roketal **3** (R_f 0.39, 62%): δ0.85 [d, 3, J = 6.8 Hz, 26a-CH_3], 0.92 [d, 3, J = 6.6 Hz, 24-CH_3], 0.96 [t, 3, J = 6.9 Hz, 28-CH_3], 1.27 [q, 1, J = 12 Hz, C18–$H_{(a)}$], 1.34 [t, 1, J = 12 Hz, C20–$H_{(a)}$], 1.40–2.15 [m, H on C16, C18(e), C20(e), C22, C24, C26, C27], 3.65 [dd, 1, J = 1.4 and 10.4 Hz, C25–H], 3.88 [m, H on C15, C17, and C23], 3.96 [tt, 1, J = 11 Hz; C19–H]; M^+ 302, m/e 284, 266, 257, 245, 239, 227, 221, 209, 187, 169, 161, and 111.

2 (20 mg) was stirred in 0.5 mL of 1% methanolic H_2SO_4. After 18 h, TLC (SiO_2, $CHCl_3$–CH_3OH 9:1) revealed three products of R_f 0.47, 0.46 (α- and β-methyl oleandroside), and 0.16. The solution was neutralized with solid $NaHCO_3$, filtered, and evaporated and the products were isolated by preparative TLC. By repeated chromatography, the epimeric 2-methyl pentanetriols could be separated. Tentative assignments of the 14*S* configuration to the minor and of the 14*R* configuration to the major isomer were based on Felkin's rules.[13] (14*S*)-Triol **4**: ^1H NMR [$CDCl_3$] δ 0.88 [d, 3, J = 7.0 Hz, 12a-CH_3], 1.22 [d, 3, J = 7.0 Hz, 14a-CH_3], 1.57 [OH], ~1.92 [m, C12–H], 2.53 [OH], 3.08 [OH], 3.61 [dd, 1, J = 3.7 and 7.3 Hz, C13–H], 3.69 [t, 1, J = 7.4 Hz, C11–H], ~3.95 [dq, 1, J = 4.1 and 6.2 Hz, C14–H]; M^+ 134.0936 (measured on tri-TMSi derivative), m/e 133, 116, 103, 89, 75, 71, 59, 45, and 32. (14*R*)-Triol **4**: ^1H NMR δ 0.99 [d, 3, J = 7.0 Hz, 12a-CH_3], 1.26 [d, 3, J = 7.0 Hz, 14a-CH_3], 1.57 [OH], 1.90 [m, C12–H], 2.53 [OH], 3.08 [OH], 3.35 [dd, 1, J = 3.0 and 5.6 Hz, C13–H], 3.70 [dd, 1, J = 3.7 and 10.7 Hz, C11–H], 3.79 [dd, 1, J = 3.7 and 10.7 Hz, C11–H], 3.91 [dq, 1, J = 3.1 and 6.2 Hz, C14–H].

(13) M. Cherest, H. Felkin, and N. Prudent, *Tetrahedron Lett.*, 2199–2204 (1968).

The Absolute Stereochemistry and Conformation of Avermectin B_{2a} Aglycon and Avermectin B_{1a}

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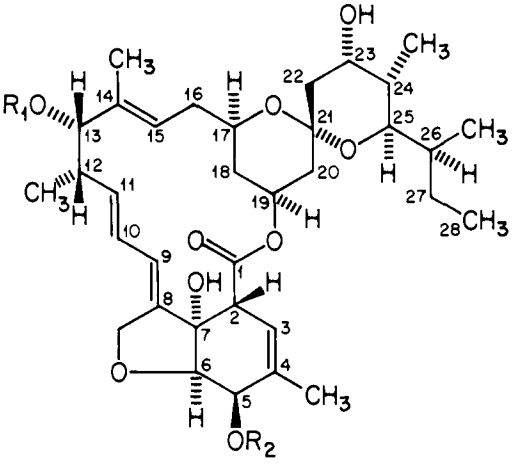
Contribution from the Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065. *Received April 9, 1979. Revised Manuscript Received February 6, 1981*

Abstract: The crystal structures of the potent antiparasitic agents avermectin B_{2a} aglycon (**1b**) [a = 15.061 (8), b = 9.005 (2), c = 14.624 (7) Å, β = 96.37 (4)°, $P2_1$, Z = 2, $C_{34}H_{50}O_9$] and avermectin B_{1a} (**3**) [a = 39.362 (13), b = 9.500 (3), c = 14.694 (2) Å, β = 106.43(2)°, $C2$, Z = 4, $C_{48}H_{72}O_{14}$] were solved to establish both the relative and absolute stereochemistry of these *Streptomyces* metabolites. Detailed ^1H NMR analyses showed that the solution conformation of the basic avermectin skeleton is virtually identical with the solid state conformation.

The avermectins are a previously undescribed series of compounds isolated from *Streptomyces avermitilis* with potent anthelmintic as well as ectoparasitic activity.[1] Initial isolation as well as structural and biological characterization indicated that at least eight related compounds possessed this remarkable activity. To unambiguously define the absolute stereochemistry as well as the conformation of the avermectins, single-crystal X-ray diffraction experiments of avermectin B_{2a} aglycon (**1b**) and avermectin B_{1a} (**3**) were undertaken.

Experimental and Methods

Avermectin B_{2a} Aglycon (1b). One of the major components, avermectin B_{2a} (**1a**), was subjected to acidic methanolic hydrolysis conditions to yield avermectin B_{2a} aglycon (**1b**).[1a] The aglycon ($C_{34}H_{50}O_9$) was recrystallized from methanol to give colorless crystals of symmetry $P2_1$ with cell unit dimensions of a = 15.061 (8), b = 9.005(2), and c = 14.624 (7) Å and β = 96.37 (4)°. The calculated density was 1.18 g/cm³ for



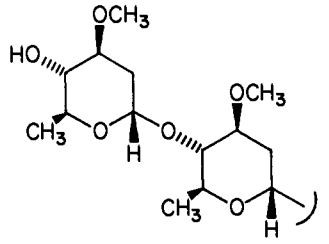
1a : R_1 = $C_{14}H_{25}O_6$ (disaccharide 2)
R_2 = H
b : R_1 = H; R_2 = H
c : R_1 = H; R_2 = CH_3

Z = 2 with six molecules of methanol in the unit cell (vide infra). A suitable crystal was mounted in a Lindemann glass capillary with mother liquor. Of the 2902 unique reflections measured ($2\theta \leq 115°$) with graphite monochromated Cu Kα radiation (λ = 1.5418 Å), 2510 (86%) were significant ($I \geq 3\sigma I$). These observed reflections were corrected for

(1) (a) Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlanta, Georgia, October, 1–4 1978. Avermectins: A new family of potent anthelmintic agents. Abstract No. 462: Producing Organisms and Fermentation, R. W. Burg, B. M. Miller, E. E. Baker, J. Birnbaum, S. A. Currie, R. Hartman, Y.-L. Kong, R. L. Monoghan, G. Olson, I. Putter, J. B. Tunac, H. Wallick, E. O. Stapley, R. Oiwa, and S. Omura. Abstract No. 463: Isolation and Chromatographic Properties, T. W. Miller, L. Chaiet, D. J. Cole, L. J. Cole, J. E. Flor, R. T. Goegelman, V. P. Gullo, A. J. Kempf, W. R. Krellwitz, R. L. Monoghan, R. E. Ormond, K. E. Wilson, G. Albers-Schonberg, and I. Putter. Abstract No. 464: Structure Determination, G. Albers-Schonberg, B. H. Arison, J. C. Chabala, A. W. Douglas, P. Eskola, M. H. Fisher, J. M. Hirshfield, K. Hoogsteen, A. Lusi, H. Mrozik, J. L. Smith, J. P. Springer, and R. L. Tolman. Abstract No. 465: Efficacy of the B_{1a} component, J. R. Egerton, D. A. Ostlind, L. S. Blair, C. H. Eary, D. Suhayda, R. F. Riek, and W. C. Campbell. Full papers to Abstracts No. 462, 463, and 465 have appeared in *Antimicrob. Agents Chemother.*, **15**, 361, 368, 372 (1979). (b) G. Albers-Schonberg, B. H. Arison, J. C. Chabala, A. W. Douglas, P. Eskola, M. H. Fisher, A. Lusi, H. Mrozik, J. L. Smith, and R. L. Tolman, *J. Am. Chem. Soc.*, preceding paper in this issue.

0002-7863/81/1503-4221$01.25/0

Lorentz, polarization, and background effects. The structure was solved by using a multisolution tangent formula approach[2] and Fourier techniques. From difference Fourier maps, three molecules of methanol were found cocrystallized with each molecule of the aglycone **1b**. Initial stages of structure refinement were undertaken with a block-diagonal least-squares technique[3] with final positions found by using full-matrix techniques. The function minimized was $\sum\omega(|F_o| - |F_c|)^2$ with $\omega = (1/\sigma F_o)^2$. All nonhydrogen atoms were refined with anisotropic temperature factors; hydrogens were added in calculated positions with isotropic temperature factors assigned corresponding to the temperature parameters of the atoms to which they were bound. The positions of these hydrogens were subsequently refined with the use of additional cycles of least-squares refinements giving a final unweighted residual of 0.072 for the 2510 observed reflections. Tables I, II, and III containing the fractional coordinates, bond distances, and bond angles, respectively, for **1b** may be found in the Supplementary Material. Figure 1 is a computer-generated drawing of the solid-state conformation of avermectin B_{2a} aglycon (**1b**).[4]



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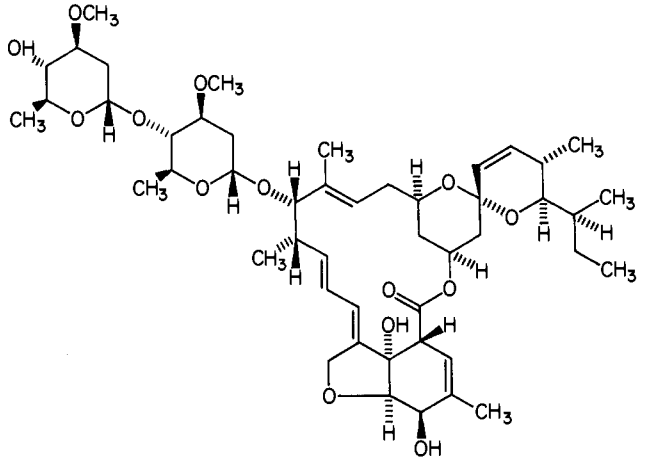

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Avermectin B_{1a} (3). Avermectin B_{1a} (**3**) ($C_{48}H_{72}O_{14}$) was recrystallized from methanol solutions to give large, clear parallelepipeds. The symmetry of the unit cell was *C*2 with $a = 39.362$ (13), $b = 9.500$ (3), $c = 14.694$ (2) Å and $\beta = 106.43$ (2)°, thus making $Z = 4$. Of the 3814 unique reflections measured with $\theta \leq 57°$, 3309 (87%) were above background ($I \geq 3\sigma I$). Graphite monochromated Cu Kα radiation ($\lambda = 1.5418$ Å) was used throughout the experiments. The observed reflections were corrected for Lorentz, polarization, and background effects.

The structure was solved with the use of a multisolution tangent formula approach[2] coupled with a tangent formula recycling procedure.[5] From Fourier difference maps a number of moderately high regions of electron density were found near and on the twofold axes of symmetry. This sort of arrangement is caused by channels of solvent molecules of methanol in the crystal structure. However, the methanol was badly disordered within the region and only two atoms, corresponding to oxygen atoms of methanol, could be identified with any degree of certainty. After least-squares refinements of the nonhydrogen atoms with anisotropic temperature factors,[3] hydrogens were added with isotropic temperature parameters corresponding to the atoms to which they are bonded. Additional full-matrix least-squares cycles varying positions of the hydrogens lowered the unweighted *R* factor to its present value of 0.073. Figure 2 is a computer-generated drawing[4] of avermectin B_{1a} (**3**) in the solid state. Tables IV, V, and VI contain the fractional coordinates, bond distances, and bond angles for avermectin B_{1a} (**3**). These tables may be found in the Supplementary Material. Bond distances between some of the atoms on the periphery of the molecule are shorter than normal because of rotary oscillation[6] but were not corrected.

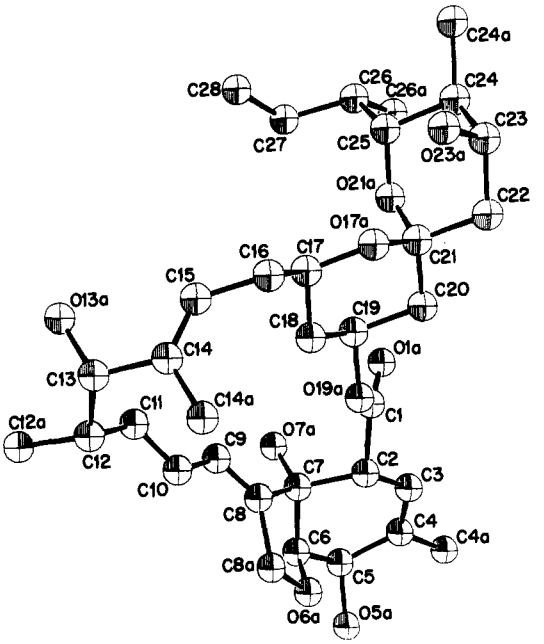


Figure 1. A computer-generated drawing of avermectin B_{2a} aglycon (**1b**) with hydrogens omitted for clarity.

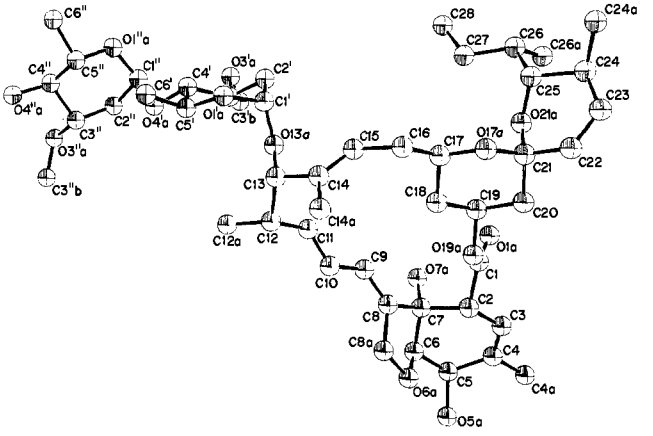


Figure 2. A computer-generated drawing of avermectin B_{1a} (**3**) with hydrogens omitted for clarity.

Discussion

X-ray Diffraction Results. Avermectin B_{2a} aglycon (**1b**) contains a 16-membered lactone ring, a spiroketal couched in two 6-membered rings, as well as a cyclohexenediol fused to a 5-membered cyclic ether. Because of the rigidity of the total molecule the double bond of the cyclohexene ring is not completely planar but has a torsional angle of 12°. Consequently, the cyclohexene ring has a chair conformation with the vertex at C4 quite flattened. The substituents C1 and O5a are equatorial while O6a and O7a

(2) G. Germain, P. Main, and M. M. Woolfson, *Acta Crystallogr., Sect. B*, **B26**, 274 (1970).
(3) J. M. Stewart, G. J. Kruger, H. L. Ammon, C. Dickinson, and S. R. Hall, "The X-ray System, Version of June 1972". TR-192, Computer Science Center, University of Maryland, College Park, Maryland, 1972.
(4) C. K. Johnson, "ORTEP-II: A Fortran Thermal-Ellipsoid Plot Program for Crystal Structure Illustrations", U.S. Atomic Energy Commission, Report ORNL-3794 (2nd Revision, with Supplemental Instructions), Oak Ridge National Laboratory, Oak Ridge, Tenn., 1970.
(5) J. Karle, *Acta Crystallogr., Sect. B*, **B24**, 182 (1968).
(6) G. H. Stout and L. H. Jensen, "X-ray structure determination", MacMillan, New York, 1968, pp 413 and 414.

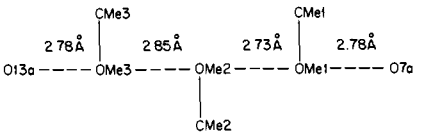


Figure 3. A schematic drawing showing the intramolecular network of hydrogen bonds for the cocrystallized methanol in the crystal lattice of **1b**.

are axial thereby implying that the five-membered cyclic ether ring is cis fused to the cyclohexene ring. The five-membered ring has close-to-an-envelope conformation with C6 being 0.67 Å out of the least-squares plane formed by the other four atoms. The two six-membered rings of the spiroketal system exist in chair conformations with C16, O19a, and C22 equatorial to one ring while C20, H23, C24a, and C26 are equatorial to the other. The transoid diene component of the 16-membered ring is 3° from planarity. As judged from models, the 16-membered lactone is quite rigid and from a detailed analysis of the ^{1}H NMR spectra of a related molecule the conformation in solution is virtually identical with the conformation in the solid state (vide infra).

An interesting arrangement of hydrogen bonds was found in this crystal lattice. The three molecules of methanol which cocrystallized with each molecule of the aglycon **1b** were found to intramolecularly link O7a to O13a, which are a distance of 7.9 Å apart, with a series of four hydrogen bonds. A schematic drawing is shown in Figure 3. In addition, intermolecular hydrogen bonds exist between O13a and O23a of 2.72 Å and O5a and O7a of 2.89 Å, while the possibility exists for an additional intramolecular hydrogen bond between O1a and O7a of 3.18 Å. Positions for hydrogens attached to oxygens were not determined.

Not surprisingly, the overall conformations of **1b** and **3** are quite similar but not identical. The cyclohexene ring in **3** has flattened into a sofa conformation as evidenced by the lowering of the torsion angle of the double bond to 2°. C7 is 0.64 Å remote from the best plane formed from the other five atoms of the ring. The cis-fused five-membered ring of **3** is a more distorted envelope conformation than **1b** with C6 0.61 Å out of the best plane formed from O6a, C7, C8, and O8a. In the spiroketal system the saturated ring is also in a chair conformation, but because of the introduction of the double bond between C22 and C23 the other ring has a half-chair conformation with O21a and C25 −0.31 Å and 0.45 Å, respectively, from the best plane formed from C21, C22, C23, and C24. The transoid diene is only 1° remote from planarity. The two oleandrose moieties attached as a disaccharide to C13 both have chair conformations with O13a axial and O3′a, O4′a, and C6′ equatorial to the first ring while O4′a is axial and O3″a, C4″a, and C6″ are equatorial to the second. The 6-membered ring of the first sugar is approximately perpendicular to the second sugar as well as the 16-membered lactone.

As would be expected, an extensive hydrogen-bonding network is important in fixing the molecules of **3** in the crystal lattice. Intermolecular hydrogen bonds have been determined based on the following oxygen–oxygen distances: O5a–O3″a, 3.14 Å; O7a–O4″a, 2.85 Å; O6a–OMe2 (methanol 2), 3.10 Å; O7a–OMe1 (methanol 1), 2.65 Å;[7] and OMe1 (methanol 1)–OMe2 (methanol 2), 2.43 Å.[7] In addition the possibility exists for an intramolecular hydrogen bond between O1a and O7a of 2.93 Å. Positions for hydrogens attached to oxygens were not determined.

^{1}H NMR Results. The sterochemical features of avermectin A_{2a} aglycon (**1c**), deduced from the ^{1}H NMR data,[1b] agree in all respects with the X-ray crystallographic results which virtually assures that the solution and solid-state conformations of the basic avermectin skeleton are the same. The fact, for example, that a large coupling constant in **1c** is associated with H17, H19, H24, and H25 is compelling evidence that these protons are axial. An equatorial designation for H23 is indicated from the small coupling constants with the neighboring H24 and H22. A torsional angle of about 35° for H5–C5–C6–H6 is estimated from the unmodified Karplus curve based upon the observed 5.5 Hz vicinal coupling

(7) Too short because of poor positions for the methanols.

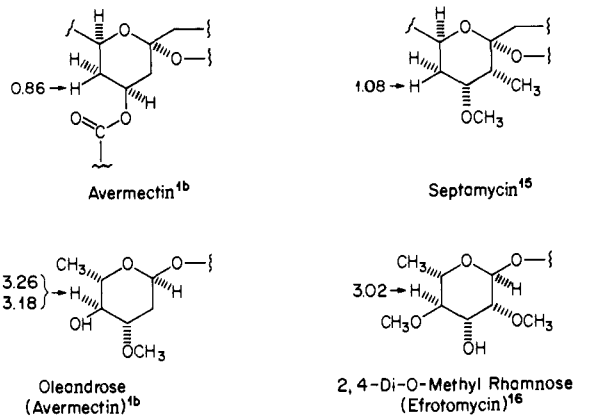


Figure 4. Shielded protons in tetrahydropyran derivatives.

constant. When electronegativity corrections are applied with the use of the equation $J_{vic} = J^0_{vic}(1 - 0.07\Delta X)$[8a] where $J^0_{vic} = 8$ Hz, $J_{5,6}$ becomes 6.0 Hz which corresponds to a dihedral angle of 30°. This compares with the value of 34 ± 2° obtained by the X-ray measurements. Good agreement between the NMR and X-ray results is also noted for the H12–C12–C13–H13 torsional angle where an electronegativity corrected approximate value of 50°, calculated from the NMR data ($J(obsd)_{13,12} = 2.8$ Hz), compares favorably to the 48 ± 2° value from the X-ray determination. The vicinal coupling constants associated with the diene system ($J_{10,11} = 14.3$ and $J_{9,10} = 10.0$ Hz) strongly suggest an all-trans relationship. A figure of 10 Hz is typical for trans vinyl protons coupled through a formal single bond.[8b]

On a more subtle level, the X-ray results place the H16 that is trans to the axial H17 near the plane of the C14–C15 double bond. This would account for the NMR observation which reveals this proton to be at lower field than the geminal H16. Normally, axial or pseudoaxial protons absorb at higher fields than their equatorial counterparts. The reversal of the usual relationship can be attributed to the deshielding influence of the double bond on protons lying close to and in the plane of the unsaturated system.

Addition of a trace of CD_3OD to the $CDCl_3$ solution of the A_{2a} aglycon (**1c**) induced a surprisingly large 0.06 ppm upfield shift for H19 suggesting that the methanol was localized in the vicinity of the carbonyl group presumably via hydrogen bonding. This inference is given weight by the X-ray results that avermectin B_{2a} aglycon (**1b**) cocrystallizes with three molecules of methanol which straddle the region between the lactone and O13a. The solvent molecule closest to the lactone is in a favorable location for hydrogen bonding with O1a and/or O7a.

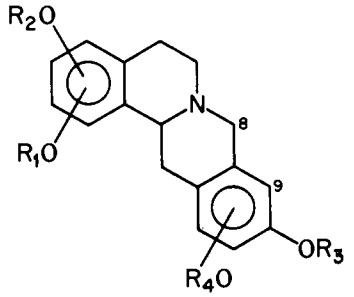
The shifts induced by benzene relative to $CDCl_3$ in **1c** are largely unexceptional (0.1 ppm downfield to 0.3 ppm upfield) aside from the 0.42 ppm downfield displacement of H19. Downfield shifts in benzene are limited to instances where a proton is near one or two negative groups.[9-11] A zone of high electron density in the vicinity of the affected proton seems to be a requirement. One would therefore conclude, in agreement with the X-ray results, that the carbonyl oxygen, O1a, and H19 are on the same side of the macrocyclic ring and are in reasonably close proximity.

An apparent disagreement between the NMR and X-ray analyses of **1c** concerned the description of the local environment of the axial H18. NMR evidence suggested that it was located close to and above the plane of an unsaturated grouping because of its distinctive high-field chemical shift of δ 0.86. This view conflicted with the X-ray results which indicated that the H18 was not suitably positioned to experience long-range shielding from

(8) (a) A. A. Bothner-By, "Advances in Magnetic Resonance", Vol. 1, Academic Press, New York, **1965**, p 202. (b) F. A. Bovey, "Nuclear Magnetic Resonance Spectroscopy", Academic Press, New York, 1969, p 360.
(9) D. H. Williams and D. A. Wilson, *J. Chem. Soc. B*, 144 (1966).
(10) J. H. Bowie, J. Ronayne, and D. H. Williams, *J. Chem. Soc. B* 535 (1967).
(11) R. G. Wilson and D. H. Williams, *Tetrahedron*, **25**, 155 (1969).

the carbonyl group or from any of the unsaturated moieties. The issue was resolved in favor of the X-ray results after it was observed that comparable shielding effects occurred in some tetrahydropyran derivatives without the intervention of unsaturated groups. This suggested that the displacements had their origin in oxygen lone-pair-electron interactions which are believed to be significant in the examples depicted in Figure 4 because of increased rigidity of the heterocyclic ring. The importance of rigidity is indicated by the absence of upfield shifts in tetrahydropyran, the 2-methyl, 2-(hydroxymethyl), and 2-(tetrahydrofurfuryloxy) analogues.[12] The high-field methylene signals in these simpler and presumably more flexible molecules all center around δ 1.5 with no absorbance above δ 1.4.

It is apparent that all of the highly shielded axial protons in Figure 4 are in similar environments relative to the oxygens of the substituted tetrahydropyran ring. While by no means established, it is felt that the endo-cyclic oxygen fulfills the proposed requirement for a rigid shielding source and probably effects the upfield displacements via a syn-axial relationship between the lone pair and the affected proton. Shielding by a lone pair has been invoked to account for the upfield displacements of axial protons which are α to the nitrogen in piperidines.[13] There appears to be no reason why a similar phenomenon would not be observed for oxygen although pertinent references are difficult to find. A possible example is in the tetrahydroprotoberberine alkaloids (**4**) in which the C8 methylene protons appear as a broad singlet in analogues unsubstituted at C9. Introduction of a 9-oxygenated substituent results in an upfield shift of ca. 0.4 ppm of the axial H8 and a comparable deshielding of the equatorial H8.[14] Models



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suggest that the shielded axial proton and the oxygen lone pair bear a similar stereochemical relationship as in the avermectins. Newman projections predict that similar diamagnetic shifts in avermectin should also be experienced by the axial H20 and by the axial H2 in the two oleandrose moieties. The data in Table III of ref 1b are consistent with this view in that the pertinent axial protons are upfield from their equatorial counterparts by $\geq$0.5 ppm.

Oxygen lone pair shielding may also account for the anomalously large 0.5 ppm upfield shift of the oleandrose H4″ that accompanies alkylation of the O4a″ hydroxyl. It is reasonable to suppose that the introduction of the equatorial alkyl group between the two existing equatorial substituents could alter the chair conformation of the tetrahydropyran ring which in turn could affect the lone pair/H4″ interaction.

The two sugars have been independently shown to be both α-L-oleandrose.[1b] Consequently, in addition to defining the relative stereochemistry of **3**, the crystal structure of **3** establishes the absolute configuration of the avermectins. The twelve asymmetric centers of **1b** are as follows: C2–R, C5–R, C6–R, C7–S, C12–S, C13–S, C17–R, C19–S, C21–R, C23–S, C24–S, C25–R, and C26–S. In addition, circular dichroism (CD) measurements have shown that the avermectins and the milbemycins (vide infra) have identical absolute configurations.[17] All figures and drawings show this correct absolute configuration.

Comparison with the Milbemycins. The structures of the avermectins were found to be closely related to those of the milbemycins,[18] a series of pesticidal metabolites from another *Streptomyces* strain. The experiments described above have shown that the relative as well as the absolute configuration of all corresponding stereochemical centers are identical. One of the principal differences between the avermectins and the milbemycins is that the former have *sec*-butyl and isopropyl side chains attached to C25 while the milbemycins have methyl and ethyl groups. The other major difference is that the avermectins have a disaccharide attached through an oxygen bonded to C13 while none of the milbemycins has an oxygen at C13.

Acknowledgment. We are indebted to W. C. Randall and T. W. Miller for obtaining CD measurements and to G. Albers-Schonberg and O. D. Hensens for helpful discussions.

Supplementary Material Available: Crystallographic data for avermectin B_{2a} aglycon (**1b**) and avermectin B_{1a} (**3**) (14 pages). Ordering information is given on any current masthead page.

(12) "Aldrich Catalog of NMR Spectra", Vol. 1 pp 152, 153, and 156.
(13) H. P. Hamlow, S. Okuda, and N. Nakagawa, *Tetrahedron Lett.* 2553 (1964).
(14) C. Y. Chen and D. B. MacLean, *Can J. Chem.*, **46**, 2501 (1968).
(15) N. A. Rodios and M. J. O. Anteunis, *J. Antibiot.*, **31**, 294 (1978).
(16) R. Wax, W. Maiese, R. Weston, and J. Birnbaum, *J. Antibiot.* **29(6)**, 670 (1976).
(17) T. W. Miller and W. C. Randall, Merck Sharp & Dohme Research Laboratory, Rahway, New Jersey, and West Point, Pennsylvania, private communications.
(18) Complete references are given in *J. Antibiot.* **29** (1976), Index of Compounds from Actinomycetes Nos. 14–16 and 35–42.