_{22}–C_{23}–C_{24}–C_{19}	−53	−56
C_{23}–C_{24}–C_{19}–O_{20}	58	62
C_{24}–C_{19}–O_{20}–C_{21}	−65	−67

[a] The angle is designated positive if, when viewed from B to C, atom A has to be rotated clockwise to eclipse atom D.

The six-membered rings adopt the chair conformation in both molecules, with O_{20} and C_{23} lying −0.71 and +0.65 Å, and $O_{20'}$ and $C_{23'}$ lying −0.65 and +0.64 Å out of the best planes through the other four atoms in the ring. In both molecules, C_{39} and O_{40} are *axial*, while C_{16} and C_{41} are *equatorial*. Even the orientations of the side chains are quite similar in the two molecules, *e.g.*, $\tau(C_{13}C_{16}C_{36}C_{37})$ is 154 and 160°, $\tau(C_{14}C_{13}C_{34}C_{35})$ is −179 and −160° in the two molecules, while the $C_{11}C_{32}$ and $C_{34}C_{35}$ bonds are aligned parallel to each other and perpendicular to the plane of the ketone group (C_{12}═O_{33}) in each case. The similarities in conformation can be recognized by the close approach to a noncrystallographic twofold axis relating the two molecules (for example, in Figure 2, this axis would be vertical). If, however, the position C_{42a} represents the major site for C_{42}, there is a very significant difference in the position of the terminal carbon atom of the ethyl group ($C_{41}C_{42}$) in the two molecules. The $C_{21}C_{22}C_{41}C_{42a}$ torsion angle is 179°, while that for $C_{21'}C_{22'}C_{41'}C_{42'}$ is −60°; the angle $C_{21}C_{22}C_{41}C_{42b}$ is −100°.

The Ba^{2+} ion approaches O_{20} in an *axial* direction, while the $Ba^{2+}\cdots O_{15}$ vector lies *equatorially*[35] with respect to the five-membered ring. In the primed molecule, the water molecule is positional *axially* with respect to $O_{20'}$ and *equatorially* with respect to $O_{15'}$. Donohue[34] has recently questioned that there are directional requirements for hydrogen bond acceptors, although Sundaralingam[30] has found a preferred orientation for hydrogen bond acceptors in pyranosides.

The low solubility of the barium salt in water and the high solubility in organic solvents can be understood in structural terms as virtually all the oxygen atoms are "buried" or "hidden" in the interior of the complex, and the exterior surface of the complex is hydrophobic. There is no interpenetration of side groups from the two antibiotic anions. The overall shape of the complex is

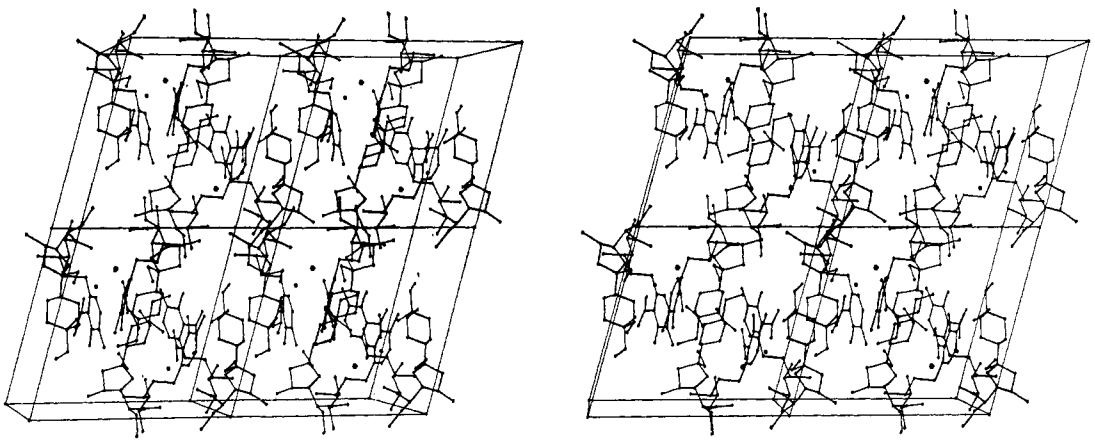


Figure 7. Stereoscopic view of the contents of four unit cells. The view is down the *b* axis, with the *a* axis horizontal and the *c* axis almost vertical. The origin is at the furthest corner of the upper left of the drawing. To avoid complicating the drawing the atom numbering is not shown, but the view of the molecules is the same as shown in Figure 3.

Table IV. Comparison of the Torsion Angles in the Tetrahydrofuran Rings in the Present Structure with Those in Ophiobolin Methoxybromide and Sucrose

	C_{18}–C_{14}[a]	C_{14}–O_{15}	O_{15}–C_{16}	C_{16}–C_{17}	C_{17}–C_{18}
Unprimed molecule	−31	15	6	−26	34
Primed molecule	−45	32	−6	−22	40
Ophiobolin methoxybromide[b]	−36	31	−12	−12	29
	(C_{15}–C_{14})	(C_{14}–O_1)	(O_1–C_{17})	(C_{17}–C_{16})	(C_{16}–C_{15})
Fructofuranosyl ring in sucrose[c]	−31	15	8	−27	35
	($C_{3'}$–$C_{2'}$)	($C_{2'}$–$O_{2'}$)	($O_{2'}$–$C_{5'}$)	($C_{5'}$–$C_{4'}$)	($C_{4'}$–$C_{3'}$)

[a] The numbering systems used in the studies of ophiobolin methoxybromide and sucrose are given in parentheses below the values for the torsion angles. [b] Calculated by us from data presented in ref 37. [c] Taken from Table VI in ref 36.

substantially distorted from a sphere with the axis in the direction normal to the antibiotic anion "circles" being compressed. Some important intermolecular contacts are listed in Table V, and Figure 7 shows some

Table V. Some Intermolecular Contacts (Å) Less Than 3.80 Å

Intracomplex Intermolecular Distances[a]			
C_{36}---$C_{1'}$	3.76	C_{21}---$O_{28'}$	3.53
C_{37}---$C_{4'}$	3.78	C_{39}---$O_{28'}$	3.35
C_{36}---$C_{6'}$	3.79	C_{35}---$O_{33'}$	3.63
C_1---$C_{21'}$	3.56	C_2---$C_{39'}$	3.61
C_{25}---$C_{21'}$	3.68	O_{27}---$C_{42'}$	3.63
O_{26}---$C_{21'}$	3.68	O_{40}---$C_{42'}$	3.72
O_{20}---$C_{25'}$	3.38	C_{42b}---$C_{42'}$	3.27[b]
C_{21}---$O_{27'}$	3.56	C_{42c}---$C_{42'}$	2.73[b]
Intercomplex Intermolecular Distances			
C_{35}---C_{42a}^{I}	3.55	C_{35}---C_8^{III}	3.71
$C_{35'}$---O_{28}^{I}	3.46	C_{36}---C_{32}^{III}	3.75
$C_{35'}$---$C_{42'}^{I}$	3.68	C_{37}---C_{32}^{III}	3.71
$O_{28'}$---$C_{37'}^{II}$	3.69	C_{17}---O_{28}^{IV}	3.51
$C_{29'}$---$C_{24'}^{II}$	3.73	C_{37}---C_{42a}^{IV}	3.40
C_{14}---C_9^{III}	3.78	C_{39}---C_8^{IV}	3.69

[a] This table does not include Ba---O and O---O contacts previously listed in Table II. I, $x, y, 1 + z$; II, $1 - x, 1/2 + y, 1 - z$; III, $2 - x, 1/2 + y, 2 - z$; IV, $2 - x, 1/2 + y, 1 - z$. [b] See Experimental Section for a discussion of these contacts.

aspects of the molecular packing in the crystal. Apart from the problems already referred to regarding C_{42}, the shortest intracomplex intermolecular distances are O_{33}---$O_{33'}$ 3.09 Å, O_{20}---$C_{25'}$ 3.38 Å, and C_{39}---$O_{28'}$ 3.35 Å and the shortest intercomplex distances are $C_{35'}$---O_{28}^{I} 3.46 Å and C_{17}---O_{28}^{IV} 3.51 Å.

Clearly this antibiotic represents another member of the general family to which the previously described monensic acid[4] and nigericin[7,8] belong. The high oxygen content, most of which is involved in complex formation, the general circular shape, and the mainly hydrophobic exterior are common features. Similarities also exist between this class of structures and the macrotetrolides which as neutral molecules can coordinate metal ions *via* ethereal and carbonyl oxygen atoms. The crystal structure of a macrotetrolide, the K^+ ion complex of nonactin,[39] and that of a cyclohexadepsipeptide, the K^+ ion complex of enniatin B,[40] have been described. In the latter structure, coordination of the depsipeptide to K^+ is entirely through oxygen. Model studies on different sizes of cyclic polyethers with the ability to complex various types of metal ions have also been reported,[41] and X-ray studies on some of these synthetic compounds are in progress.[42] Structural studies of the Ag^+ salt of X-537A are in progress in our laboratory.[43,44]

(39) B. T. Kilbourn, J. D. Dunitz, L. A. R. Pioda, and W. Simon, *J. Mol. Biol.*, **30**, 559 (1967); M. Dobler, J. D. Dunitz, and B. T. Kilbourn, *Helv. Chim. Acta*, **52**, 2573 (1969).
(40) M. Dobler, J. D. Dunitz, and J. Krajewski, *J. Mol. Biol.*, **42**, 603 (1969).
(41) C. J. Pedersen, *J. Amer. Chem. Soc.*, **89**, 7017 (1967); **92**, 386, 391 (1970).
(42) D. Bright and M. R. Truter, *Nature*, **225**, 176 (1970).
(43) C. A. Maier and I. C. Paul, work in progress.
(44) NOTE ADDED IN PROOF. Two further relevant structures have been reported; that of a K^+ complex of the cyclododecadepsipeptide, valinomycin (M. Pinkerton, L. K. Steinrauf, and P. Dawkins, *Biochem. Biophys. Res. Commun.*, **35**, 512 (1969)) and that of a Rb^+ "cryptate" (B. Metz, D. Moras, and R. Weiss, *Chem. Commun.*, 217, 572 (1970)).

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Carbon Isotope Effects on the Enzymatic Decarboxylation of Glutamic Acid[1,2]

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Abstract: Steady-state kinetic parameters and carbon isotope effects have been measured for the enzymatic decarboxylation of glutamic acid from pH 3.6 to 5.5. Over this range the maximum velocity changes by less than 50%, reaching a maximum at pH 4.4. The carbon isotope effect varies from a minimum of $k^{12}/k^{13} = 1.014$ at pH 4.0 to a maximum of 1.022 at pH 5.5. These data indicate that the energy barriers for decarboxylation and for formation of the enzyme–substrate complex are very similar. Decomposition of the enzyme–substrate complex to reform enzyme and substrate occurs with general base or general acid–specific base catalysis.

The enzymatic decarboxylation of amino acids usually requires the assistance of pyridoxal 5′-phosphate and proceeds according to the general mechanism of Scheme I.[4,5] Although this mechanism is qualitatively correct, it does not specify many important details of this sequence. Except for the binding of the coenzyme to an ε-amino group of a lysine residue of the enzyme,[6] little is known about the detailed role of the enzyme. Other catalytic groups are presumably involved, but this has not been demonstrated. As a start toward elucidation of this mechanism we have used isotope effects and steady-state kinetics to investigate the pH dependences and relative rates of the individual steps in the reaction sequence.

Carbon isotope effects have been used extensively in studies of organic reaction mechanisms.[7-11] Large isotope effects are observed only in cases where a bond to the isotopic atom is formed or broken in or before a slow step. Thus the decarboxylations of azulene-1-carboxylic acid[10] and of 4-methoxyanthranilic acid[11] show no carbon isotope effects at low acidity (protonation of the starting material is rate determining) and a "full" isotope effect ($k^{12}/k^{13} = 1.04$) at high acidity (decarboxylation is rate determining). The carboxyl carbon isotope effect on an enzymatic decarboxylation should provide information about the relative rate of the decarboxylation step in the overall reaction sequence. If decarboxylation is rate determining the reaction should exhibit a substantial carbon isotope effect.

Few carbon isotope effects on enzymatic reactions have been reported. Seltzer, Hamilton, and Westheimer[12] reported that there is no isotope effect on the enzymatic decarboxylation of oxalacetic acid, whereas the metal-catalyzed reaction shows an isotope effect (k^{12}/k^{13}) of 1.06. Studies of carbon isotope effects on the urease reaction[13] are not easily interpretable because of unexplained variations in the observed isotope effect with various enzyme preparations. Other studies[14] are deficient because of insufficient precision in the measurement of isotopic abundances.

Bacterial L-glutamate decarboxylase is a particularly suitable object for a detailed kinetic and isotope effect

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