$$Me_{4-n}Ph_nSn + RLi \underset{}{\overset{K_{eq}}{\rightleftharpoons}} \underset{\mathbf{1}}{Me_{5-m}Ph_mSn^-Li^+} \quad (1)$$
$$R = Ph, Me$$

THF/HMPA.[7] Again, the signal multiplicities and intensities were fully consistent with the structures expected from 1:1 complexation of organolithium reagent and organostannane.

We believe that our organotin "ate" complexes have trigonal-bipyramidal structures as do most main group hypervalent pentacoordinate compounds.[8] Each compound showed only a single set of phenyl and methyl resonances in the ^{13}C spectra at temperatures down to −80 °C, although two methyl or phenyl signals would have been expected for all except $Ph_2Me_3Sn^-Li^+$ if the compounds were not fluxional. Pseudorotation must therefore be fast on the NMR time scale.[9] Values of $^1J_{CSn}$ and δ were calculated by assigning optimal J and δ values to apical and equatorial groups and averaging the appropriate number of each type of ligand. This simple model provided predictions that agreed well with the experimental values listed in Table I. Reasonable results were obtained only when the phenyl group was assigned a higher apicophilicity than methyl.[10] A tetragonal-pyramidal model did not fit the observed data. The calculated $^1J_{CSn}$ values for both methyl and phenyl carbons were as expected: small coupling to apical (low s character) and large coupling to equatorial carbons.[11] The phenyl ipso carbon shifts were also interesting: the equatorial shifts are unexceptional while the apical phenyls are at 180 ppm, close to the value for phenyllithium.[12,13]

Some qualitative observations on the equilibrium constants between the various "ate" complexes and their dissociated species (eq 1) have been made. The fraction of "ate" complex increases as the number of phenyl ligands is increased: the relative concentration of higher phenyl homologues (**1**, m = 3–5) is substantial even in pure THF solution, but the lower phenyl homologues (m < 3) need HMPA to form.[14]

The observation of tin "ate" complexes under conditions that have typically been used in the preparation of lithium reagents via the Li/Sn exchange raises the question of whether these complexes or the lithium reagents in equilibrium with them are the actual reactive species.[3] We cannot as yet answer this question, but both qualitative and quantitative reactivity tests indicate that the "ate" complexes are substantially less reactive than lithium reagents toward electrophiles such as trimethylsilyl chloride, n-butyl iodide, and dimethyl disulfide.

Recent spectroscopic[3a,b] and chemical[3c] studies of (α-alkoxyalkyl)tin systems directed at the question of stable tin "ate" complexes have given negative or indefinite results. This is not surprising, since these studies were carried out in ether, THF, or DME solution with tributyltin compounds, conditions that we now know are not favorable for the formation of "ate" complexes in NMR-detectable amounts. The earliest evidence for a pentaorganostannate that we have been able to find is a 1954 report by Beermann and Hartmann[15] who proposed the formation of sodium triphenyldiethynylstannate during reaction of triphenylethynyltin with sodium acetylide. Several tetraorganostannate complexes have been recently described.[16]

Our studies provide solid evidence for the existence of hypervalent pentaorgano tin "ate" complexes and implicate them as intermediates in the Li/Sn exchange. These findings coupled with our earlier evidence for a similar intermediate in the Li/I exchange of aryl iodides[1a] suggest that hypervalent "ate" complexes may be generally involed in lithium-metalloid exchange reactions.[17]

Acknowledgment. We thank the Petroleum Research Fund, administered by the American Chemical Society, the National Science Foundation, and PPG Industries for support of our work. Dr. Bruce Adams provided valuable assistance in the design and execution of NMR experiments.

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Crystal and Molecular Structure of Methotrexate

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Methotrexate is a potent inhibitor of the reduction of dihydrofolate to tetrahydrofolate by dihydrofolate reductase (DHFR), an essential step in the synthesis of nucleotide bases, and is consequently a successful and widely used antitumour drug.[1] For some years a major effort has been directed toward the rational design of improved inhibitors of DHFR action.[2] It is believed that the greatly enhanced binding of methotrexate to DHFR compared to that of the natural substrate, dihydrofolate, is associated with protonation of different conformations of these ligands.[3] Thus, a knowledge of the ground-state structure and conformation of both inhibitors and substrates is required for this rational design of new inhibitors to be pursued. Considerable work has gone into establishing the preferred conformations of dihydrofolate reductase ligands by experimental and theoretical methods.[4,5] Despite many attempts to crystallize and structurally characterize such molecules, to date, none has been successful.

We report herein the crystallization and the determination of the crystal structure of methotrexate. The conformation is similar in some respects to that seen for methotrexate bound to DHFR

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† School of Chemistry.
‡ Department of Pharmacy.

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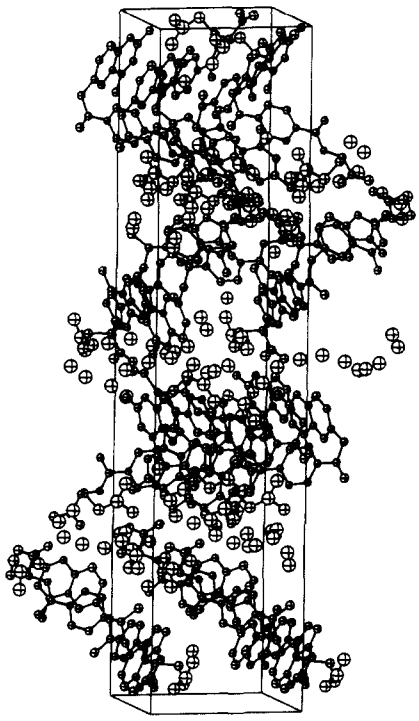


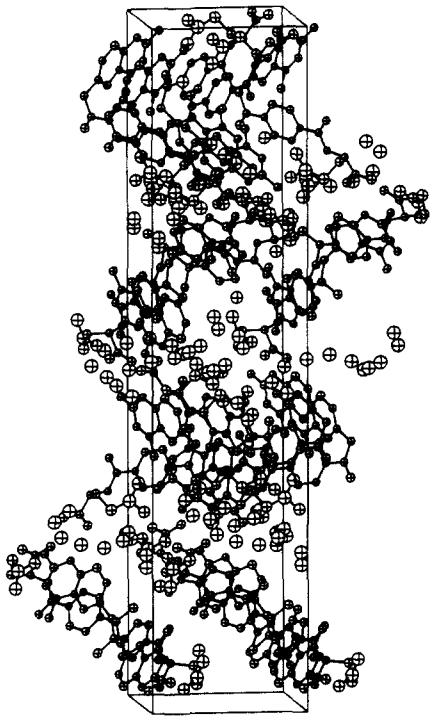
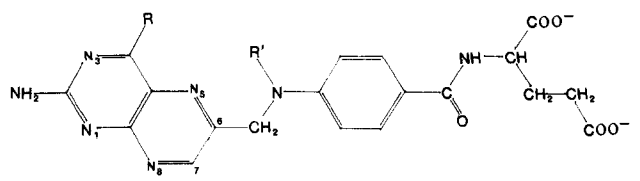


Figure 1. Stereoview of the unit cell of methotrexate.

but is even more similar to the conformation that has been proposed for dihydrofolate bound to DHFR.[3] It is concluded that binding may not impose as much strain on these ligands as had been previously suggested.[4,5] Methotrexate (I) from the manufacturer (Lederle Laboratories, American Cyanamid Co., Pearl River, NY) is a powder that has a low degree of crystallinity as evidenced by broad peaks in X-ray powder diffraction patterns. Recently, while attempting to modify the solid form of methotrexate, we obtained crystals sufficiently large for single-crystal X-ray diffraction: the parent material was dissolved in hot water containing 1% Tween 80 (pH 3.7) and allowed to cool to room temperature. An X-ray powder diffraction pattern of the material thus formed was distinctly different from that of the parent material. However, spectroscopic tests (UV, IR, ^{1}H NMR, and mass spectroscopy) confirmed that the material was methotrexate with solvent of crystallization.



I, methotrexate: R = NH_2, R′ = CH_3
II, dihydrofolate: R = O, R′ = H. C_7 and N_8 positions hydrogenated

A crystal of dimensions 0.20 × 0.10 × 0.10 mm was obtained and found to be tetragonal, space group $P4_12_12$ with a = 10.343 (2) Å and c = 45.521 (6) Å. X-ray diffractometer data were collected using Mo Kα radiation to a 2θ angle of 40°. The structure was solved by direct methods, which revealed the p-aminobenzoyl group, extended by subsequent difference Fourier synthesis, and refined by full-matrix least-squares methods to an R factor (on 983 F, $I > 2.0\sigma(I)$) of 0.083.

The structure consists of layers of hydrophobic and hydrophilic character alternating along the c axis (Figure 1). The hydrophobic layers accomodate the pteridine and benzoyl groups stacked so that they overlay benzoyl and pteridine groups, respectively, of adjacent molecules. The carboxylate groups are disposed into the hydrophilic layers which also accommodate disordered water molecules. The layers are linked by a very strong pair of hydrogen bonds from the two oxygens of the α-carboxylate to N1 and the 2-amino group (N2) of the pteridine (N···O, 2.62 (3) and 2.77 (3) Å). It is interesting that an identical hydrogen bond pair between a carboxylate of an aspartate and the pteridine is observed in the binding of methotrexate to DHFR.[3] The enhanced binding of methotrexate over dihydrofolate (II) is largely due to the ability of methotrexate to form this strong interaction which is in turn due to the greater basicity of N1.[6] It is not possible to establish, with the limited diffraction data currently available, whether N1 is protonated. However, the formation of a hydrogen bond between N1 and an α-carboxylate oxygen atom implies that one of these two atoms is protonated and the pK_a's, which are 5.7 and 2.7, respectively,[7] indicate it is N1 which is protonated. The bond lengths and planarity of the pteridine group are not significantly different than those observed in pteridine itself,[8] though again the resolution of the present structure limits the implications that can be drawn from this similarity. There is a strong hydrogen bond between the 4-amino group and the benzoyl oxygen, also reminiscent of the interaction between this amine and a peptide oxygen of DHFR.[3]

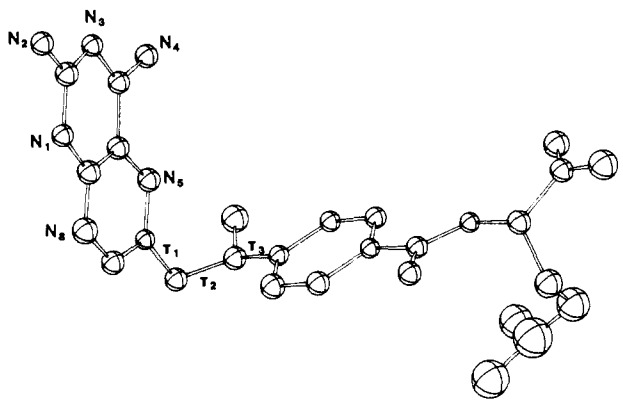


Figure 2. Methotrexate molecule and atom numbering.

The conformation of methotrexate (Figure 2) is best described as two planar groups; the first defined by the 2,4-diaminopteridine

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group and the second by the (*p*-aminobenzoyl)-L-glutamate. These planes meet at an angle of 84°. The α-carboxylate lies nearly in the latter plane while the γ-carboxylate is folded back and lies roughly parallel to the same plane. The conformation of the glutamate portion of methotrexate is different than that seen for the bound forms.[3] Considerable variability is observed in the confomation of the bound forms so there is evidently a large degree of conformational freedom in this part of the molecule.

The conformational parameters of most direct relevance to the binding of methotrexate are the three torsion angles (τ_1, τ_2, and τ_3) through the pteridine to *p*-aminobenzoyl linkage. Two of these angles, τ_2 and τ_3, are similar to those observed in methotrexate bound to DHFR while τ_1 is approximately 180° different.[3] Thus, the conformation observed here, for methotrexate, is similar to that suggested for bound dihydrofolate[3] that is, with the pteridine ring flipped over. It is possible that the conformation in the crystal is stabilized by the ring stacking or hydrogen bonding interactions described above. However, the constancy of the τ_2 and τ_3 torsion angles over three independent determinations of methotrexate bound to DHFR[3] and in the present structure suggests that this conformation is a potential energy minimum. Likewise, the two alternative dispositions of the pteridine ring appear to represent preferred geometries. These results are in disagreement with theoretical studies,[4,5] and are therefore of importance to those attempting to design new inhibitors of DHFR.

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Supplementary Material Available: Tables of least-squares coordinates and thermal parameters for the non-hydrogen atoms of methotrexate (2 pages). Ordering information is given on any current masthead page.

Model for the Polyepoxide Cyclization Route to Polyether Antibiotics

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The polyepoxide cyclization mechanism of Westley and Cane for the biosynthesis of polyether antibiotics is appealing because of the simplicity with which the stereochemically complex polytetrahydrofuranoid segments are assembled from a basically achiral polyolefinic precursor.[1] While several previous synthesis of these materials have used such cyclizations in a stepwise manner,[2] the most effective route of this type would require only two steps from polyolefin, namely, polyepoxidation and polycyclization. In this paper we describe the direct preparation of a triepoxide closely related to the C9–C23 segment of monensin B from a triene precursor and its acid-catalyzed polycyclization to tristetrahydrofuranoid material (Scheme I).[3]

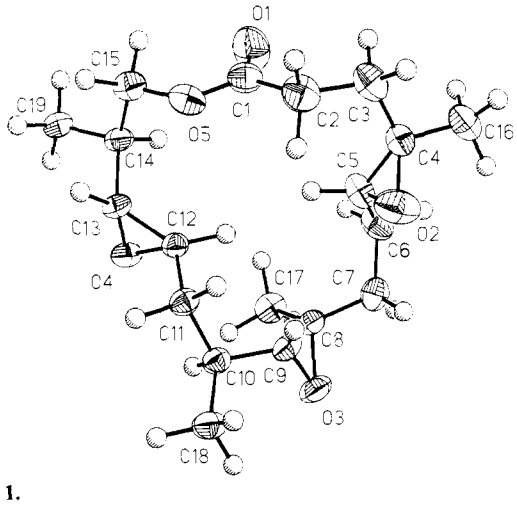
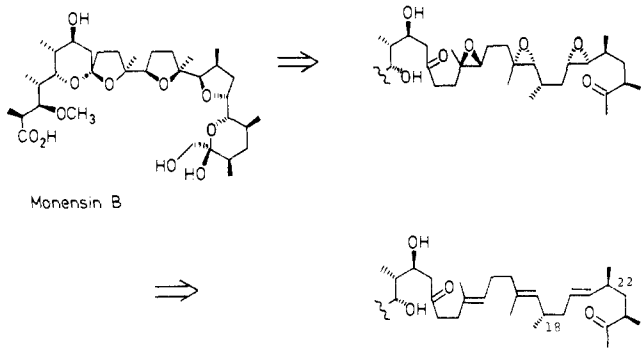
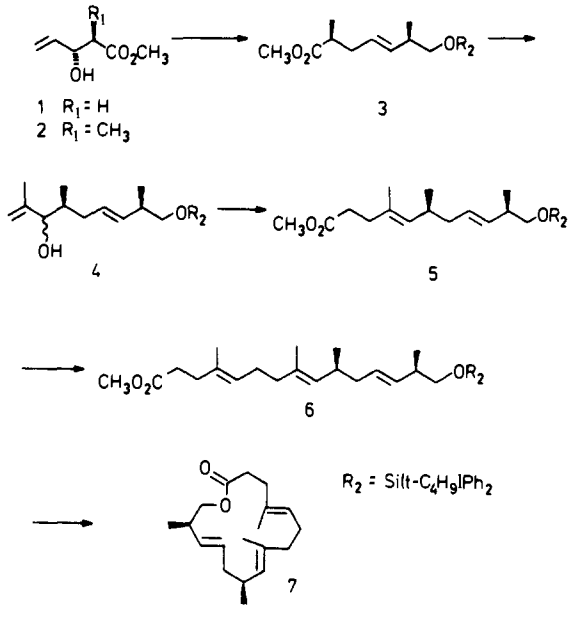


Figure 1.

Scheme I



Scheme II



The key synthetic problem of the polyene–polyepoxide–polyether approach is stereochemical control of the polyepoxidation. While enantioselective epoxidation offers a potential solution, current chiral epoxidation methodology necessitates adjacent hydroxyl groups and the fact that the disubstituted C20–C21 epoxide differs from the two trisubstituted epoxides enantiomerically in monensin makes such a scheme impractical. A more effective approach would use resident chirality at C18 and C22 of the monensin B triene for epoxidation stereocontrol.

Preparation of the appropriate triene follows established methodology and begins with hydroxypentenoic ester **1**. The second chiral center is established by β-hydroxy ester dianion

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