potential utility of this approach. The role of polyamines in trypanosomes and their probable dependence on ODC as sole putrescine source emphasize their likely vulnerability to specific inhibitors of polyamine biosynthesis. Inhibitors of ODC may be particularly useful both as sole agents and in combination with currently available trypanocidal compounds.

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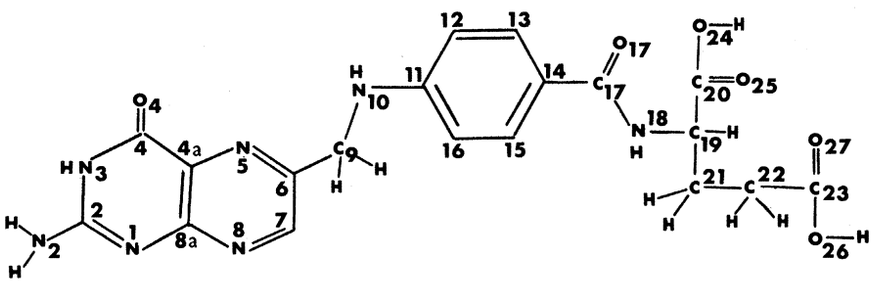
Folic Acid: Crystal Structure and Implications for Enzyme Binding

Abstract. *The crystal and molecular structure of folic acid dihydrate has been determined by x-ray diffraction. Folic acid is in an extended conformation with the pteridine ring in the keto form. The C(4) oxygen and N(10) atoms are on the same side of the molecule, hydrogen-bonded to the same water. This conformation has the pteridine rotated approximately 180° away from the orientation of the pteridine ring of methotrexate bound to dihydrofolate reductase. The folic acid pteridine and phenyl rings interact in a stacking manner which is suggestive of the type of associations these groups could form in a complex of folate, dihydrofolate reductase, and reduced nicotinamide adenine dinucleotide phosphate.*

The B vitamin folic acid is a necessary cell growth factor; the reduced form of the vitamin, 5,6,7,8-tetrahydrofolate, is an essential cofactor in a large number of enzyme reactions involving one-carbon transfers, including the synthesis of thymidylate from deoxyuridylate. Since a lack of thymidylate stops DNA synthesis and cell division, blockade of tetrahydrofolate formation generally leads to cell death. In mammalian cells folic acid is reduced first to 7,8-dihydrofolate and then to tetrahydrofolate, both steps being catalyzed by the enzyme dihydrofolate reductase (DHFR). This system has been the focus of a great deal of



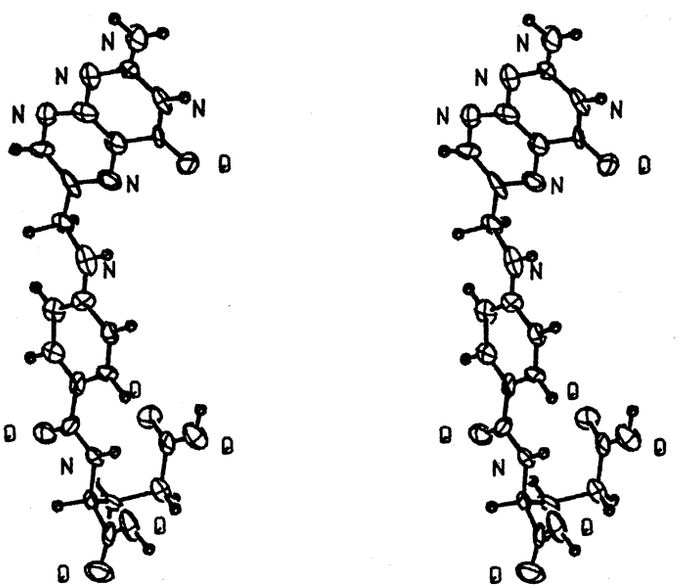


Fig. 1 (above). Folic acid chemical structure and atom numbering scheme. Fig. 2 (right). Stereoscopic drawing of the folic acid molecular structure.

research aimed at developing anticancer agents. If very selective folic acid antagonists could be developed which could exploit evolutionary differences between the DHFR enzyme or folate transport systems of normal and tumor cells and inhibit tumor cell DHFR preferentially, they would be extremely valuable anticancer drugs. Much effort has been expended on the development of such antagonists, but, until now, without benefit of detailed stereochemical information on folates. We report here the three-dimensional molecular conformation of folic acid determined from its crystal structure.

Crystals of folic acid dihydrate ($C_{19}H_{19}N_7O_6 \cdot 2H_2O$) were grown from a mixture of dimethyl sulfoxide, water, and ethanol. They are orthorhombic, space group $P2_12_12_1$, with unit cell dimensions a = 7.295(2) Å, b = 8.655(3) Å, and c = 32.545(15) Å; Z = 4 molecules in the unit cell; and a calculated density of 1.54 g/cm^3. A very thin platelike crystal (0.75 by 0.15 by 0.01 mm) was used to measure intensities of 1525 independent x-ray reflections with a FACS-I diffractometer, using nickel-filtered copper radiation to a 2θ limit of 110°. Of these, 845 reflections were found to have intensities greater than twice their standard deviation and were classified as observed.

The structure was solved by direct methods. An electron density (E) map clearly showed 27 of the 32 nonhydrogen atoms of the folic acid molecule. The remaining atoms, including two water oxygens and all but one hydrogen, were located from subsequent least-squares refinements and difference Fourier syntheses. The hydrogens were given isotropic temperature factors equal to the values for the atoms to which they are bonded and were held stationary during refinement. All heavy atoms except the water oxygens were given anisotropic thermal parameters and the 297 variables including the scale factor were refined by least-square procedures, using all 1525 reflections, to a discrepancy (R) index of 0.184. The R value based on the observed data is 0.146.

Figure 1 shows a line drawing of the molecule with the numbering scheme which will be used in the description of the structure. A stereoscopic view of the molecule (Fig. 2) shows folic acid to be in an extended conformation. The substituted pterin and p-aminobenzoyl groups are planar to within 0.06 and 0.03 Å, respectively, and the dihedral angle between their planes is 27°. The two substituent atoms N(2) and C(9) are 0.11 Å from the least-squares plane of the pteridine ring system; atoms N(18) and C(19) are 0.01 and 0.03 Å, respectively, out of the plane of the p-aminobenzoyl group. The values of the following selected torsion angles indicate the extended nature of the conformation: N(5)-C(6)-C(9)-N(10), 31°; C(6)-C(9)-N(10)-C(11), 180°; C(13)-C(14)-C(17)-N(18), 175°; and C(14)-C(17)-N(18)-C(19), −178°.

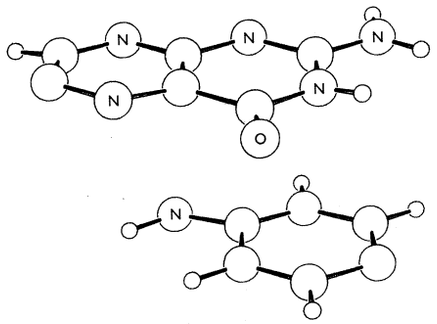


Fig. 3. Intermolecular stacking arrangement of the pteridine and phenyl rings in the folic acid crystal structure. Perpendicular separation of the two ring planes is 3.28 Å.

The observed C(4)-O(4) bond distance is 1.23 ± 0.03 Å; this value plus location of the hydrogen attached to N(3) clearly establishes the keto rather than the enol form of the folic acid molecule in the crystal. This result supports other studies which have shown the keto-hydroxy equilibrium to lie strongly on the keto side (*1*).

Each folic acid is hydrogen-bonded to two other symmetry-related molecules and to the two water molecules. Two hydrogen bonds are formed between the N(2) hydrogen and N(1) atoms and the alpha carboxyl group of the glutamic acid end of a symmetry-related molecule. Another pair of hydrogen bonds is observed between the N(3) hydrogen and O(4) atoms of the pteridine ring and the gamma carboxyl group of the glutamic acid end of a second symmetry-related folic acid. This second symmetry-related molecule is positioned so that its p-aminobenzoyl group is below the pteridine ring. The two rings are almost exactly parallel (the dihedral angle between them is only 2.7°), and the shortest intermolecular contacts occur between C(8a) and N(10) (3.47 Å), C(2) and C(16) (3.33 Å), and C(4) and C(12) (3.33 Å). The perpendicular separation between the ring planes is 3.28 Å. Figure 3 illustrates the stacking arrangement of the pteridine and phenyl rings. The interplanar separation and the parallel alignment of the two groups indicate that there is significant interaction between the delocalized pi electrons of the pteridine and p-aminobenzoyl rings. A hydrogen-bonding scheme similar to that observed in this structure and the ability to participate in such stacking interactions may be of importance in the binding of folates at the DHFR active site.

The orientation of the pteridine group relative to the rest of the molecule is stabilized by hydrogen bonds between a water molecule and the C(4) oxygen and N(10) atoms (Fig. 4). It is possible that a similar hydrogen-bonded conformation could be formed in the folate-DHFR complex through participation of a serine, threonine, or tyrosine hydroxyl.

Although there have been no crystallographic studies to date on folate or dihydrofolate bound to DHFR, crystal structure analyses of DHFR from *Escherichia coli* and from *Lactobacillus casei* have been published. In the first study (*2*) the folate antagonist methotrexate is bound to the DHFR; the second structure (*3*) is a ternary complex of the enzyme, methotrexate, and reduced nicotinamide adenine dinucleotide phosphate (NADPH). Methotrexate is a potent DHFR inhibitor that differs structurally from folic acid only by substitution of an amino group for the oxygen bonded to C(4) and replacement of the hydrogen at N(10) by a methyl group. There are some noteworthy similarities and differences between the conformation of folic acid in its crystal structure and the conformation of methotrexate bound to DHFR. Folic acid is in an extended conformation with the torsion angle C(6)-C(9)-N(10)-C(11) equal to 180° (Fig. 2), while methotrexate binds to DHFR in a somewhat bent conformation with the analogous torsion angle close to 90°.

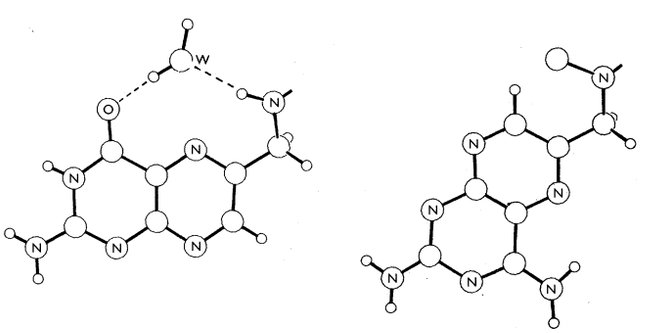


Fig. 4. Comparison of the orientation of the pteridine ring in the folic acid crystal structure (left) and in the methotrexate-DHFR complex (right). The water molecule (W) hydrogen-bonded to the O(4) and N(10) atoms of folic acid is also shown.

More importantly, in terms of the possible mode of substrate-enzyme binding, in folic acid the C(4) oxygen and N(10) hydrogen are on the same side of the pteridine ring, hydrogen-bonded through a water molecule, whereas in the enzyme-inhibitor complex the methotrexate C(4) amino group and N(10) methyl are on opposite sides of the ring. Thus the orientation of the pteridine ring in the methotrexate-enzyme complex differs from the orientation of the same ring in folic acid by an ~180° rotation about the C(6)-C(9) bond. This rotational difference is illustrated in Fig. 4. A number of spectroscopic observations have indicated that there are differences in the way methotrexate and folate bind to DHFR. Matthews *et al.* (*3*) found from model-fitting experiments that the DHFR active site could accommodate a molecule with the orientation of the pteridine ring opposite to that found for methotrexate; strong evidence for such an altered orientation for folate has recently been provided by Charlton *et al.* (*4*). The conformation of folic acid in the crystal adds further support for this concept and provides a model at the atomic resolution level for what that orientation may be.

The intermolecular hydrophobic interaction between the pteridine and phenyl rings in the folic acid crystal structure (Fig. 3) is also suggestive of conformational arrangements that could occur in the enzyme-folate-NADPH complex. The phenyl ring of methotrexate participates in hydrophobic interactions in both DHFR complexes: in *Escherichia coli* with an isoleucine and in the *Lactobacillus casei* complex through a parallel stacking arrangement with the aromatic ring of a phenylalanine. It is likely that the folate *p*-aminobenzoyl ring binds in a similar manner. In the *L. casei* ternary complex the methotrexate pteridine ring is inclined at about 45° to the plane of the NADPH nicotinamide. It may be that folate-DHFR productive binding and reduction of folate by hydride transfer from NADPH in the ternary complex are facilitated by a parallel association of the pteridine and nicotinamide rings similar to the stacking interaction observed in the folic acid crystal.

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JH Zero: New Naturally Occurring Insect Juvenile Hormone from Developing Embryos of the Tobacco Hornworm

Abstract. *A new insect juvenile hormone was isolated from developing embryos of the tobacco hornworm moth,* Manduca sexta. *The new hormone was found with juvenile hormone I and is a 1-carbon homolog of this substance. The assigned structure is methyl (2*E*,6*E*,10-*cis*)-10,11-epoxy-3,7-diethyl-11-methyl-2,6-tridecadienoate, which constitutes a trishomosesquiterpenoid skeleton. This is the first chemical identification of any juvenile hormone from insect eggs.*

Three insect juvenile hormones (JH) have been isolated and characterized from various species representing several insect orders (*1*, *2*). Biosynthetically the most "conventional" (*3*) of the JH's, JH III (structure **4** in Fig. 1) is found in at least one stage of development in nearly all insects surveyed to date (*1*). The homosesquiterpenoid and bishomosesquiterpenoid, JH II (**3**) and JH I (**2**), occur mainly in Lepidoptera, although both have been reported in one species of cockroach (*4*). The latter JH's, arising biosynthetically from propionate and acetate precursors (*3*), are produced by pathways that now appear to be peculiar to insects. Theoretically, organisms that produce JH I could have the biosynthetic capability of elaborating even higher sesquiterpenoid homologs to serve potentially in a hormonal capacity. We now report the identification of JH 0 (**1**), isolated together with its biosynthetic cognate JH I from eggs of the tobacco hornworm *Manduca sexta*. Comparison of mass spectral and chromatographic data from JH 0 and two of its derivatives with synthetic standards confirmed the assignment of structure as a homolog of JH I, bearing an ethyl group at C-3. Approximately 200 ng of natural material was available for all of the structure elucidation work performed.

We recently have studied JH titers throughout the life cycle of *M. sexta* (*5*) using coupled gas chromatography (GC)–mass spectroscopy (MS). By means of a multiple peak-scanning technique, selected ion monitoring, we use the mass spectrometer as a sensitive and selective GC detector, monitoring one (or more) prominent ions produced by electron impact fragmentation of a JH derivative resulting from d_3-methanolysis (**1a** to **4a** in Fig. 1). Such analysis of a purified and appropriately derivatized extract allows quantitative and qualitative analysis of insect JH titers (*6*).

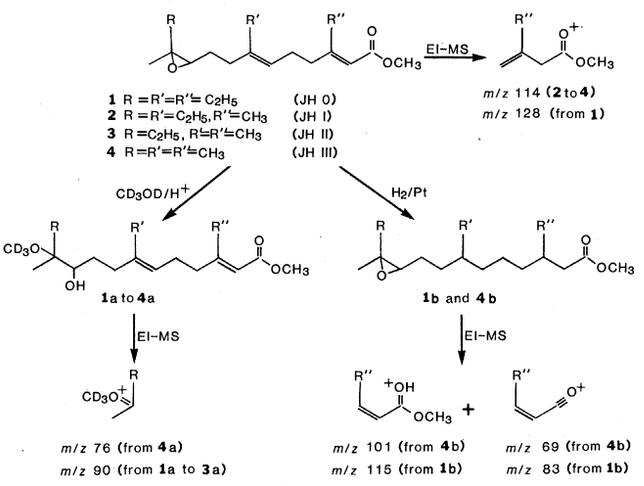

Fig. 1. Important mass spectral fragment ions of the juvenile hormones (**1** to **4**), their methoxy-d_3-hydrin derivatives (**1a** to **4a**), and their tetrahydro derivatives (**1b** and **4b**); *EI*, electron impact.

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