Crystal and Molecular Structure of Kromycin

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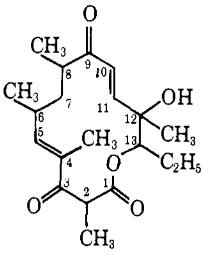
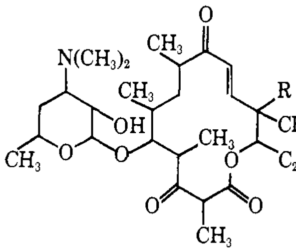
Abstract: Kromycin, the anhydro aglycone of the macrolide antibiotic, pikromycin, crystallizes in the space group $P2_12_12_1$ with $a = 8.218 \pm 0.007$, $b = 11.92 \pm 0.01$, $c = 21.93 \pm 0.02$ Å, $\rho_{\text{calcd}} = 1.084$ g cm^{-3}, and $Z = 4$. A total of 1539 independent diffraction intensities were recorded with Mo Kα radiation on a four-circle diffractometer. The crystal structure was solved by direct methods using a multiple solution procedure. Full-matrix, least-squares refinement yielded a conventional residual, $R = 0.088$. Kromycin has a 14-membered lactone ring structure containing two double bonds in trans configurations. One at C_{10}–C_{11} is a common structural feature in pikromycin, narbomycin, and methymycin. The second, at C_4–C_5, results from the removal of the glycone at C_5 in pikromycin. The absolute chiralities of the five asymmetrically substituted carbon atoms are unambiguously assigned.

Kromycin is the anhydro aglycone of the macrolide antibiotic pikromycin; the latter has long been thought[1,2] to contain a 12-membered lactone ring structure differing from methymycin[3] only in the location of the attached desosamine. It was recently proposed[4] that kromycin has structure 1 and that pikromycin (2) is a hydroxylated narbomycin (3) containing a 14-membered ring. More recently, we presented a brief report[5] of an X-ray diffraction study of kromycin that fully corroborated structure 1 and provided a complete configurational and conformational picture of the molecule. We provide herein a detailed account of the results of this study.



1



2, R = OH
3, R = H

Experimental Section

A sample of kromycin was prepared from pikromycin and purified by Muxfeldt.[4] Weissenberg and precession photographs of crystals obtained from ethyl ether by slow evaporation displayed orthorhombic symmetry and systematic extinctions which uniquely corresponded to the space group $P2_12_12_1$. The lattice constants, determined from a least-squares refinement of the setting angles of 40 reflections with Mo Kα radiation on a Picker FACS-I four-circle diffractometer, are $a = 8.218 \pm 0.007$, $b = 11.92 \pm 0.01$, and $c = 21.93 \pm 0.02$ Å and the calculated density is $\rho_{\text{calcd}} = 1.084$ g cm^{-3} ($\rho_{\text{obsd}} = 1.08$ g cm^{-3}) for $C_{20}H_{30}O_3$ with $Z = 4$.

The diffraction intensities of a 0.38 × 0.30 × 0.19 mm parallelepiped crystal were measured on the diffractometer in a θ–2θ scan mode with Mo Kα radiation, monochromated with a highly oriented graphite crystal. Each scan (1°/min) included a variable increment in angle above the 2.0° minimum to allow for spectral dispersion; background counts of 40-sec duration were taken at both limits of each scan. Of the 1737 independent reflections measured ($2\theta < 50.7°$), a total of 1539 were retained as objectively observed with the criterion $|F_o| \geq 1.35\sigma_F$. The standard deviation was obtained from $\sigma_F{}^2 = (C + k^2B)/(4|F_o|^2L_p{}^2) + (0.025 \cdot |F_o|)^2$, wherein C is the total count from the scan, k is the ratio of the scanning time to the total background counting time for the total background count B, and L_p is the Lorentz polarization correction. In order to monitor alignment and possible deterioration of the crystal, three standard reflections were measured at intervals of 100 reflections throughout the 7-day data collection period; a slow monotonic decrease of nearly 10% of initial intensity was observed in all three reflections. A least-squares analysis of this linear decay provided individual correction factors that were applied to each reflection in the data set. Lorentz polarization corrections were also applied, but test calculations revealed that neither absorption nor extinction corrections were required.

Structure Determination and Refinement. The structure was solved by direct methods[6,7] using a computerized multiple solution procedure and tangent formula[8] phase refinement. From a total of 154 reflections with $|E| \geq 1.4$, the phase determination was initiated with the starting sets of phases for six reflections as listed in Table I. Sixteen different starting phase combinations were expanded in a computer formalism using the relation $\varphi_h = \langle\varphi_k + \varphi_{h-k}\rangle$ and the tangent formula. The resulting expanded sets of phased normalized structure factors, $|E_h|$, were tested against a consistency index,[9] $\langle C\rangle$, a statistical index,[6] $\langle\alpha\rangle$, and a residual index,[6] R, where

$$\langle C\rangle = \langle(A^2 + B^2)^{1/2}/\Sigma|E_kE_{h-k}|\rangle$$

$$\langle\alpha\rangle = \langle 2\sigma_3\sigma_2{}^{-3/2}|E_h|(A^2 + B^2)^{1/2}\rangle$$

and

$$R = (\Sigma||E_h| - |E_{h\ \text{calcd}}||)/\Sigma|E_h|$$

In these expressions

$$|E_{h\ \text{calcd}}| = [(\langle A\rangle^2 + \langle B\rangle^2)\Sigma|E_h|^2/\Sigma(\langle A\rangle^2 + \langle B\rangle^2]^{1/2}$$

$$A = \Sigma|E_kE_{h-k}\cos(\varphi_k + \varphi_{h-k})|$$

Table I. Phase Assignments for Starting Set of Reflections

h	k	l	$\|E\|$	Phase, radians
7	3	0	2.54	$-\pi/2$ origin fixing
0	3	1	2.19	$\pi/2$ origin fixing
3	4	0	1.86	$-\pi/2$ origin fixing
0	7	3	2.25	$\pm\pi/2$
1	1	1	1.88	$\pm\pi/2$
2	2	8	1.80	$\pm\pi/4$, $\pm3\pi/4$

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[1] H. Brockmann and R. Oster, *Chem. Ber.*, 90, 605 (1957).

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[3] C. Djerassi and J. A. Zderic, *J. Amer. Chem. Soc.*, 78, 6390 (1956).

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[6] J. Karle and I. L. Karle, *Acta Crystallogr.*, **21**, 849 (1966).

[7] J. Karle, *ibid.*, *Sect. B*, 24, 182 (1968).

[8] J. Karle and H. Hauptman, *ibid.*, **9**, 635 (1956).

[9] M. G. B. Drew, D. H. Templeton, and A. Zalkin, *ibid.*, *Sect. B*, **25**, 261 (1969).

Table II. Fractional Atomic Coordinates for Oxygen and Carbon Atoms in Kromycin[a]

Atom	10^4x	10^4y	10^4z
O_1	2625 (4)	2940 (3)	3103 (3)
O_{1a}	335 (5)	2098 (4)	2772 (2)
O_{3a}	−1992 (5)	4234 (4)	2246 (2)
O_{9a}	5710 (10)	398 (8)	924 (3)
O_{12a}	5060 (5)	242 (4)	3184 (2)
C_1	1190 (7)	2958 (5)	2832 (2)
C_2	717 (7)	4178 (5)	2632 (3)
C_3	−561 (8)	4060 (5)	2125 (3)
C_4	−48 (7)	3669 (5)	1515 (3)
C_5	1481 (7)	3801 (5)	1321 (3)
C_6	2206 (8)	3514 (5)	707 (3)
C_7	3663 (8)	2697 (6)	788 (3)
C_8	3268 (9)	1478 (7)	1004 (3)
C_9	4717 (11)	892 (8)	1253 (4)
C_{10}	5055 (10)	868 (9)	1900 (4)
C_{11}	4362 (8)	1456 (5)	2322 (3)
C_{12}	4701 (8)	1428 (6)	2997 (3)
C_{13}	3176 (7)	1829 (5)	3353 (3)
C_{2a}	66 (9)	4847 (6)	3199 (3)
C_{4a}	−1395 (8)	3137 (8)	1136 (4)
C_{6a}	2704 (10)	4698 (7)	391 (3)
C_{8a}	2534 (14)	683 (8)	493 (4)
C_{12a}	6199 (8)	2150 (7)	3153 (4)
C_{13a}	3418 (10)	2039 (7)	4040 (3)
C_{13b}	1804 (12)	2204 (8)	4385 (3)

[a] Estimated standard deviations (in parentheses) correspond to the last significant digits.

Table III. Thermal Parameters for Oxygen and Carbon Atoms in Kromycin

A. For Atoms Refined with Anisotropic Thermal Parameters[a]

Atom	$10^4\beta_{11}$	$10^4\beta_{22}$	$10^4\beta_{33}$	$10^4\beta_{12}$	$10^4\beta_{13}$	$10^4\beta_{23}$	B, Å^2 [c]
O_1	141 (7)	74 (3)	25 (1)	8 (5)	−18 (2)	0 (1)	3.97
O_{1a}	135 (7)	95 (4)	43 (1)	−3 (5)	−17 (3)	6 (2)	5.17
O_{3a}	139 (7)	132 (5)	4 (1)	32 (5)	5 (3)	8 (2)	5.47
O_{9a}	698 (26)	334 (13)	43 (2)	275 (16)	92 (6)	24 (4)	10.76
O_{12a}	157 (7)	98 (4)	42 (1)	12 (5)	−27 (3)	9 (2)	5.26
C_9	382 (22)	191 (11)	32 (2)	86 (14)	67 (7)	10 (4)	7.02
C_{10}	311 (19)	223 (12)	35 (2)	152 (14)	32 (6)	26 (5)	7.26
C_{2a}	296 (16)	123 (7)	25 (2)	80 (10)	9 (5)	−8 (3)	5.76
C_{4a}	172 (13)	197 (10)	37 (2)	−33 (10)	−12 (4)	−6 (4)	6.78
C_{6a}	311 (18)	169 (9)	28 (2)	−21 (12)	−9 (5)	2 (4)	7.22
C_{8a}	557 (30)	167 (10)	39 (2)	−91 (16)	48 (8)	−19 (4)	9.14
C_{12a}	139 (11)	130 (8)	59 (3)	−43 (9)	−16 (5)	8 (4)	6.25
C_{13a}	331 (19)	136 (8)	27 (2)	42 (12)	−23 (5)	10 (3)	6.54
C_{13b}	401 (21)	199 (11)	29 (2)	54 (15)	27 (6)	11 (4)	8.18

B. For Atoms Refined with Isotropic Thermal Parameters[b]

Atom	B, Å^2	Atom	B, Å^2	Atom	B, Å^2
C_1	3.66 (11)	C_5	4.63 (13)	C_{11}	4.79 (13)
C_2	3.99 (12)	C_6	4.93 (14)	C_{12}	4.74 (14)
C_3	4.23 (12)	C_7	5.31 (14)	C_{13}	4.52 (13)
C_4	4.40 (12)	C_8	6.18 (17)		

[a] The anisotropic thermal parameters are in the form $\exp[-(h^2\beta_{11} + k^2\beta_{22} + l^2\beta_{33} + 2hk\beta_{12} + 2hl\beta_{13} + 2kl\beta_{23})]$. [b] The isotropic thermal parameters are in the form $\exp[-B \sin^2 \theta/\lambda^2]$. [c] The isotropic equivalent of the anisotropic thermal parameters obtained from: $B = 4.0\,[V^2\det(\beta_{ij})]^{1/3}$.

and

$$B = \Sigma|E_kE_{h-k} \sin (\varphi_k + \varphi_{h-k})|$$

The set with the highest consistency index ($\langle C\rangle = 0.57$), the highest $\langle\alpha\rangle$ index ($\langle\alpha\rangle = 19.0$), and the lowest residual ($R = 0.27$) was clearly the best choice. This set, which corresponds to the starting phase sequence $-\pi/2$, $\pi/2$, $-\pi/2$, $\pi/2$, $-\pi/2$, $-3\pi/4$ in Table I, contained 145 statistically acceptable phases. Three cycles of tangent formula refinement with 279 reflections ($|E| \geq 1.3$) expanded this set to a total of 253 phase reflections. An E synthesis based upon these phases was structurally interpretable; coordinates of the thirteen highest peaks were used to calculate the initial phases for about 130 reflections with $|E| \geq 1.3$. This set was expanded with the tangent formula to 261 phases ($|E| \geq 1.3$) and all oxygen and carbon atom positions were clearly defined in the resulting E synthesis.

Three cycles of full-matrix, least-squares refinement[10] of the atomic coordinates and isotropic thermal parameters reduced $R_1 = (\Sigma||F_o| - |F_c||)/\Sigma|F_o|$ from 0.298 to 0.157 for 1066 unit weighted reflections. Atomic scattering factors were calculated for neutral atoms from the expansion formula and coefficients given by Cromer and Mann.[11] After additional refinement cycles, hydrogen atom positions were located by difference Fourier analysis; some of the methyl and ethyl hydrogens were poorly resolved. Final coordinates and thermal parameters for all carbon and oxygen atoms (Tables II and III) were determined from three final full-matrix refinement cycles minimizing $\Sigma w(|F_o| - |F_c|)^2$. Hydrogen atom positions were fixed and isotropic thermal parameters were assigned (Table IV); the thermal parameters of all oxygen atoms, all methyl and ethyl carbon atoms, and atoms C_9 and C_{10} were refined anisotropically. The final conventional residual for the 1539 reflections was $R_1 = 0.088$ and the weighted residual, $R_2 = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2]^{1/2}$, with $w = 1/\sigma_F{}^2$, was 0.080. The standard deviation of an observation of unit weight was 1.33. A final listing of observed and calculated structure factors is available.[12]

Results and Discussion

A perspective view of the structure of the kromycin molecule is presented in Figure 1. The labeling system is a simple extension of the formal ring labels in **1**. The stereographic conformation shown in Figure 2

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(10) Following W. R. Busing, K. O. Martin, and H. A. Levy, "ORFLS, A Fortran Crystallographic Least-Squares Program," ORNL-TM-305, Oak Ridge National Laboratory, Oak Ridge, Tenn, 1962. Estimated standard deviations in these distances and angles were calculated following W. R. Busing, K. O. Martin, and H. A. Levy, "OR-FFE, A Fortran Crystallographic Function and Error Program," ORNL-TM-306, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1964.

(11) D. T. Cromer and J. B. Mann, *Acta Crystallogr., Sect. A*, **24**, 321 (1968).

(12) A table of observed and calculated structure amplitudes from the final refinement, a table of all important nonbonded interatomic contacts in the crystal structure, and a stereographic packing drawing have been deposited as Document No. NAPS-01556 with the ASIS National Auxiliary Publications Service, c/o CCM Information Corp., 909 3rd Ave., New York, N. Y. 10022. A copy may be secured by citing the document number and by remitting $5.00 for photocopies or $2.00 for microfiche. Advance payment is required. Make check or money order payable to ASIS–NAPS.

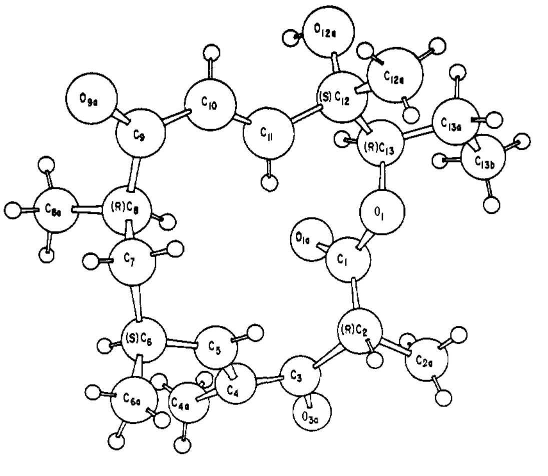

Figure 1. A perspective view of the structure of the kromycin molecule.

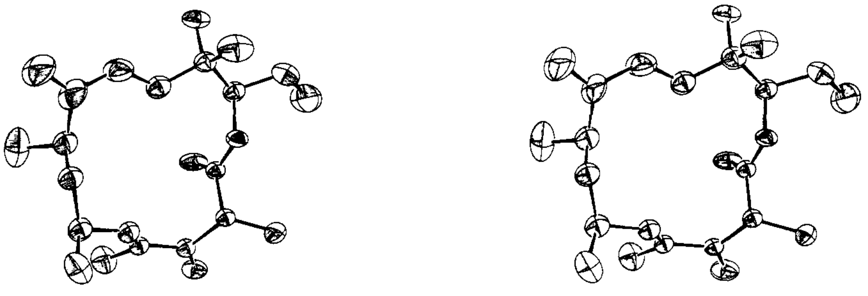

Figure 2. A stereoscopic representation of the conformation of kromycin.

was computer generated[13] from the atom coordinates; for clarity, hydrogen atoms have been eliminated.

All significant bond lengths, bond angles, and dihedral angles in the molecule are presented in Tables V, VI, and VII, respectively; they are accompanied by standard deviations derived from the least-squares analysis. For further reference, a complete listing of all of the shortest nonbonded interatomic contacts in the crystal structure is available;[12] the listing describes all intramolecular contacts shorter than 3.10 Å and all intermolecular contacts less than 3.70 Å.

The structure analysis completely corroborates all of the essential structural features deduced by Muxfeldt, *et al.*,[4] in a chemical and spectroscopic study; moreover, in addition to this confirmation of chemical composition, it provides a detailed description of the configurational and conformational properties of the molecule.

The absolute configuration of the (+)-lactonic acid produced upon oxidation of pikromycin has been determined chemically.[14] Since kromycin yields the same lactonic acid,[15] the structure analysis provides an absolute assignment of chirality to all asymmetric carbon atoms in the molecule. As is indicated in Figure 1, the chiral centers are (R)-C_2, (S)-C_6, (R)-C_8, (S)-C_{12}, and (R)-C_{13}. A detailed comparison[5] with similar results on erythromycin suggests that pikromycin and erythromycin have equivalent configurations at all common asymmetric centers. Moreover, chemical and spectroscopic evidence[4,15,16] indicates that kromycin and narbomycin (**3**) possess related configurations and, in large measure, identical elements of a rigid ring structure.

The double bond at C_{10}–C_{11} is in a trans configuration; the sequence C_9–C_{10}–C_{11}–C_{12} is quite planar (dihedral angle, 179.6°) but the bond angles $C_9C_{10}C_{11}$ (128.2°) and $C_{10}C_{11}C_{12}$ (128.4°) indicate a considerable degree of strain at this point in the ring. The conjugation between C_{10}–C_{11} and C_9–O_{9a} must surely be somewhat affected by ring strain; the average dihedral angle of 11.4° around C_9–C_{10} represents a considerable twist between the two planar sp^2 groups.

The double bond length (1.273 Å) is remarkably short (*cf.* a normal[17] value of 1.335 for a simple double bond and 1.36 Å for a double bond in a C═C—C═O sequence). In contrast to this inexplicable result, all of the associated bonds in this region of the ring are of normal length. Thus, on one side, the bond C_{11}–C_{12} (1.507 Å) is exactly the average length[17] for a single bond in the sequence C—C═C, and the C_{12}–O_{12a} bond length, 1.432 Å, precisely corresponds to the average[17] (1.426 Å) for a wide variety of alcohols. On the other side, the bond length of 1.447 Å for C_9–C_{10} is, once again, at the average value[17] (1.44 Å) for a single bond in a C═C—C═O sequence. Similarly, the carbonyl bond, C_9–O_{9a} (1.224 Å), is exactly at the average length[17] for the C═O bond in the same sequence. Finally, the single bond C_8–C_9 (1.468 Å) is slightly shorter than the expected value[17] (1.506 Å) but part of this difference may be due to a foreshortening effect of thermal vibrations which maximize at that point in the ring (*vide infra*).

Thus, there appears to be no simple explanation for the very short double bond at C_{10}–C_{11}. A number of additional refinement cycles were calculated in order to test the sensitivity of the results to changes in the refinement model. Neither alternative weighting schemes nor the inclusion of higher angle (lower quality) data nor alternative assignments of isotropic and anisotropic parameters resulted in any significant changes in the bond lengths or in the residuals.

The double bond at C_4–C_5 is also in a trans configuration; the bond exhibits a slight twist (the dihedral

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[13] C. K. Johnson, "ORTEP, A Fortran Thermal-Ellipsoid Plot Program for Crystal Structure Illustrations," ORNL-TM-3794, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965.

[14] C. Djerassi, O. Halpern, D. I. Wilkinson, and E. J. Eisenbraun, *Tetrahedron*, 4, 369 (1958).

[15] R. Anliker, D. Dvornik, K. Gubler, H. Heusser, and V. Prelog, *Helv. Chim. Acta*, 39, 1785 (1956).

[16] V. Prelog, A. M. Gold, G. Talbot, and A. Zamojski, *ibid.*, 45, 4 (1962).

[17] L. E. Sutton, Ed., "Tables of Interatomic Distances and Configurations in Molecules and Ions, Supplement 1956–1959," The Chemical Society, London, 1965, pp S14s–S23s.

Table IV. Fractional Atomic Coordinates for the Hydrogen Atoms with the Assigned Isotropic Thermal Parameters

Atom	10^3x	10^3y	10^3z	B, Å^2	Bonded to
H_1	403	−35	312	7.0	O_{12a}
H_2	169	467	249	5.0	C_2
H_3	238	437	158	5.0	C_5
H_4	142	300	48	5.0	C_6
H_5	429	283	38	5.0	C_7
H_6	453	306	107	5.0	C_7
H_7	246	164	136	7.0	C_8
H_8	595	30	210	7.0	C_{10}
H_9	347	216	215	5.0	C_{11}
H_{10}	253	112	328	5.0	C_{13}
H_{11}	110	490	351	7.0	C_{2a}
H_{12}	−75	430	345	7.0	C_{2a}
H_{13}	−50	545	300	7.0	C_{2a}
H_{14}	−85	278	78	7.0	C_{4a}
H_{15}	−187	402	100	7.0	C_{4a}
H_{16}	−223	259	140	7.0	C_{4a}
H_{17}	324	440	−2	7.0	C_{6a}
H_{18}	340	520	65	7.0	C_{6a}
H_{19}	165	512	45	7.0	C_{6a}
H_{20}	325	78	16	8.0	C_{8a}
H_{21}	228	22	55	8.0	C_{8a}
H_{22}	145	115	42	8.0	C_{8a}
H_{23}	595	300	315	8.0	C_{12a}
H_{24}	680	180	350	8.0	C_{12a}
H_{25}	723	217	280	8.0	C_{12a}
H_{26}	403	133	421	5.0	C_{13a}
H_{27}	425	270	410	5.0	C_{13a}
H_{28}	155	210	485	7.0	C_{13b}
H_{29}	115	310	425	7.0	C_{13b}
H_{30}	101	145	420	7.0	C_{13b}

Table V. Bond Lengths in Kromycin with Estimated Standard Deviations from Least-Squares Analysis

Bond	Length, Å	Bond	Length, Å
O_1–C_1	1.321 (6)	C_6–C_{6a}	1.560 (10)
O_1–C_{13}	1.443 (7)	C_7–C_8	1.491 (9)
O_{1a}–C_1	1.207 (7)	C_8–C_9	1.468 (11)
O_{3a}–C_3	1.222 (7)	C_8–C_{8a}	1.558 (11)
O_{9a}–C_9	1.224 (9)	C_9–C_{10}	1.447 (11)
O_{12a}–C_{12}	1.432 (8)	C_{10}–C_{11}	1.273 (9)
C_1–C_2	1.497 (8)	C_{11}–C_{12}	1.507 (8)
C_2–C_3	1.536 (8)	C_{12}–C_{13}	1.545 (9)
C_2–C_{2a}	1.550 (8)	C_{12}–C_{12a}	1.515 (9)
C_3–C_4	1.470 (8)	C_{13}–C_{13a}	1.538 (9)
C_4–C_5	1.335 (8)	C_{13}–C_{13b}	1.539 (12)
C_4–C_{4a}	1.510 (9)		
C_5–C_6	1.508 (8)	O_5–H_1	1.09
C_6–C_7	1.522 (9)	C–H average	1.05
		C–H range	0.93–1.18

Table VI. Bond Angles in Kromycin with Estimated Standard Deviations from Least-Squares Analysis

Atoms	Degrees	Atoms	Degrees
$C_1O_1C_{13}$	117.7 (4)	$C_7C_8C_9$	111.0 (6)
$O_1C_1O_{1a}$	123.8 (5)	$C_7C_8C_{8a}$	112.8 (6)
$O_1C_1C_2$	112.2 (5)	$C_9C_8C_{8a}$	108.8 (7)
$O_{1a}C_1C_2$	123.9 (5)	$C_8C_9C_{9a}$	116.3 (9)
$C_1C_2C_3$	108.0 (5)	$C_8C_9C_{10}$	121.9 (7)
$C_1C_2C_{2a}$	107.6 (5)	$C_{9a}C_9C_{10}$	121.8 (8)
$C_3C_2C_{2a}$	112.9 (5)	$C_9C_{10}C_{11}$	128.2 (7)
$C_2C_3O_{3a}$	119.0 (5)	$C_{10}C_{11}C_{12}$	128.4 (6)
$C_2C_3C_4$	119.3 (5)	$C_{11}C_{12}O_{12a}$	109.8 (5)
$O_{3a}C_3C_4$	121.6 (5)	$C_{11}C_{12}C_{13}$	109.9 (5)
$C_3C_4C_5$	121.8 (6)	$C_{11}C_{12}C_{12a}$	111.2 (6)
$C_3C_4C_{4a}$	114.2 (5)	$O_{12a}C_{12}C_{13}$	107.3 (5)
$C_5C_4C_{4a}$	124.0 (6)	$O_{12a}C_{12}C_{12a}$	105.7 (5)
$C_4C_5C_6$	129.3 (6)	$C_{13}C_{12}C_{12a}$	112.8 (5)
$C_5C_6C_7$	109.7 (5)	$C_{12}C_{13}O_1$	108.5 (5)
$C_5C_6C_{6a}$	108.4 (5)	$C_{12}C_{13}C_{13a}$	115.7 (6)
$C_7C_6C_{6a}$	111.4 (6)	$O_1C_{13}C_{13a}$	106.2 (5)
$C_6C_7C_8$	115.2 (6)	$C_{13}C_{13a}C_{13b}$	112.9 (7)

Table VII. Dihedral Angles[a] in Kromycin with Estimated Standard Deviations from Least-Squares Analysis

Atoms	Degrees	Atoms	Degrees
$O_1C_1C_2C_3$	158.3 (0.4)	$C_{8a}C_8C_9C_{10}$	−138.3 (0.9)
$O_1C_1C_2C_{2a}$	−79.6 (0.6)	$C_{8a}C_8C_9O_{9a}$	41.9 (1.1)
$O_{1a}C_1C_2C_3$	−24.1 (0.8)	$C_8C_9C_{10}C_{11}$	−11.3 (1.6)
$O_{1a}C_1C_2C_{2a}$	98.0 (0.6)	$O_{9a}C_9C_{10}C_{11}$	168.6 (1.0)
$C_1C_2C_3O_{3a}$	102.8 (0.6)	$C_9C_{10}C_{11}C_{12}$	179.6 (0.9)
$C_1C_2C_3C_4$	−73.5 (0.6)	$C_{10}C_{11}C_{12}C_{13}$	−155.7 (0.8)
$C_{2a}C_2C_3O_{3a}$	−16.1 (0.8)	$C_{10}C_{11}C_{12}O_{12a}$	−38.0 (1.0)
$C_{2a}C_2C_3C_4$	167.6 (0.5)	$C_{10}C_{11}C_{12}C_{12a}$	78.7 (1.0)
$C_2C_3C_4C_5$	−23.5 (0.9)	$C_{11}C_{12}C_{13}O_1$	−51.3 (0.6)
$C_2C_3C_4C_{4a}$	157.5 (0.6)	$C_{11}C_{12}C_{13}C_{13a}$	−170.5 (0.6)
$O_{3a}C_3C_4C_5$	160.3 (0.6)	$O_{12a}C_{12}C_{13}O_1$	−170.6 (0.4)
$O_{3a}C_3C_4C_{4a}$	−18.7 (0.9)	$O_{12a}C_{12}C_{13}C_{13a}$	70.2 (0.7)
$C_3C_4C_5C_6$	−176.6 (0.6)	$C_{12a}C_{12}C_{13}O_1$	73.4 (0.7)
$C_{4a}C_4C_5C_6$	2.3 (1.1)	$C_{12a}C_{12}C_{13}C_{13a}$	−45.8 (0.8)
$C_4C_5C_6C_7$	−125.4 (0.7)	$C_{12}C_{13}O_1C_1$	115.0 (0.5)
$C_4C_5C_6C_{6a}$	112.8 (0.8)	$C_{13a}C_{13}O_1C_1$	−119.9 (0.6)
$C_5C_6C_7C_8$	68.3 (0.7)	$C_{13}O_1C_1C_2$	176.0 (0.5)
$C_{6a}C_6C_7C_8$	−171.7 (0.6)	$C_{13}O_1C_1O_{1a}$	−1.6 (0.8)
$C_6C_7C_8C_9$	−161.9 (0.6)	$C_{12}C_{13}C_{13a}C_{13b}$	−169.1 (0.6)
$C_6C_7C_8C_{8a}$	75.8 (0.8)	$C_{13b}C_{13a}C_{13}O_1$	70.4 (0.8)
$C_7C_8C_9C_{10}$	97.1 (0.9)		
$C_7C_8C_9O_{9a}$	−82.7 (1.1)		

[a] An arbitrary but self-consistent set defined for the sequence a–b–c–d as the positive clockwise rotation from a to d in the projection of the array down the line b–c.

angle $C_3C_4C_5C_6$ is 176.6°) and the atoms C_3, C_4, C_5, and C_6 are at a mean distance of ±0.014 Å from a least-squares plane. Again, there is evidence of ring strain; the bond angle $C_4C_5C_6$ is 129.3° and the methyl group C_{4a} has bond angles of 124.0 and 114.2° at C_4. Conjugation between C_4–C_5 and the carbonyl group C_3–O_{3a} must be seriously affected by the average twist angle of 21.1° between the two sp^2 systems at C_3 and C_4.

The double bond at C_4–C_5 is precisely the average[17] length (1.335 Å) of a normal double bond and the carbonyl group C_3–O_{3a} is also of normal[17] length (1.222 Å). The bond C_3–C_4 (1.470 Å) has an appropriate length for a single bond in a somewhat twisted sequence C=C—C=O. Similarly, C_5–C_6 (1.508 Å) is exactly the right length for a single bond next to a double bond.

The ester group at C_1 has normal bond lengths and bond angles and it links into the ring through normal bonds. In fact, all other ring bonds are normal except for C_7–C_8 which is curiously short (1.491 Å); the associated bond angle, C_6–C_7–C_8, is mildly strained at 115.2°. The average length of all C–CH_3 bonds in the structure is 1.54 Å.

The molecular packing in the crystal structure has no unusual features; a stereographic representation of the structure is available.[12] There are no abnormally short intermolecular or intramolecular nonbonded contacts. The shortest intermolecular distances of each kind are O···O (2.92 Å), O···C (3.31 Å), C···C (3.70 Å), O···H (2.38 Å), C···H (2.97 Å), and H···H (2.25 Å); all of these contacts are close to the sum of the appropriate van der Waals contact distances. There is no evidence of hydrogen bonding at O_{12a}.

A detailed interpretation of the anisotropic thermal vibrations of a molecule as complex as kromycin would be of value only if exceptionally good data were available in a high data–parameter ratio. Nevertheless, the principal features of the molecular vibration are clear. The most rigid section of the ring is centered in the ester group at C_1; the thermal parameters at O_1, C_1, and C_2 are less than 4 Å^2, the lowest values in the structure. This observation supports the suggestion[4] that C_2 behaves like a bridgehead carbon atom in a relatively rigid ring. This proposal was advanced to explain the difficulty of deuterium exchange at C_2; the rigidity of the ring was indicated by the temperature independence of the nmr spectrum of the molecule.

The thermal parameters of the groups on either side of the ester group are also relatively low. In fact, all ring atoms in the sequence C_6, C_5, ..., C_{12}, C_{11} have values of B less than 5 Å^2. The parameters increase markedly at C_9 and C_{10} and, not unexpectedly, are even larger at the pendant atoms O_{9a} and C_{8a}. In a very rigid organic structure, atomic thermal parameters are about 2.0–3.5 Å^2, and in a very soft structure, values of B range from 6 to 12 Å^2 and even higher. Unfortunately, it is rarely possible to distinguish the effects of intramolecular vibrations and group oscillations from overall rigid-body libration of the molecule. In the present case, the evidence for moderate ring strain and the absence of strong intermolecular interactions indicates that the ring is fairly rigid and that it librates with a maximum amplitude of oscillation at O_{9a} and C_{8a}.

Correction of bond lengths for apparent foreshortening due to molecular vibration scarcely seems warranted in the present case. Typically, the corrections are about 0.01 Å; even if the effect is somehow curiously focused at C_{10}–C_{11}, it is doubtful that it would exceed 0.02 Å at this point. This would still leave this bond inexplicably short. Moreover, since all other bonds in that region of the molecule appear to be of normal length, large libration effects are not indicated.

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Crystal and Molecular Structure of 5,12a-Diacetyloxytetracycline

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Abstract: A crystal structure analysis of 5,12a-diacetyloxytetracycline has revealed a conformation of the tetracycline ring structure that differs markedly from the one observed in 5-hydroxytetracycline and in 7-chlorotetracycline. The principal difference between the two conformations involves a drastic twist of 108.9° about C_{4a}–C_{12a} and associated rotations about all contiguous bonds. The detailed geometries at C_4, C_{4a}, C_5, C_{5a}, and C_{12a} are consistent with the interpretation of recent nmr studies of oxytetracyclines in nonaqueous solvents. The structure was solved by direct methods analysis of data from a crystal with space group $P2_12_12_1$ and $a = 18.896 \pm 0.010$, $b = 14.229 \pm 0.007$, $c = 9.406 \pm 0.006$ Å, $Z = 4$, and density, $\rho_{\text{calcd}} = 1.43$ g cm^{-3}. An anisotropic least-squares refinement converged to a conventional residual of $R = 0.076$ for 3124 independent reflections recorded with Mo $K\alpha$ radiation on an automatic four-circle diffractometer.

The chemistry of tetracycline derivatives has been reviewed at length in a recent report.[1] The range of conformations accessible to the basic four-ring system and the relative stabilities of different conformers are matters of considerable importance in the formulation and interpretations of detailed reaction mechanisms. Although nmr[2] studies of tetracycline and oxytetracycline derivatives in solution suggest that more than one conformation exists, the crystal structures of two different derivatives were found to be virtually identical; a single molecular conformation emerged from the analyses of the isomorphous hydrochloride salt structures of 7-chlorotetracycline[3,4a,b] (Aureomycin[4c]) (**1**) and 5-hydroxytetracycline[5,6a] (Terramycin[6b]) (**2**) and, as well, from circular dichroism studies[7] in dilute aqueous solutions. Since the unit cell was clearly different for crystals of the free base, 5,12a-diacetyloxytetracycline[8] (**3**), the present study was undertaken with the expectation that a different molecular packing and a new molecular conformation would be revealed. Moreover, a large number of high quality diffraction data were accessible and this provided an opportunity to establish the mo-

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