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Absolute Configuration of Biological Tetrahydrofolates. A Crystallographic Determination

Sir:

Derivatives of tetrahydrofolic acid are involved in a large number of enzyme-mediated biological reactions.[1] These optically active derivatives all contain an asymmetric carbon atom at the 6 position of the reduced pteridine ring, plus the asymmetric centers in the L-glutamic acid residues. Most of the enzymic reactions that involve tetrahydrofolates are stereospecific and require one particular configuration at atom C-6. However, the absolute configuration of the C-6 position has not been determined, largely because of difficulties involved in the de novo chemical synthesis of specific stereoisomers of tetrahydrofolates, and in efforts to grow folate crystals that are suitable for X-ray analysis.

We have succeeded in crystallizing 5,10-methenyl-5,6,7,8-tetrahydrofolic acid (5,10-CH-THF)$^+$, which is an intermediate in the chemical and enzymic conversions of 5- and 10-formyl-5,6,7,8-tetrahydrofolic acids (5-CHO-THF and 10-CHO-THF); is produced by enzymic transformations of 5-formimino- and 5,10-methylene-5,6,7,8-tetrahydrofolic acids; and is a cofactor in the formylation of glycinamide ribonucleotide by the transformylase (E.C. 2.1.2.2). Here we present the crystal structure of the natural diastereomer of (5,10-CH-THF)$^+$, and the crystal structure of the diastereomer with the unnatural configuration at the C-6 position. The results of these two crystallographic analyses indicate the absolute configuration at atom C-6 in the tetrahydrofolates of biological systems.

A small amount of pure (−)-L-5-CHO-THF was isolated during the large-scale preparation of *dl*-L-5-CHO-THF from *dl*-L-THF via the methenyl compound.[2] This material has the natural configuration at the C-6 position, as evidenced by the finding that it is about twice as effective as the corresponding racemic mixture in supporting bacterial growth in liquid media, and in reversing in vivo methotrexate toxicity.[3] A crystalline sample of (+)-L-(5,10-CH-THF)$^+$Cl$^-$·HCl·H_2O was obtained by treating (−)-L-5-CHO-THF with dilute hydrochloric acid.[2] Crystals of the bromide hydrobromide salt of (5,10-CH-THF)$^+$ were grown by dissolving the chloride salt in 48% (w/w) aqueous hydrobromic acid and equilibrating this solution, by vapor diffusion, against a large excess of 29% (w/w) aqueous hydrobromic acid. The crystals, which grow as large yellow plates, are monoclinic, space group $P2_1$, with a = 12.696 (1), b = 14.487 (2), c = 6.990 (1) Å; β = 100.80 (1)°. Three-dimensional intensities for 2162 independent reflections, measured on an automated diffractometer, were used for the structural analysis. A trial structure was obtained by the heavy-atom method and was refined by full-matrix least squares to an R index ($\Sigma||F_o| - |F_c||/\Sigma|F_o|$) of 0.082. The absolute configuration, which was originally assigned from the fact that the glutamate residue has the L configuration, was confirmed by the use of anomalous dispersion data. The absolute configuration and conformation of this natural diastereomer of (5,10-CH-THF)$^+$ are shown in Figure 1a.

The unnatural diastereomer of 5,10-CH_2-THF was prepared from (±)-5,10-CH_2-THF by the enzymic depletion (thymidylate synthetase)[5] of the natural diastereomer, (+)-5,10-CH_2-THF. This material was converted into (−)-(5,10-CH-THF)$^+$ by treatment with formic acid containing 2-mercaptoethanol.[6,7] Treatment with dilute hydrochloric acid then converted the compound into the chloride hydrochloride salt, which was purified by column chromatography. Crystals of the bromide hydrobromide salt were prepared by the same technique described for (+)-(5,10-CH-THF)$^+$. The yellow, platelike crystals are monoclinic, space group $P2_1$, with a = 12.459 (1), b = 14.528 (4), c = 7.006 (2) Å; β = 96.06 (2)°. X-ray intensity data were collected for 2358 independent reflections. A trial structure was obtained by the heavy-atom method and was refined by full-matrix least squares to R = 0.056. Anomalous dispersion effects again were used to confirm the absolute configuration. The structure of this unnatural diastereomer is shown in Figure 1b.

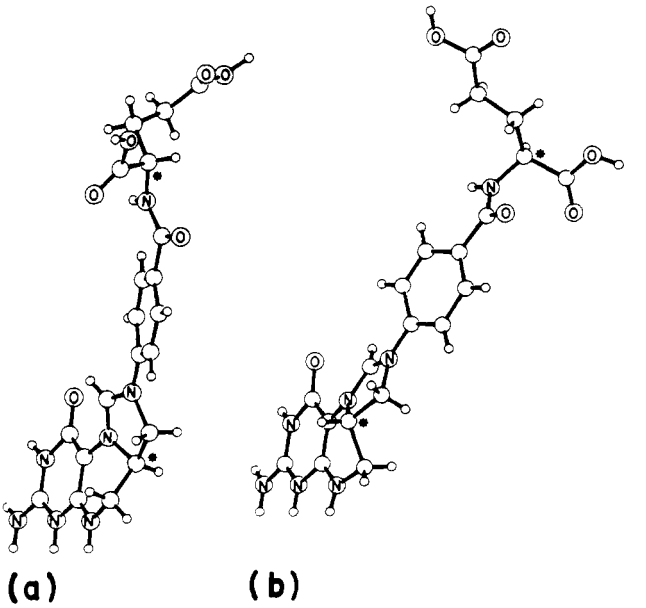

Figure 1. Structures of the N-1-protonated derivatives of (a) (+)-(5,10-CH-THF)$^+$, the natural diastereomer, and (b) (−)-(5,10-CH-THF)$^+$, the unnatural diastereomer. The asymmetric carbon atoms are designated by asterisks. This drawing was prepared by use of the computer program ORTEP.[4]

As can be seen from Figure 1, the major difference between these two diastereomers involves the configuration at the C-6 position. For the natural diastereomer (Figure 1a) the absolute configuration of atom C-6 in the reduced pyrazine ring is R, which corresponds to the *S* configuration for 5,6,7,8-tetrahydrofolic acid. Those portions of the molecules which include the pyrimidine, tetrahydropyrazine, imidazole, and benzene rings, plus the carbonyl group, are nearly mirror images in the crystal structures of the two diastereomers. However, the conformations of the L-glutamate residues are different in the two structures. In both diastereomers the benzene and heterocyclic rings form a nearly planar arrangement with all component atoms lying within 0.35 Å of a common plane; the observed bond lengths indicate that there is a resonating, conjugated system through the pyrimidine ring, the upper portion of tetrahydropyrazine and imidazole rings, and the benzene ring.[3]

In summary, our crystallographic results indicate that the configuration of natural (5,10-CH-THF)$^+$ is the one depicted in Figure 1a. Since all tetrahydrofolic acid derivatives in biological systems are interconvertible through enzymic and chemical reactions that retain the stereochemistry at C-6, it is clear that the absolute configuration depicted in Figure 1a is the one that would be expected for other natural derivatives of tetrahydrofolic acid.[1] Knowledge of this configuration should be of immediate use in efforts to understand enzymic reactions that require folate cofactors. For example, a recent

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crystallographic study by Matthews et al.[8] of the ternary complex that *Lactobacillus casei* dihydrofolate reductase (DHFR) forms with NADPH and methotrexate (MTX) indicates that the tetrahydrofolic acid produced by DHFR would have the unnatural *R* configuration at C-6, if dihydrofolic acid (DHF) binds to the enzyme in the same orientation found for MTX. However, these authors have suggested that MTX and DHF might bind to the enzyme in different orientations, with the pteridine rings rotated 180° relative to each other; this alternative orientation would lead to the *S* configuration at atom C-6 of THF, as required by our results.

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Pentacarbonyl(7-η^1-cycloheptatrienyl)rhenium. Synthesis and Fluxional Behavior of a *Monohapto*cycloheptatrienyl Derivative of a Transition Metal

Sir:

Although the η^3, η^5, and η^7 bonding modes of the cycloheptatrienyl ligand are well established, no *monohapto*-7-cycloheptatrienyl derivative of a transition metal is known.[1] The lack of such compounds is noteworthy, since they would provide the simplest cyclic system in which a 1,5 sigmatropic shift (as expected from orbital symmetry rules[2] for "ordinary" migrating groups) could be distinguished from the 1,2 (least motion) pathway. For the intensively studied η^1-C_5H_5 derivatives,[3] these pathways are of course not distinguishable. We now report the synthesis of (7-η^1-C_7H_7)$Re(CO)_5$, the first *monohapto*-7-cycloheptatrienyl derivative of a transition metal, and a study of its fluxional character which establishes a 1,2 shift as the only observable migration pathway.

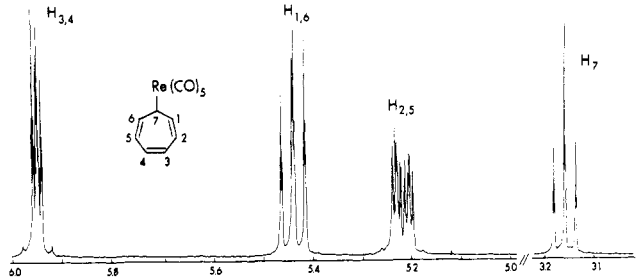
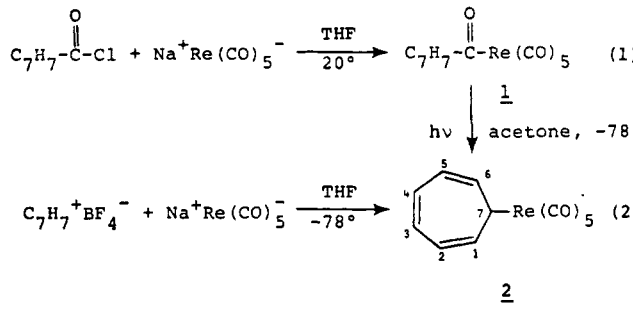


Figure 1. 400-MHz ^1H NMR spectrum of (7-η^1-C_7H_7)$Re(CO)_5$ (**2**) in methylcyclohexane-d_{14} at 30 °C. Scale is in parts per million from Me_4Si = 0. Precise chemical shifts and assignments are given in note 7. Irradiation at δ 3.16 ppm causes an intensity decrease in the signal at δ 5.44 ppm.

The title compound has been prepared in two ways. In the first (eq 1), addition of 7-cycloheptatrienylacyl chloride[4] to



a tetrahydrofuran (THF) solution of $Na^+Re(CO)_5^-$ affords the acyl **1**.[5] Decarbonylation of **1** under ultraviolet light[6] affords the 7-η^1-cycloheptatrienyl derivative **2** as orange, air-stable needles.[7,8] In the second method (eq 2) reaction of tropylium cation with $Na^+Re(CO)_5^-$ affords **2** in 90% yield; this facile reaction is surprising in view of earlier reports of carbonyl anion–tropylium cation reactions in which metal carbonyl dimers and ditropyl are formed,[10] or one instance where a trihapto derivative was formed in low yield.[11] We attribute the difference to the strength of rhenium–carbon bonds.[12]

The fluxional behavior of **2** was studied using the spin saturation transfer technique[13] in dioxane-d_8.[14] Irradiation of H_7 (see Figure 1) in the 25–37° range caused the $H_{1,6}$ resonance to decrease in intensity[15] while the other olefinic resonances were unaffected. This result is a clear, qualitative indication that H_7 is exchanging with $H_{1,6}$ but not with $H_{2,5}$ or $H_{3,4}$, i.e., that a 1,2 shift is taking place.[16] Quantitative results are summarized in Table I, from which activation parameters[17] for the 1,2 shift in **2** are as follows: $\Delta G^{\ddagger}_{300}$ = 19.8 ± 0.1 kcal mol^{-1}, $\Delta H^{\ddagger}$ = 18.1 ± 1.9 kcal mol^{-1}, $\Delta S^{\ddagger}$ = −5.7 ± 2 eu.[18]

It is of interest to compare the 1,2 or least motion shift established here for **2** with the 1,5 shift observed[19] and recently confirmed[20] in (7-η^1-C_7H_7)$Sn(C_6H_5)_3$ (**3**), for which $\Delta G^{\ddagger}_{300}$ = 15.44 ± 0.14 kcal mol^{-1}.[20] Migration in the tin derivative conforms to the ordinary symmetry rules, while in the transition metal derivative it does not. It has occasionally been suggested[20,21] that orbital symmetry restrictions might be relaxed when the migrating group possesses valence-shell d orbitals; the present case provides the first test of this possi-

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